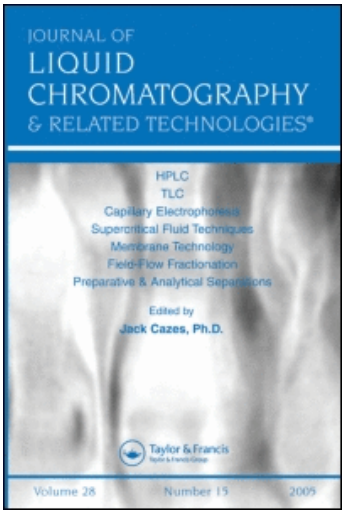

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Quantitative Structure Retention Relationship Studies of Some Basic Antimalarial Compounds

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To cite this Article Taylor, R. B. , Ochekpe, N. A. and Wangboonskul, J.(1989) 'Quantitative Structure Retention Relationship Studies of Some Basic Antimalarial Compounds', Journal of Liquid Chromatography & Related Technologies, 12: 9, 1645 — 1668
To link to this Article: DOI: 10.1080/01483918908049532
URL: http://dx.doi.org/10.1080/01483918908049532

JOURNAL OF LIQUID CHROMATOGRAPHY, 12(9), 1645-1668 (1989)

QUANTITATIVE STRUCTURE RETENTION RELATIONSHIP STUDIES OF SOME BASIC ANTIMALARIAL COMPOUNDS

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ABSTRACT

Studies have been made of the correlation between chromatographic retention parameter, k' and solute hydrophobicity as determined by the oil-in-water partition coefficient. Using a set of 29 basic antimalarial drugs and analogues such correlations have been made under ion pairing and non ion pairing conditions. The capacity factors have been obtained extrapolated to pure water and in the case of ion pairing conditions, the k'max values have been used.

It is shown that better correlations can be obtained under the ion pairing conditions. The use of oppositely charged ion pairing agents can provide an alternative to the inclusion of hydrophobic alkylamines in order to overcome putative effects of residual silanols.

INTRODUCTION

There have been numerous attempts to describe the interrelationship between the oil-in-water partition coefficient (P) and the chromatographic column capacity

factor (k') in reversed phase HPLC (1,2,3). The purposes of establishing such correlations have included more rapid determination of hydrophobicity parameters for use in quantitative structure activity analysis of drugs, the prediction of retention when log P values are known as an aid in analytical method development and also the investigation of processes occurring during chromatography using reversed phase systems.

Using various particular eluting solvents with usually octadecylsilica linear correlations between log k' and log P have been obtained for several sets of neutral solutes and for acidic solutes. To overcome the variety of mobile phases which have been chosen the capacity factor k'_w obtained by extrapolation to an eluting solvent of pure water has been increasingly used (3,4,5). For basic solutes in general poor correlations have been achieved (6). This has been attributed to ion exchange processes occurring between wholly or partly ionised basic solutes and acidic silanol groups remaining on the C-18 surface. Better correlations have been established for such solutes following the addition of hydrophobic alkylamines to the eluting solvent (7,8). Such alkylamines are believed to operate by preferentially interacting with the residual silanol groups.

An alternative approach is to minimise the ionic nature of the basic solutes by the addition of hydrophobic anionic species, that is, compounds which are generally used as ion pairing agents. Little work has been published on this approach and such work has used arbitrary concentrations of pairing ion and organic modifier (6).

It has been demonstrated that for many solute systems under ion-pair conditions the k' value increases to a maximum and then decreases as eluant pairing ion concentration increases (9,10,11). It is possible that the maximum k' obtainable is a characteristic chromatographic property related to solute hydrophobicity. The ion-exchange retention is being fully accommodated by using the basic solute in the fully protonated state as a result of the presence of adequate concentrations of pairing ion.

It is the purpose of the present work to compare correlations obtained between the capacity factor k'_w in absence and presence of pairing ion using the maximum k'_w (k'_wMax) obtained under ion pairing conditions.

