ELSEVIER

Journal of Chromatography A, 697 (1995) 219–227

JOURNAL OF CHROMATOGRAPHY A

Chromatographic behaviour of positional isomers on porous graphitic carbon

Qian H. Wan, P. Nicholas Shaw, Martyn C. Davies, David A. Barrett*

Department of Pharmaceutical Sciences, University Park, University of Nottingham, Nottingham, NG7 2RD, UK

Abstract

Retention characteristics of six sets of ionizable substituted benzene isomers have been measured on a porous graphitic carbon column using buffered aqueous eluents containing 35% acetonitrile. The elution orders varied with the different types of substituents on the benzene ring and were not readily predictable from the known structures of the isomers. Over the range of mobile phase between pH 2 to 9.4, the retention of the solutes was correlated directly with their degree of ionization, with the ionized form being least retained. An interpretation of these data was suggested based on the solute molecular orientation effect induced by the competing interactions of solute with adsorbent and solvent.

1. Introduction

The retention behaviour of positional isomers in liquid chromatography (LC) has been a subject of recurring interest because the separation of such compounds is of primary importance in pharmaceutical analysis. In addition, the investigation of the separation behaviour of isomers can impart some valuable insights into the molecular mechanism of retention as well as providing useful data to test the accuracy of existing retention models. In the case of positional isomers of disubstituted benzenes, the principal factors which determine the elution order of *ortho*-, *meta*-, and *para*-isomers have been extensively studied for both normal- and reversed-phase LC. Elution orders on alumina were first established by Snyder [1] and interpreted in terms of various physicochemical effects, including intramolecular hydrogen bonding, electronic activation, steric repulsion and solute molecular orientation. Studies by Kiselev et al. [2] of retentive behaviour of isomers with polar substituents on hydroxylated silica largely confirmed the observations made by Snyder; that is, the elution orders varied for different sets of isomers, depending upon the nature and position of the substituent groups, their influence on the electron density distribution in the benzene ring, the possibility of the formation of intra- and inter-molecular hydrogen bonds and the orientation of solute molecules relative to the adsorbent surface.

The retentive behaviour of isomeric alkylbenzenes in reversed-phase LC has been studied by several groups and shown to be difficult to predict. Smith [3] showed that the connectivity index, derived from the topology of the molecule, fails to correlate with the relative retentions of isomeric compounds separated on three reversed-phase silica columns. With similar columns, Barman and Martire [4] examined the

* Corresponding author.

0021-9673/95/$09.50
SSDI 0021-9673(94)00813-2

dependence of solute retention on the polarity of the mobile phase, solute dipole moment and column temperature for isomeric xylenes, ethyltoluenes and diethylbenzenes. They found that the elution order of the isomers is consistent with a change in dipole moment. Knox et al. [5] noted that the elution order of isomeric xylenes separated on porous graphitic carbon (PGC) is the opposite to that observed on reversed-phase silica and they attributed this to the unique surface properties of the graphitic carbon.

Being the only LC support with a crystalline surface, PGC can be expected to show improved separation of positional isomers [6]. First, the energy of solute–adsorbent interactions is very much dependent upon the distance between the surface and the force centres in the solute molecule, so the planar surface of graphitic carbon may conveniently be exploited to separate isomers which differ only in geometrical structure [7]. Secondly, unlike bonded reversed-phase silica, which shows persistent residual effects arising from the underivatized silanol groups, PGC possesses an energetically homogeneous surface with minimal active sites on the edges of the graphitic sheets [8]. These properties makes PGC an ideal material for investigation of the retentive behaviour of ionizable compounds and for the further studies of the mechanism underlying the separation of isomers.

This study examines the retention behaviour of isomeric benzenes with ionizable substituents in a reversed-phase liquid chromatographic (RPLC) system consisting of PGC columns and acetonitrile–buffer eluents over a range of pH values. The elution orders of different sets of isomers and the relative retentions at various mobile phase pH values have been examined in order to elucidate the role of solute molecular orientation in determining the elution order of isomers.

