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Quantitative structure–retention relationships for secondary interactions in cation-exchange liquid chromatography

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ABSTRACT

Eighty-three aryl alkyl amines with a range of substituents in the aromatic ring were chromatographed on a silica stationary phase in the ion-exchange mode using a non-polar eluent which promotes hydrogen bonding interactions. The increase in retention due to the presence of the polar substituents was quantified using the functional group contribution value τ. The correlation between τ and a range of substituent physical and quantum chemical parameters was investigated using multiple regression.

The increase in retention could be attributed to a hydrogen bonding interaction between the polar substituents and unionised silanols. This could be quantified in terms of the hydrogen bonding acceptor properties of the substituent (log K_β) and it size, although the quality of the correlations was poor. A superior correlation was obtained using the lipophilicity (π) of the substituent. The correlations, which gave an R^2 of 0.8423 at best were unsuited to accurate retention prediction.

INTRODUCTION

The findings presented here arose from chance observations in other areas of analytical work carried out in this laboratory [1,2]. Firstly, in the development of a rational approach to solid-phase extraction for drugs [1], using nominally reversed-phase cartridges, we observed anomalies in the elution order of some compounds which we attributed to a hydrogen bonding interaction between acceptor atoms in the solute and donor groups (presumably unbonded unionised silanols) on the stationary phase.

The second observation arose from our interest in the use of silica as a cation exchanger for the analysis of basic compounds [2]. Although it has been shown that retention is controlled in the main by the pK_a of the solute [3], to account for the good separation between compounds of similar pK_a, necessitated invoking some, as yet, unexplained interaction. For example [2], the four β-blockers propranolol, atenolol, alprenolol and practolol all have the same oxypropanolamine side chain and pK_a values within 0.05 units of 9.5 [4]. On silica however they show good separation with k' ranging from 1.5 to 2.5. Examination of the data in the light of the above findings [1] indicated that the two compounds showing the greatest retention (atenolol and practolol) possessed an amide substituent; a strong hydrogen bond acceptor group in the aromatic ring. In contrast, propranolol has an unsubstituted naphthalene and alprenolol a simple allyl substituted phenyl ring, neither of which are strong hydrogen bond acceptors. It seemed possible therefore that the longer retention of the more polar compounds (atenolol and prac-

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tolol) was due to some form of hydrogen bonding. The silica cation-exchange system, with its methanol rich eluent which favours hydrogen bonding between the solute and the stationary phase [5], appeared to be a good vehicle to allow the study of these secondary hydrogen bond interactions.

The recent publication [6] of hydrogen bond acceptor values (log K_β) for a wide range of structural fragments commonly used in drug design, facilitated this investigation. These values were used with some caution since they were generated under conditions which are quite different to the chromatographic conditions employed here.

We generated retention data for 83 compounds with substituents covering a wide range of log K_β values with the aim of correlating the increase in retention with the hydrogen bond acceptor potential of the molecules. In so doing we hoped to gain a clearer understanding of the factors controlling retention on silica when used with methanol–aqueous eluents. This information could then be used for the prediction of retention, thus minimising method development work.

EXPERIMENTAL

Equipment

The HPLC system consisted of a Perkin-Elmer 250 pump, a 135 variable-wavelength UV detector, and a ISS 200 autosampler. Chromatographic data were recorded using a Hewlett-Packard HP 3392A integrator.

Materials

Acetic acid and ammonia (25%, w/v) were AnalaR grade from BDH (Liverpool, UK), methanol was HPLC grade from Fisons (Loughborough, UK). The test solutes were obtained from the ICI compound collection and were used as received. These compounds were of four structural types. Types I–III were β-blockers and type IV were phenethylamine derivatives, the structures of which are shown in Fig. I. With the exception of the four parent compounds they were all substituted in the 4-position of the aromatic ring. The substituents studied are given in Table I.