A set of compounds based largely on analogues of metabolites of the antimalarial drug proguanil are used as solutes together with some other antimalarial drugs of diverse chemical structures. As part of an ongoing

programme of work related to assay of such antimalarial drugs (12,13), it is also intended that this quantitative structure retention study will provide information useful in assay development for antimalarial drugs using ion pairing systems.

EXPERIMENTAL

Materials and Equipment

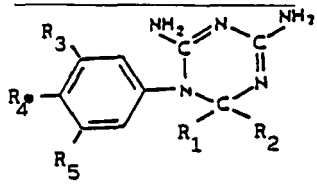
The 29 basic compounds used as test solutes comprised three distinct groups. Eleven of these were analogues of cycloguanil, the major metabolite of proguanil and 15 were analogues of 4-chlorophenylbiguanide, the second metabolite of this drug. These compounds were kindly made available by Imperial Chemical Industries plc. The structures of these compounds and analogues are shown in Tables 1 and 2. Chloroquine was obtained as a gift from Wallace Chemist as was pyrimethamine from Roche and quinine from Aldrich Chemicals.

The chromatograph used consisted of a Waters Associates M6000A pump and fixed wavelength (254 nm) UV detector Model 441. Injection was via a Rheodyne 7125 valve fitted with a 20 ul loop. The chromatographic columns used were 100 x 2 mm I.D. and 40 x 2 mm I.D., depending upon the magnitude of the capacity factor and were stainless steel slurry packed with 5 um ODS Hypersil obtained from Shandon. Acetonitrile was

TABLE 1

Showing structures of cycloguanil analogues and listing antimalarial drugs used.

A. CYCLOGUANIL SERIES



Compound No	R_1	R_2	R_3	R_4	R_5
1	CH_3	CH_3	H	Cl	H
2	CH_3	C_2H_5	H	Cl	H
3	CH_3	CH_3	H	Cl	Cl
4	CH_3	CH_3	H	Br	H
5	CH_3	CH_3	H	I	H
6	H	CH_3	H	Cl	H
7	CH_3	CH_3	Cl	H	Cl
8	CH_3	CH_3	Br	Cl	H
9	CH_3	CH_3	Cl	I	H
10	H	CH_3	Cl	H	H
11	CH_3	CH_3	H	Cl	Cl

B. ANTIMALARIAL DRUGS

19 proguanil

20 chlorproguanil

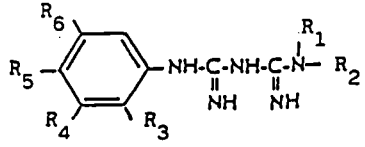
27 chloroquine

28 pyrimethamine

29 quinine

TABLE 2

Showing structures of 4-chlorophenyl-biguanide analogues.



Compound No	R_1	R_2	R_3	R_4	R_5	R_6
12	CH_3	C_3H_7	H	Cl	I	H
13	H	C_3H_7	H	Br	Br	H
14	CH_3	$CH(CH_3)_2$	H	Br	I	H
15	CH_3	$CH(CH_3)_2$	H	Cl	Cl	H
16	H	C_4H_9	H	Cl	Cl	H
17	H	H	H	H	Cl	H
18	H	H	H	Cl	Cl	H
19	H	$CH(CH_3)_2$	H	H	Cl	H
20	H	$CH(CH_3)_2$	H	Cl	Cl	H
21	H	H	H	H	Cl	H
22	H	H	H	H	Cl	H
23	H	H	Cl	H	H	Cl
24	H	H	R	Cl	H	H
25	H	H	F	H	H	H
26	H	H	H	H	F	H

obtained from Rathburn Chemicals and sodium lauryl sulphate from Fisons. Water was purified using a Millipore Milli-Q system.

Procedure

The retention times of all solutes were determined at acetonitrile-water mixtures comprising 20, 30, 40, 50 and 60%v/v acetonitrile. In each of these solvents the sodium laurylsulphate concentration was varied from zero to a maximum of 0.24 mol dm^{-3}. This was done by adding solid pairing ion salt to the appropriate concentration of acetonitrile water. The pH of the system was maintained constant at 2.5 using phosphate buffer.

The column hold up time was determined by measuring the first refractive index peak due to the aqueous solvent in which all solutes were injected.