2. Experimental

2.1. Chemicals

Orthophosphoric acid of analytical grade was obtained from Sigma (Dorset, UK) and triethylamine (HPLC grade) was purchased from Romil Chemicals (Loughborough, UK). Acetonitrile (HPLC grade), sodium dihydrogenphosphate and disodium hydrogenphosphate were obtained from Fisons (Loughborough, UK). Deionised, purified water was produced in-house with an Elgastat water purification system (Elga, High Wycombe, UK). All mobile phases were carefully degassed by helium purge before use.

The substituted benzenes used as test compounds were of analytical grade or better. Six series of ionizable positional isomers of benzene were selected to include acidic, basic and amphoteric compounds. The cresol isomers were included in order to relate the data to previous studies. The structures of all the test compounds are shown in Table 1.

2.2. Equipment

The chromatographic system consisted of a Gilson 305 pump (Villiers le Bel, France), a Gilson 805 manometric module, a Gilson 231 XL sampling injector, a Gilson 401 diluter and an ABI 759A absorbance detector (Foster City, CA, USA) connected to a Gilson HPLC 715 system controller via a Gilson 506B interface. All pH measurements were carried out with a Corning Model 7 pH meter equipped with automatic temperature compensation. The electrode was calibrated with pH 4.0, 7.0 or 10.0 standard solutions, depending on the range investigated.

2.3. Chromatographic experiments

A Hypercarb column (100 × 4.6 mm I.D., particle diameter 7 μm) was supplied by Shandon HPLC (Runcorn, UK). All separations were performed isocratically at ambient temperature with a flow-rate of 1 ml/min. UV detection at 254 nm was used. The mobile phase consisted of aqueous buffer–acetonitrile in 65:35 volume ratio. The pH value of the buffer was adjusted by adding an appropriate amount of triethylamine or orthophosphoric acid to 0.01 *M* orthophosphoric acid solution. When a change in mobile phase pH was made, equilibration with the new mobile phase was carried out for at least 30 min before the retention data were taken for

Table 1
Retention and selectivity data for disubstituted benzene isomers separated on PGC

Compound	Substituent		k	α	pH
	X	Y			
o-Aminobenzoic acid	H_2N	COOH	7.57	1.96	2.0
m-Aminobenzoic acid	H_2N	COOH	0.28	1.00	2.0
p-Aminobenzoic acid	H_2N	COOH	3.86	13.78	2.0
o-Cresol	CH_3	OH	4.64	1.08	2.0
m-Cresol	CH_3	OH	3.86	1.00	2.0
p-Cresol	CH_3	OH	4.28	1.11	2.0
o-Anisic acid	CH_3O	COOH	7.57	1.00	2.0
m-Anisic acid	CH_3O	COOH	17.28	2.28	2.0
p-Anisic acid	CH_3O	COOH	20.43	2.70	2.0
o-Toluic acid	CH_3	COOH	11.00	1.00	2.0
m-Toluic acid	CH_3	COOH	13.28	1.21	2.0
p-Toluic acid	CH_3	COOH	16.14	1.47	2.0
o-Anisidine	CH_3O	NH_2	1.43	10.21	3.3
m-Anisidine	CH_3O	NH_2	1.93	1.35	3.3
p-Anisidine	CH_3O	NH_2	0.14	1.00	3.3
o-Phenetidine	C_2H_5O	NH_2	4.28	8.50	3.3
m-Phenetidine	C_2H_5O	NH_2	5.07	1.18	3.3
p-Phenetidine	C_2H_5O	NH_2	0.50	1.00	3.3

the next sample. The solute solutions were prepared by dissolving the test compounds in the mobile phase (aqueous buffer–acetonitrile, 65:35) to give a concentration of 1–10 μg/ml. Injections of 1–10 μl of these mixtures produced satisfactory chromatographic peaks. All isomer solutes were analysed separately (in duplicate) to determine the elution order and then the isomer mixtures were analysed. The mobile phase hold-up time, t_M, was taken as the time from injection to the moment when the trace for the solvent disturbance crossed the baseline. The solvent disturbance peak was generated by acetonitrile in which the samples were dissolved. The mean retention factors, k and separation factors, α, were calculated from multiple injections of the isomer mixtures. The effect of the mobile phase on retention of all the isomer mixtures was studied over the pH range from pH 2.0 to pH 9.4.