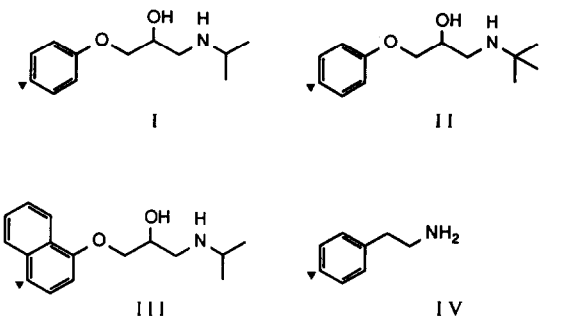

Fig. 1. The parent structures for the four sets of compounds studied (I to IV). The site of substitution is indicated by a closed triangle

Chromatography

The column was 100 × 4.6 mm I.D. packed with Spherisorb S5W silica (Phase Separations, Deeside, UK). The eluent was methanol–aqueous ammonium acetate buffer (9:1), pH 9.1. The buffer was prepared from ammonia (65 ml, 25%), acetic acid (11 ml) and water (924 ml).

Methods

The test solutes were dissolved in methanol (*ca.* 0.1 mg/ml) and a minimum of duplicate injections (1 to 10 μl) made to give k' data with reproducibility of better than 1%. Diphenylamine was used as the t_0 marker in the calculation of k' as described previously [3]. Hydrogen bond acceptor values (log K_β) were obtained from the literature [6] or from other sources [7]. Where the substituent had more that one hydrogen bond acceptor atom *e.g.* 11 and 36, only the log K_β of the stronger hydrogen bond acceptor was considered.

Computational work was carried out using a Vax computer running an "in-house" molecular modelling system, based on MOPAC and AMPAC software. The following parameters were calculated for the 4-substituents; charge on the donor atom (QM), fragment volume (FV), fragment polarisability (FPO), moment of fragment polarisability (FPM), fragment dipole (DCM), the dipole vectors (DCX, DCY and DCZ), the Verloop steric parameters (L, B1, B2,

TABLE I

HPLC RETENTION DATA AND SUBSTITUENT π AND LOG K_β VALUES FOR THE 83 COMPOUNDS STUDIED

NA = Not applicable.