The log P values for all the solutes were estimated using the theoretical calculation method of Rekker (14). Fragmental constants and correction values used were obtained from literature sources (14,15,16).

RESULTS AND DISCUSSION

Non ion-pairing situation

Different extrapolation procedures have been reported in the literature to allow determination of

log k'_w (2,3,4). In the present work under the non ion-pairing condition it was found that logarithmic transformation of both the k' and the organic modifier concentration was required to provide an adequately linear extrapolation. Table 3 shows the characteristics of the best line fit obtained following the sum of squares regression analysis of log k' on log percent acetonitrile concentration for the 29 solutes studied. The log k'_w values quoted represent the intercepts of these lines at zero acetonitrile concentration. The slopes of these regression lines are seen to vary considerably from compound to compound, values from 0.645 to 6.333 being obtained. This is in marked contrast to the situation prevailing under ion-pairing conditions which will be described below and indicates that solute-solvent interactions differ markedly among compounds under these conditions.

Table 3 also shows the log P values calculated for the various compounds. The correlations obtained between log k'_w and log P for the compounds are shown graphically in Figures 1 to 3, together with the linear regression lines obtained. Figure 1 shows the data for the set of 11 cycloguanil derivatives. Figure 2, the corresponding data for the compounds based on 4-chlorophenylbiguanide and Figure 3 the data for the complete set including the structurally different

TABLE 3

Showing the slope and intercept (log k'w) of the best line for the plot of log k' versus log acetonitrile concentration, and the log P for the compounds.

Compound No	log P	log k'w	SLOPE	SD	R
1	-0.550	3.141	1.797	0.271	0.936
2	-0.83	4.739	2.605	0.158	0.986
3	1.440	5.739	3.117	0.429	0.963
4	-0.340	3.565	2.053	0.210	0.969
5	-0.073	4.224	2.413	0.309	0.953
6	-1.094	2.799	1.599	0.287	0.912
7	0.122	4.220	2.356	0.330	0.994
8	0.890	4.290	2.427	0.364	0.937
9	1.320	4.142	2.240	0.254	0.975
10	-1.094	2.630	1.547	0.308	-.894
11	0.220	4.975	2.762	0.176	0.988
12	2.736	9.713	5.000	0.706	0.980
13	3.122	6.597	3.487	0.558	0.951
14	2.945	12.150	6.333	1.411	0.953
15	2.210	7.969	4.328	0.393	0.984
16	3.234	8.179	4.268	0.586	0.964
17	1.658	2.971	1.685	0.225	0.949
18	2.330	4.792	2.699	0.376	0.945
19	2.039	5.439	2.955	0.267	0.976
20	2.704	6.132	3.254	0.289	0.977
21	0.934	2.296	1.249	0.314	0.841
22	1.867	3.027	1.721	0.286	0.923
23	1.462	4.038	2.284	0.267	0.960
24	1.658	2.643	1.534	0.369	0.852
25	0.274	1.347	0.778	0.046	0.993
26	1.135	1.082	0.645	0.089	0.962
27	2.850	4.472	2.587	0.276	0.967
28	0.350	5.243	2.951	0.232	0.981
29	2.645	3.801	2.293	0.198	0.978