2.4. Data analysis

The separation factor of a pair of isomers is given as a ratio of the retention factor of the later eluted species to that of the first eluted one, for adjacent peaks.

The retention factor of any ionizable solute as a function of mobile phase pH can be expressed by the general form of Horvath's equation [9]:

$$k = \Sigma x_i k_i$$

where x_i and k_i are the mole fraction and the retention factor of the solute in the ith form, respectively. x_i, as a function of pH, can be readily derived from the known dissociation equilibria. For monoprotic acids and bases, the retention factor is given by

$$k = [k_1 + k_2 \exp(\mathrm{pH} - \mathrm{p}K_{a(1)})]/[1 + \exp(\mathrm{pH} - \mathrm{p}K_{a(1)})]$$

where $pK_{a(1)}$ is the negative logarithm of the acid dissociation constant in the mobile phase and k_1 and k_2 refer to the retention factors of the acidic and basic forms of the solute, respectively.

Similarly, the retention factor of amphoteric substances, such as aminobenzoic acids, is given by

$$k = k_1/[1 + \exp(\mathrm{pH} - \mathrm{p}K_{a(1)}) + \exp(2\mathrm{pH} - \mathrm{p}K_{a(1)} - \mathrm{p}K_{a(2)})] + k_2/[1 + \exp(\mathrm{p}K_{a(1)} - \mathrm{pH}) + \exp(\mathrm{pH} - \mathrm{p}K_{a(2)})] + k_3/[1 + \exp(\mathrm{p}K_{a(2)} - \mathrm{pH}) + \exp(\mathrm{p}K_{a(1)} + \mathrm{p}K_{a(2)} + 2\mathrm{pH})]$$

where k_1, k_2 and k_3 are the retention factors of the cationic, the zwitterionic and the anionic forms of the amphoteric solute and $K_{a(1)}$ and $K_{a(2)}$ are the corresponding dissociation constants, respectively.

The pH and k values are known from the experimental data and the remaining unknown parameters ($\mathrm{p}K_{a(1)}$, $\mathrm{p}K_{a(2)}$, k_1, k_2 and k_3) in the above equations can be found by using a non-linear least squares technique to fit an appropriate model to the data (software package, MINIM 2.0.2 (R.D. Purves, Department of Pharmacology, University of Otago, New Zealand).

3. Result and discussion

3.1. Choice of support material

Consideration of the thermodynamics of chromatographic retention indicates that the interaction energy which is responsible for solute retention is governed by three competing effects: solute–adsorbent interactions, solvent–solute interactions and solvent–adsorbent interactions. As the solute–adsorbent interactions are the only positive contribution to the retention, the surface properties of porous graphitic carbon (PGC) are relevant to this study.

PGC is a spherical microparticulate packing material made by a "template" method invented by Knox and Gilbert [10] in 1979. Spherical silica gel particles are used as a porous template and filled with a polymer. After pyrolysing the polymer in an inert atmosphere, the template silica is dissolved out to leave a porous glassy carbon. The material is then further heated to induce graphitisation. The product thus obtained has the two-dimensional graphitic structure which has been established by an X-ray diffraction analysis [5] and can be considered, at a molecular level, to have a flat, crystalline surface of intertwining graphitic ribbons held together by covalent bonds to form a dense carbon network. It appears that the relatively flat molecular ribbon structure of PGC offers a contrasting selectivity to the "brush like" surface structure of the traditional bonded silica materials used for HPLC analysis.

Thus the PGC material has unique properties as a chromatographic adsorbent and offers outstanding characteristics for the study of the separation of structurally similar compounds by reversed-phase liquid chromatography.

3.2. Effect of substituents

For each series of benzene isomers the mobile phase pH was optimised over the range of pH 2.0 to 9.4 to give the best separation factors and the resulting retention data are listed in Table 1. Figs. 1–3 show the chromatograms of *o*-, *m*- and *p*-isomers of the acidic, basic and amphoteric substituted benzenes obtained at optimum pH values, from which the retention factors, k and separation factors, α, were determined.