No.	Substituent	k'	τ	π^a	Log $K_\beta{}^b$	Core structure
1	H	0.97	NA	0.00	NA	I
2	CH_3	0.97	0.00	0.56	−1.3	I
3	OCH_2CHCH_2	1.02	0.02	0.43	−0.8	I
4	Cl	0.99	0.01	0.71	−0.7	I
5	OCH_2CCH	0.97	0.00	−0.43	0.3	I
6	OCH_3	1.09	0.05	−0.02	0.3	I
7	NO_2	1.22	0.10	−0.28	0.7	I
8	NH_2	1.40	0.16	−1.23	1.0	I
9	CN	1.17	0.08	−0.57	1.0	I
10	CHO	1.31	0.13	−0.65	1.2	I
11	$O(CH_2)_4CN$	1.14	0.07	−0.12	1.2	I
12	$COOCH_3$	1.02	0.02	−0.01	1.2	I
13	$COCH_3$	1.31	0.13	−0.55	1.4	I
14	OCH_2COCH_3	1.34	0.14	−0.77	1.6	I
15	CH_2COCH_3	1.22	0.10	−0.23	1.6	I
16	$NHCOCH_3$	1.28	0.12	−0.97	2.5	I
17	$NHCOCH_2CH_3$	1.17	0.08	−0.46	2.5	I
18	$NHCOCH(CH_3)_2$	1.09	0.05	−0.15	2.5	I
19	$NHCO(CH_2)_2CH_3$	1.09	0.05	0.07	2.5	I
20	$NHCO(CH_2)_3CH_3$	1.02	0.02	0.60	2.5	I
21	$CONHCH_2CH_3$	1.37	0.15	−0.76	2.8	I
22	$CONH(CH_2)_2CH_3$	1.17	0.08	−0.23	2.8	I
23	$CONHCH_2CH(CH_3)_2$	1.09	0.05	0.17	2.8	I
24	CH_2CONH_2	1.43	0.17	−1.69	3.0	I
25	OCH_2CONH_2	1.34	0.14	−1.67	3.0	I
26	$CH_2NHCOCH_3$	1.34	0.14	−1.45	3.0	I
27	$(CH_2)_2NHCOCH_3$	1.28	0.12	−1.13	3.0	I
28	$CH_2NHCOCH_2CH_3$	1.28	0.12	−0.92	3.0	I
29	$(CH_2)_2NHCOCH_2CH_3$	1.17	0.08	−0.60	3.0	I
30	$CH_2CON(CH_2CH_3)_2$	1.47	0.18	−0.12	3.0	I
31	$CH_2CONH(CH_2)_3CH_3$	1.04	0.03	0.13	3.0	I
32	$CH_2NHCONH_2$	1.43	0.17	−1.41	3.2	I
33	$NHCONH_2$	1.40	0.16	−1.30	3.2	I
34	$NHCONHCH_3$	1.37	0.15	−0.93	3.2	I
35	$NHCONHCHCH_2$	1.1	0.07	−0.68	2.8	I
36	$O(CH_2)_2NHCONHCH_3$	1.34	0.14	−0.60	3.2	I
37	$CH_2NHCONHCHCH_2$	1.14	0.07	−0.60	2.8	I
38	$NHCONHCH_2CH_3$	1.25	0.11	−0.52	3.2	I
39	$NHCONHCH(CH_3)_2$	1.11	0.06	−0.21	3.2	I
40	$NHCONH(CH_2)_2CH_3$	1.1	0.09	0.01	3.2	I
41	$CH_2NHCONH(CH_2)_2CH_3$	1.11	0.06	0.13	3.2	I
42	$(CH_2)_2NHCONH(CH_2)_2CH_3$	1.09	0.05	0.46	3.2	I
43	$NHCONH(CH_2)_3CH_3$	1.06	0.04	0.54	3.2	I
44	$CH_2NHCONH(CH_2)_3CH_3$	1.06	0.04	0.66	3.2	I
45	$NHCONH(CH_2)_5CH_3$	0.97	0.00	1.60	3.2	I
46	$CH_2NHCONH(CH_2)_5CH_3$	0.95	−0.01	1.72	3.2	I
47	H	0.91	NA	0.00	NA	II
48	Br	0.85	−0.03	0.86	−0.9	II
49	F	0.91	0	0.14	−0.7	II
50	Cl	0.91	0	0.71	−0.7	II
51	NO_2	1.07	0.07	−0.28	0.7	II
52	NH_2	1.23	0.13	−1.23	1.0	II
53	CN	1.12	0.09	−0.57	1.0	II
54	$O(CH_2)_2CN$	1.07	0.07	−0.68	1.2	II
55	CH_2CN	1.15	0.1	−0.59	1.2	II
56	$(CH_2)_2CN$	1.09	0.08	−0.43	1.2	II

(Continued on p. 20)

TABLE I (*Continued*)

No.	Substituent	k'	τ	π^a	Log K_β [b]	Core structure
57	$O(CH_2)_4CN$	1.04	0.06	−0.12	1.2	II
58	$O(CH_2)_5CN$	0.98	0.03	0.42	1.2	II
59	SO_2CH_3	1.48	0.21	−1.63	1.4	II
60	$NHSO_2CH_3$	1.26	0.14	−1.62	1.4	II
61	$NHCOCH_3$	1.12	0.09	−0.98	2.5	II
62	$CONH_2$	1.26	0.14	−1.49	2.8	II
63	$CONHCH_3$	1.26	0.14	−1.27	2.8	II
64	$N(CH_3)COCH_3$	1.41	0.19	−1.04	2.8	II
65	CH_2CONH_2	1.26	0.14	−1.69	3.0	II
66	$CH_2CONHCH_3$	1.29	0.15	−1.46	3.0	II
67	H	0.83	NA	0.00	NA	III
68	CH_3	0.79	−0.02	0.56	−1.3	III
69	Cl	0.81	−0.01	0.71	−0.7	III
70	OH	0.89	0.03	−0.69	0.2	III
71	OCH_3	0.91	0.04	−0.02	0.3	III
72	OCH_2CH_3	0.87	0.02	0.45	0.3	III
73	$COCH_3$	1.17	0.15	−0.55	1.4	III
74	$SO_2N(CH_2CH_3)_2$	0.95	0.06	0.25	1.4	III
75	$NHCOCH_3$	1.09	0.12	−0.97	2.5	III
76	$NHCOCH_2CH_3$	1.02	0.09	−0.45	2.5	III
77	OCH_2CONH_2	1.00	0.08	−1.67	3.0	III
78	H	1.58	NA	0.00	NA	IV
79	Br	1.47	−0.03	0.86	−0.9	IV
80	OH	1.73	0.04	−0.72	0.2	IV
81	OCH_3	1.77	0.05	−0.02	0.3	IV
82	NH_2	2.28	0.16	−1.23	1.0	IV
83	SO_2CH_3	2.39	0.18	−1.63	1.4	IV