Under the optimized chromatographic conditions chosen, all these isomers were separated

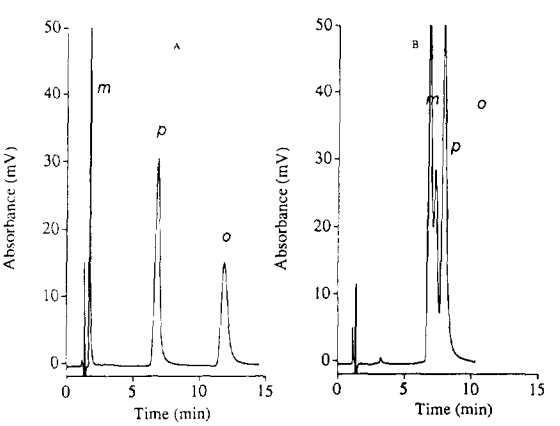

Fig. 1. Separation of disubstituted benzene isomers at pH 2.0. (A) Aminobenzoic acid; (B) cresol.

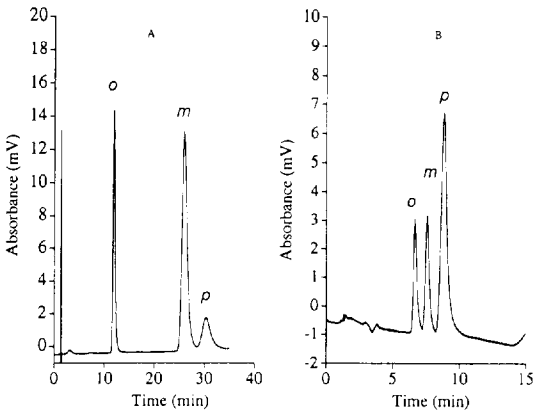

Fig. 2. Separation of disubstituted benzene isomers at pH 2.0. (A) Anisic acid; (B) toluic acid.

with a range of separation factors and elution orders. Of the isomers under examination, the aminobenzoic acid isomers show greatest separability, with the separation factors for the *p*/*m* pair and *o*/*p* pair being 13.78 and 1.96, whereas the cresol isomers were least separated, giving separation factors of 1.11 and 1.08, respectively. These results demonstrate the potential of PGC stationary phases in the separation of positional isomers of either polar or non-polar molecules. Previously, successful isomer separations have been reported for diastereoisomers of an antidepressant, geometrical isomers of cresol [11]; phenol isomers, cephalosporin isomers [12]; geometrical isomers of dothiepin [13]; anionic and cationic compounds of biomedical interest [14] and glucuronide and sulphate metabolites of morphine [15].

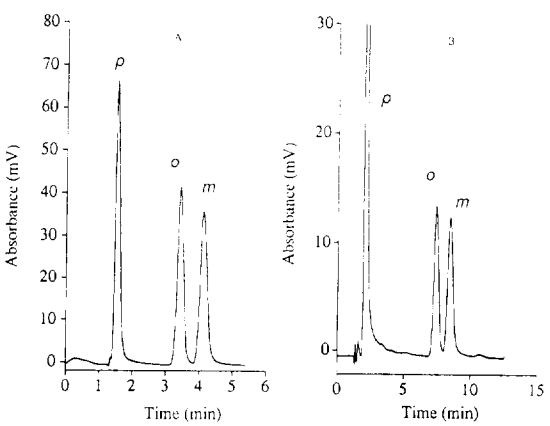

Fig. 3. Separation of disubstituted benzene isomers at pH 3.3. (A) Anisidine; (B) phenetidine.

Three types of elution order were observed for the isomers of differing substituents: $m > p > o$ for aminobenzoic acid and cresol, $o > m > p$ for toluic acid and anisic acid and $p > o > m$ for anisidine and phenetidine. The observation for the first group of isomers that the *m*-isomer elutes before the *p*- and *o*-isomers is consistent with previous results obtained for cresols and xylenes on PGC columns. It has been proposed that the elution order of the cresols was determined by the ability of the solute to undergo charge donor–acceptor interactions with the graphite surface [8]. Of the three isomers, the methyl and hydroxyl groups on the *m*-cresol disturb the electron density the most, resulting in less retention. The elution order for xylenes is accounted for by a different theory [5,7], which is essentially based on the consideration of the number of contact points available to the solute–adsorbent interactions. When *o*- and *p*-xylenes are adsorbed onto the graphite surface, four carbon atoms contact the surface (two from the methyl groups and two from the benzene ring) whereas with *m*-xylene only three carbon atoms contact the surface (two from the methyl groups and one from the benzene ring), thus the *m*-xylene is eluted before *o*- and *p*-xylenes.

However, the above theories cannot provide a satisfactory explanation for the elution orders seen for monoprotic acid and base isomers as neither of them is consistent with the predictions that the *m*-isomer should be eluted before the *o*- and *p*-isomers. Further inspection revealed that these theories assume that the aromatic compound approaches the graphite surface with its flat-side down, thus the direct contact interactions between the solute and the adsorbent surface are the sole factor determining the elution order. The assumption is reasonable in view of a linear relationship between log *k* and carbon number for the *n*-alkylbenzenes [16], but has not been established in the case of aromatic compounds with polar substituents. It is therefore speculated that the strong interaction between the polar substituent and the solvent may have

some significant influence on the alignment of the solute molecule relative to the adsorbent surface. This point will be examined in more detail in the following sections.

3.3. Effect of mobile phase pH

The pH of the mobile phase would be expected to directly influence the retention and selectivity for the separation of ionizable isomers, but the relationship between solute ionization and retention on PGC columns has not been studied in detail. Therefore experiments were carried out to determine the retention factors of the ionizable isomers over the pH range 2.0 to 9.4. In a pure reversed-phase system, with no secondary solute interactions with the stationary phase, the retention of these ionizable compounds should be dependent on the degree of ionization as indicated by the Horvath equation [9]. A PGC stationary phase, which consists predominantly of graphite sheets, might be expected to show ideal behaviour. However, on bonded silica columns it is well known that secondary interactions of residual surface silanols with basic and acidic solutes can cause anomalous retention, and ionized compounds often deviate from ideal chromatographic behaviour.

The experimental data on the ionizable test solutes demonstrated that their retention varied as a function of pH and correlated directly with the degree of ionization of the solutes, with the ionized form being least retained. Figs. 4–9 show the original retention data points for all the isomers over a range of pH 2.0 to 9.4 and a superimposed fitted curve for each individual isomer to show the goodness of fit to the Horvarth model in which solute ionisation alone controls the retention behaviour. In all cases there was an extremely high correlation between the degree of ionization and the retention of the test solutes, indicating that solute ionization is indeed the major factor influencing the retention behaviour of these simple organic acids and bases. As predicted by such a model, a major change in solute retention occurs at pH values close to the isomer pK_a.

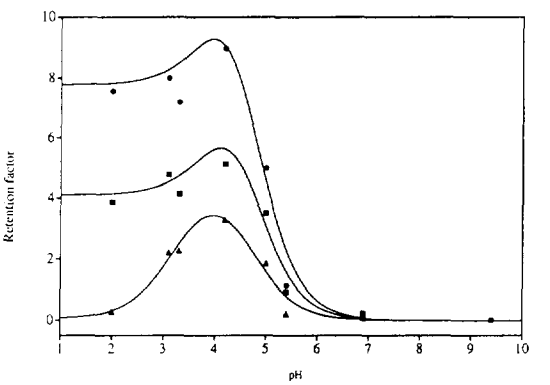

Fig. 4. Effect of pH on the retention of aminobenzoic acid isomers. (●) *ortho*, (▲) *meta*, (■) *para*.