[a] Obtained from refs. 9 and 10.
[b] Obtained from refs. 6 and 7.

B3 and B4) [8] and the charge on the acceptor atom (QM). The fragment lipophilicity (π) was obtained from the literature [9] or calculated using standard methods [10]. The pseudo cross-sectional area (CSA) was generated from FV and the Verloop length parameter L. The square of the Verloop parameters and π were also generated as has been the practice of some workers [8,11 and references cited therein].

Multiple regression analysis was carried out using the program SAS which also ran on a VAX computer.

RESULTS AND DISCUSSION

The four sets of compounds (I to IV) had clearly defined through overlapping retention ranges which were ultimately a function of the pK_a and the substituents of the basic nitrogen. The influence of the different core structures was eliminated through calculation of the retention increments or functional group contribution values (τ) for the substituents in each compound, where;

$$\tau = \log k'_s - \log k'_p$$

and the subscripts p and s refers to the parent compound (X = H, *i.e.* compounds 1, 47, 67 and 78) and the substituted compounds, respectively. This allowed the data for the four groups of compounds to be combined and analysed together. The validity of this approach was confirmed by the data in Table II which shows good agreement between τ values for compounds of differing basic type but having the same substituents.

Correlations using log K_β

Superficial examination of the raw data (Table I) showed that compounds with high log K_β

TABLE II

RETENTION INCREMENTS (τ) FOR COMPOUNDS WHERE THE SUBSTITUENT WAS COMMON TO SEVERAL OF THE CORE STRUCTURES

Substituent	τ			
	Core I	Core II	Core III	Core IV
-Cl	0.01	0.00	−0.01	NA
$-NH_2$	0.16	0.13	NA	0.16
$-NHCOCH_3$	0.12	0.09	0.12	NA
-OH	NA	NA	0.03	0.04
$-SO_2Me$	NA	0.21	NA	0.18
$-NO_2$	0.10	0.07	NA	NA
-CN	0.08	0.09	NA	NA
-Br	NA	−0.03	NA	−0.03

tended to give higher τ values, *i.e.* they showed increased retention over the parent compound. However for a range of homologues with the same log K_β, but with a range of substituent sizes (*e.g.* the amides; 16 to 20, and 21 to 23 and also the N-alkyl ureas; 38, 40, 43 and 45) the smaller substituents clearly produced a greater positive effect on retention. At the extreme the bulky N-hexyl substituent on the urea 46 completely eliminated the effect of the strong hydrogen bond acceptor such that the τ value for this compound was actually negative!

On the basis of the above observation the initial analysis of the data was based on correlations using log K_β and steric terms *e.g.* FV, L, etc. The result of this analysis for the 79 substituted compounds was only partially successful as only 57% of the variability in the data could be explained. The best correlations were observed using log K_β and L^2 ($R^2 = 0.5792$) and log K_β and L ($R^2 = 0.5724$).

Examination of the residuals showed a number of systematic deviations. The two amino compounds (8 and 52) showed higher τ values that predicted as did the sulphones (59 and 83). In other experiments [12] we have observed unusually long retention for dibasic compounds.

In the case of the sulphones it is believed that the quoted log K_β values [6] may be an underestimation of the hydrogen bond acceptor ability under the present conditions. The data of Abraham *et al.* [6] are based on a one-to-one interaction between a donor atom and the acceptor under study. Sulphones however are capable of simultaneous interaction with two donor atoms given the right conditions. It would seem probable therefore that under the present chromatographic conditions the two sulphone compounds are forming one-to-two complexes resulting in greater retention than predicted by the log K_β values.