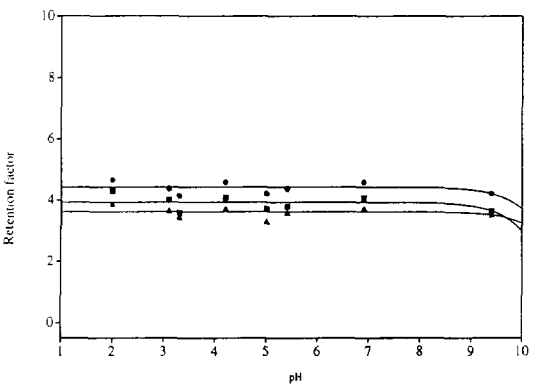

Fig. 5. Effect of pH on the retention of cresol isomers. (●) *ortho*, (▲) *meta*, (■) *para*.

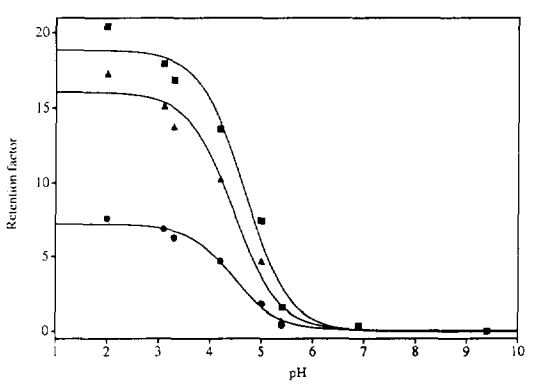

Fig. 6. Effect of pH on the retention of anisic acid isomers. (●) *ortho*, (▲) *meta*, (■) *para*.

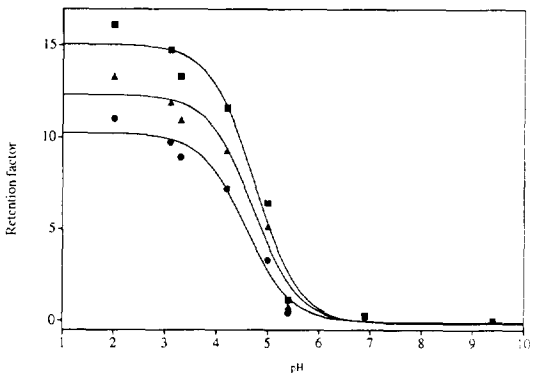

Fig. 7. Effect of pH on the retention of toluic acid isomers. (●) *ortho*, (▲) *meta*, (■) *para*.

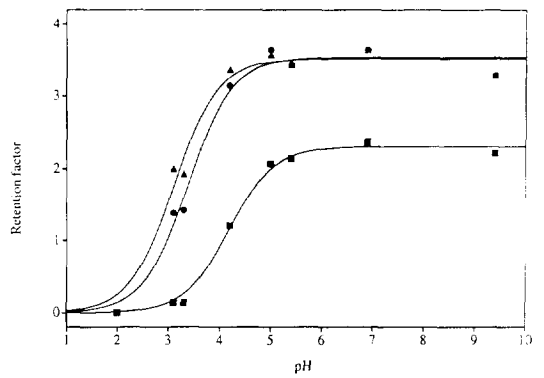

Fig. 8. Effect of pH on the retention of anisidine isomers. (●) *ortho*, (▲) *meta*, (■) *para*.

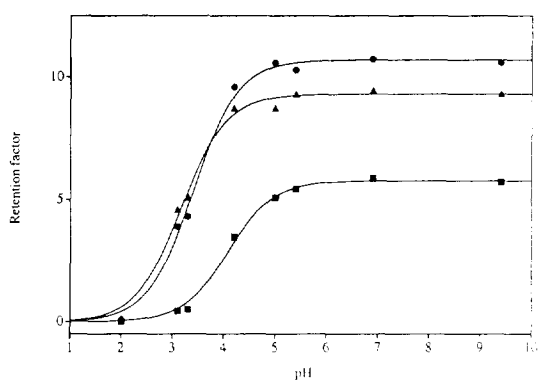

Fig. 9. Effect of pH on the retention of phenetidine isomers. (●) *ortho*, (▲) *meta*, (■) *para*.