Removal of the four compounds discussed above (8, 52, 59 and 83) led to no overall change in the analysis, although as expected the correlation coefficients improved slightly. The best equation was

$$\tau = 0.0384(\pm 0.0034)\log K_\beta - 0.00128(\pm 0.00016)L^2 + 0.0617(\pm 0.0072)$$

$$R^2 = 0.6428 \quad n = 75 \quad F = 64.8 \quad s = 0.0339$$

Correlations with the lipophilicity parameter π

It was obvious from the analysis above that the currently available log K_β values in combination with steric terms were unable to fully describe the variation in the observed retention data. Further multiple regression analysis was therefore carried out using the full set of substituent parameters described in the Methods. Using all 79 observations gave a result which initially seemed surprising. The lipophilicity of the substituents (π) was able to account for around 71% of the variability in the data. A plot of the

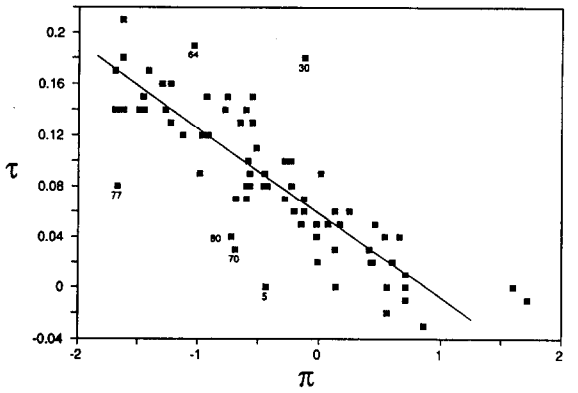

Fig. 2. A plot of τ *versus* π for the 79 substituted compounds. The identity of the six major outliers is indicated, and the best fit line excluding these compounds is shown.

correlation is shown in Fig. 2 and a number of significant outliers are highlighted. These outliers include the two phenolic compounds 70 and 80 which appear to be deviating in a systematic manner. In other work [3,12] it has been found that analytes carrying a negative charge have much reduced retention. Under the present conditions (eluent pH 9.1) the phenols would be partially ionised which could account for the observed deviation. Compounds 30, 64 and 77 were also found to be significant outliers in the correlations with log K_β. These compounds along with compound 5 all showed Studentised Residuals in excess of 2.0 and were therefore considered to be statistical outliers. Deleting these six points and repeating the analysis gave the expected improvement in the correlation and the equation shown below.

$$\tau = -0.0654(\pm 0.0035)\pi + 0.0585(\pm 0.0041)$$

$$r^2 = 0.8275 \quad n = 73 \quad F = 341 \quad s = 0.0241$$

The ability of π to describe what is essentially a polar interaction can, in retrospect, be rationalised in one of two ways. Firstly the parameter π is the lipophilicity of a molecular fragment or the 4-substituent in this particular case. These fragmental π values can be summed to generate molecular lipophilicity or log P values. It has been shown [13] that the log P can be described by an equation with three major terms, *viz.*

$$\log P = \text{cavity term} + \text{dipolar term} + \text{hydrogen bonding terms}$$

In the present context, what is of interest is the capacity term which is actually a molecular volume, either the molar volume or the intrinsic molecular volume [14] of the solute in question. The hydrogen bonding term contains a hydrogen bond acceptor contribution related to log K_β. The fact that π alone gave a similar correlation to log K_β in combination with a volume term, is therefore easily understood. It should be noted however that the coefficient in the equation relating τ and π is negative, in contrast to that for log K_β which was positive. This indicates that the effect of π is not related to a hydrophobic or reversed-phase type interaction with the stationary phase.

A second possible explanation for the observed inverse correlation with π relates to the effect and properties of the mobile phase. In the present case the eluent, which is 90% methanol is probably more lipophilic or hydrophobic than the polar ionised stationary phase. Thus it is possible that the compounds with the more lipophilic substituents (*i.e.* higher π) may partition more readily into the mobile phase and hence show more rapid elution than comparable polar compounds with substituents that have lower π values. In fact it is also possible that the exact mechanism could be a mixture of both a hydrogen bonding interaction with the stationary phase and a lipophilic interaction with the mobile phase. The present experiments however do not allow the exact mechanism to be determined.