Because there appear to be no residual effects with the PGC stationary phase, it is possible to use the curve fitting technique to estimate accurately the pK_a values of each of the isomers. Table 2 lists values of k_1, k_2, k_3, $pK_{a(1)}$ and $pK_{a(2)}$ for substituted benzene isomers, as determined from experimental data presented in Figs. 4–9. The pK_a values thus derived are compared with those in the literature [17]. There is a general agreement between experimental and literature values, with a consistent small shift in the experimental values of pK_a, which is presumably due to the reduced ionization of the solutes resulting from the presence of acetonitrile in the HPLC mobile phase.

These results suggest that the polarity or ionization level of the isomer is important in determining the separation of the isomers, with the most polar isomer eluted first and the least polar last. Using the aqueous pK_a values as an indication of the strength of the acid and basic groups on the isomers, it is possible to observe a direct correlation between order of retention and acid/base strength for the acidic isomers (anisic acid, toluic acid) and the basic isomers (anisidine, phenetidine). Since the acidity/basicity of an organic compound is strongly influenced by its electronegativity and the nature of the solvent, it is likely that both these factors will have a major effect on the order of elution of ionizable isomers.

The high stability of the PGC material over a wide range of pH values makes it feasible to use the mobile phase pH to provide an effective means of varying selectivity and optimizing separation over the pK_a range of the sample. This is best illustrated in the separation of anisidine isomers where a complete separation of three isomers can only be achieved between pH 2 and pH 5, over which range the isomers are all partly ionized. Stronger retention at higher values of pH results in the loss of resolution of *o*- and *m*-isomers. The range of pH stability of PGC columns (pH 1 to 14) will thus allow a greater flexibility in optimising isomer separations compared to the more limited pH range available on conventional bonded silica columns.

Table 2
Values of k_1, k_2, k_3, $pK_{a(1)}$ and $pK_{a(2)}$ for disubstituted benzene isomers, as determined from experimental data in Figs. 4–9

Compound	k_1	k_2	k_3^a	$pK_{a(1)}$ (experimental)	$pK_{a(2)}^a$	$pK_{a(1)}$ (literature)[b]	$pK_{a(2)}^a$
o-Aminobenzoic acid	7.60	12.53	0.00	4.00	4.73	2.108	4.946
m-Aminobenzoic acid	0.05	4.66	0.00	3.23	4.71	[c]	[c]
p-Aminobenzoic acid	4.09	8.17	0.00	4.00	4.78	2.501	4.874
o-Cresol	4.40	0.00		10.73		10.2	
m-Cresol	3.60	0.00		10.95		10.01	
p-Cresol	3.92	0.00		10.50		10.17	
o-Anisic acid	7.20	0.00		4.45		4.09	
m-Anisic acid	16.05	0.00		4.46		4.09	
p-Anisic acid	18.83	0.00		4.67		4.48	
o-Toluic acid	10.23	0.00		4.57		3.91	
m-Toluic acid	12.31	0.00		4.71		4.27	
p-Toluic acid	15.05	0.00		4.74		4.38	
o-Anisidine	0.00	3.53		3.64		4.52	
m-Anisidine	0.00	3.50		3.01		4.23	
p-Anisidine	0.00	2.30		4.14		5.34	
o-Phenetidine	0.00	10.67		3.36		4.43	
m-Phenetidine	0.00	9.25		3.11		4.18	
p-Phenetidine	0.00	5.75		4.05		5.20	

[a] Applies to aminobenzoic acid isomers only (two ionisable groups).
[b] Uncorrected literature values (in water, 25°C) [17].
[c] Data not available in the literature.

3.4. Separation mechanism

One interpretation of these data at a molecular level is offered by considering the orientation of a polar aromatic molecule when approaching the planar graphite surface of the stationary phase. The ionizable or polar side of the solute would be orientated towards the solvent/mobile phase, but the aromatic plane of the solute would tend to interact with the planar graphite surface. Thus it could be envisioned that the polar aromatic solute would not align in a parallel manner to the graphite surface, but rather be orientated at a certain angle, with the polar side of the solute tilting into the bulk of the mobile phase. The degree of the angle formed between the aromatic solute and the graphite surface would be determined by a balance between the adsorptive strength and the solvation force which, as opposed to the former, tends to lift the solute molecule from the substrate surface. From this it follows that the chromatographic resolution of a set of isomers may be improved by exploiting these two competing effects to maximize the difference in solute molecular orientation relative to the graphite surface. If this is the case then the polarity or dipole moment of the solute, which is indicative of the strength of the solvation interaction, should be a key factor along with others determining the elution order. Further experimental work will be necessary to confirm this hypothesis.

4. Conclusions

The preliminary results presented above demonstrate that porous graphitic carbon is an unique packing material which allows the use of a wide range of mobile phase pH values to optimize the separation of polar or ionizable organic isomers. Prediction of the order of elution of the polar isomers cannot be achieved using the previous theories based on solute–stationary phase interactions alone for non-polar

benzene isomers, but the data from this study indicate the major importance of solute–solvent interactions. The type of the substituents (acidic, basic, non-polar) affects the order of elution of the isomers, which appears to be consistent among each of these groups. For the ionizable compounds there is a correlation between retention of the unionized form and acid/base strength, as indicated by the pK_a. The retention behaviour of the ionizable isomers indicated a high correlation between the degree of ionization and the retention, indicating that solute ionization is the major factor determining the retention behaviour of these simple organic acids and bases. The underlying mechanism for the retention of these polar organic isomers has yet to be characterised, but this study indicates the likely importance of the orientation of polar solutes with respect to the planar graphite surface of the stationary phase.

Acknowledgements

Q.H.W. was supported by a BBSRC grant (awarded to D.A.B., M.C.D. and P.N.S.) from the Process Separations Initiative. The authors thank Shandon HPLC (Runcorn, Cheshire, UK) for the provision of materials.

References

[1] L.R. Snyder, *J. Chromatogr.*, 20 (1965) 463.
[2] A.V. Kiselev, A.A. Aratskova, T.N. Gvozdovitch and Ya.I. Yashin, *J. Chromatogr.*, 195 (1980) 205.
[3] R.M. Smith, *J. Chromatogr.*, 209 (1981) 1.
[4] B.N. Barman and D.E. Martire, *Chromatographia*, 34 (1992) 347.
[5] J.H. Knox, B. Kaur and G.R. Millward, *J. Chromatogr.*, 352 (1986) 3.
[6] J.H. Knox and B. Kaur, in P.R. Brown and R.A. Hartwick (Editors), *High Performance Liquid Chromatography*, Wiley, New York, 1989, p. 189.
[7] A.V. Kiselev and Ya.I. Yashin, *Gas Adsorption Chromatography*, J.E.S. Bradley (Translator), Plenum, London, 1969.
[8] Q.H. Wan, *PhD Thesis*, Edinburgh University, 1992.
[9] C. Horvath, W. Melander and I. Molnar, *Anal. Chem.*, 49 (1977) 142.
[10] J.H. Knox and M.T. Gilbert, *U.K. Patent*, 7939449 (1979), *U.S. Patent*, 4263268 (1979), *F.R.G. Patent*, P2946688-4 (1979).
[11] B.J. Bassler and R.A. Hartwick, *J. Chromatogr. Sci.*, 27 (1989) 162.
[12] B.J. Bassler, E. Garfunkel, R.A. Hartwick and R. Kaliszan, *J. Chromatogr.*, 461 (1989) 139.
[13] Z. Pawlak, D. Kay and B.J. Clark, *Anal. Proc.*, 27 (1990) 16.
[14] G. Gu and C.K. Lim, *J. Chromatogr.*, 515 (1990) 183.
[15] D.A. Barrett, M. Pawula, S. Taylor, Q.H. Wan and P.N. Shaw, in preparation.
[16] M.T. Gilbert, J.H. Knox and B. Kaur, *Chromatographia*, 16 (1982) 138.
[17] D.R. Lide (Editor-in-Chief), *CRC Handbook of Chemistry and Physics*, 73rd Edition, CRC Press, Boca Raton, FL, 1992.