Analysing the whole data set with all variables using stepwise multiple regression gave the equation shown below.

$$\tau = -0.0599(\pm 0.00421)\pi + 0.00878(\pm 0.00245)\log K_\beta + 0.0278(\pm 0.0079)\mathrm{B2} - 0.0118(\pm 0.0158)$$

$$R^2 = 0.7989 \quad n = 79 \quad F = 99.3 \quad s = 0.0270$$

The quality of the fit is only slightly improved over that between τ and π alone. Adding an extra two variable has only increased the R^2 by approximately 8%. Examination of the residuals

once again showed compounds 5 and 77 to be significant outliers and compounds 30 and 64 to be moderate outliers. Removing these four data points modified the equation to that shown below.

$$\tau = -0.0603(\pm 0.0036)\pi + 0.00763(\pm 0.00214)\log K_\beta + 0.0179(\pm 0.0075)\mathrm{B2} - 0.00979(\pm 0.01420)$$

$$R^2 = 0.8432 \quad n = 75 \quad F = 127 \quad s = 0.0232$$

Collinearity of variables

The above approach using log K_β and π, which contain similar information, in a single equation is only valid if the variables are totally independent. Examining the collinearity of the variables showed the correlation between π and log K_β for the full set of 79 compounds to be very poor with an r^2 of only 0.1077. This can be explained by the fact that whereas π varies more or less continuously between −1.69 and 1.72, log K_β takes discrete values, showing little variation with substitution. For example the ureas (compounds 32 to 46) have log K_β values of 3.2, or in two instances 2.8. The π values of these compounds however varies between −1.41 for compound 32 to +1.72 for compound 46. The correlation between π and the steric terms is similarly poor $r^2 < 0.1598$. In combination however certain parameters correlate reasonably well with π. For example log K_β and fragment volume (FV) gives an R^2 or 0.6232. For the most part however introducing a steric parameter into the correlation between π and log K_β has little effect. On this basis therefore it would seem justified to use π, selected steric parameters and log K_β is a single equation as carried out above.

CONCLUSIONS

The selectivity differences observed between compounds with the same pK_a when chromatographed in an ion-exchange system are due to the polarity of the substituents in the solute molecule.

Increasing polarity of the substituent generally leads to an increase in retention relative to an unsubstituted parent compound. This increase in retention correlates with the size and hydrogen bond acceptor potential (log K_β) of the substituent. Increasing log K_β leads to an increase in retention whilst increasing size of the substituent leads to a decrease in retention. As this secondary interaction involves hydrogen bonding between an acceptor atom in the substituent and unionised silanols, the effect of substituent size can be rationalised in steric terms.

The increase in retention was also correlated with the lipophilicity of the substituent (π) which was able to explain around 71% of the variability in the data. Unlike the correlations with log K_β the coefficient of the π term was negative. This suggests that a lipophilic interaction is taking place between the solute and the mobile phase. The data rules out any reversed-phase interaction with the silica stationary phase as has been suggested by other workers [14].

Overall the quality of the correlations were poor. This can be explained by a number of factors such as the inherent variability in the retention data (shown by a number of significant outliers) and the narrow retention range. More importantly however the terms log K_β and π although relatively useful are probably not a true measure of the interactions involved. The hydrogen bond acceptor values are based on one-to-one interactions, which in the case of the sulphones at least, appears to be an over simplification of the situation in the present chromatographic conditions. It is possible that other substituents are also capable of multiple interactions. Furthermore no account was taken of the substituents which had multiple hydrogen bonding atoms (*e.g.* 11 and 25).

The substituent lipophilicity values π obviously contain information that partially describes the secondary interactions observed here. However, as the concept of lipophilicity was originally designed to explain biochemical phenomenon, in particular the passage across lipid membranes [9], it is hardly surprising that π values are of only limited values in describing a phenomenon involving polar interactions.

Refinement of the data and additional studies will be necessary before retention of a novel solute can be accurately predicted.

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