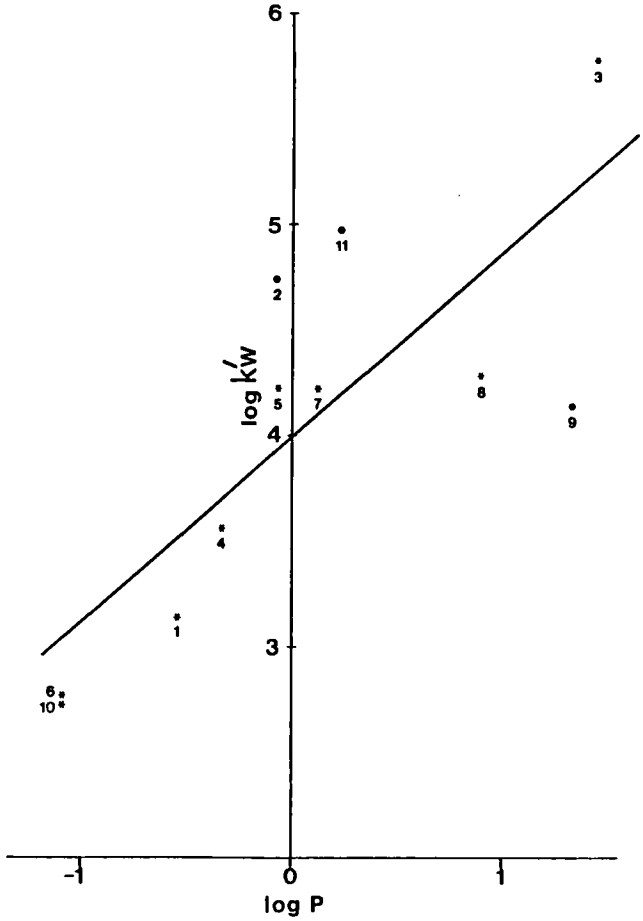


FIGURE 1 Showing the correlation between log k'_w and log P for 11 cycloguanil analogues under non ion-pairing condition. $R^2 = 0.63$; $F_{1,9} = 15.48$.
$\log k'_w = 0.85 \log P + 4.0$

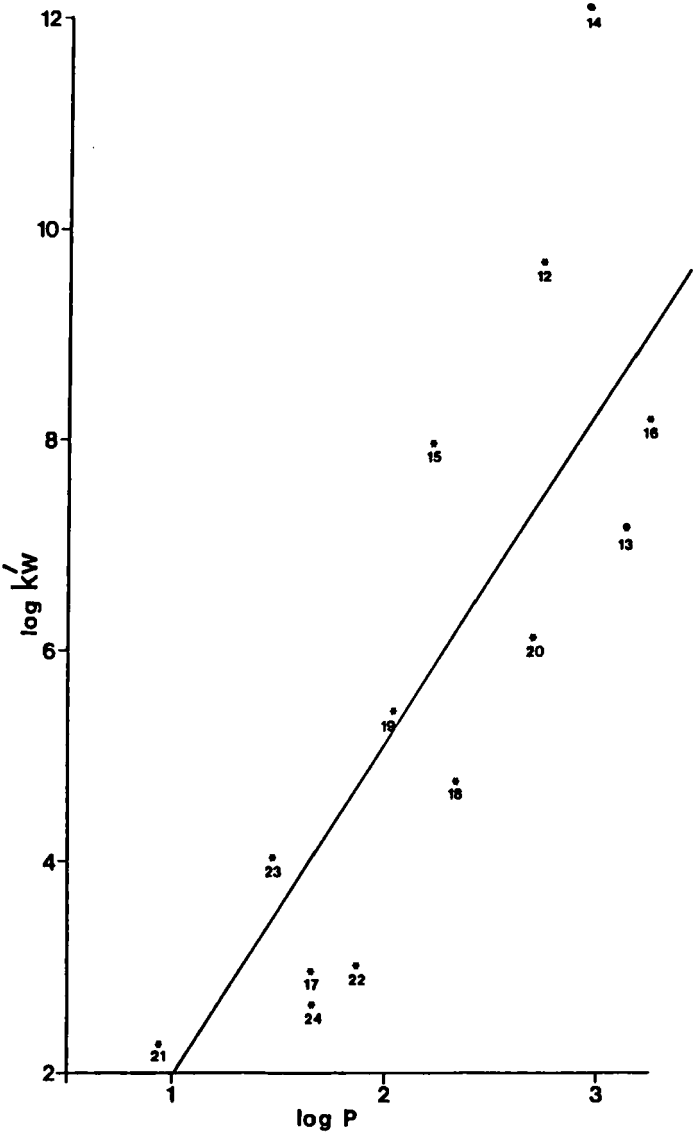


FIGURE 2 Showing the correlation between log k'_w and log P for 4-chlorophenylbiguanide analogues under non ion-pairing condition. $R^2 = 0.68$; $F_{1,13} = 28.54$.
$\log k'_w = 3.13 \log P - 1.09$

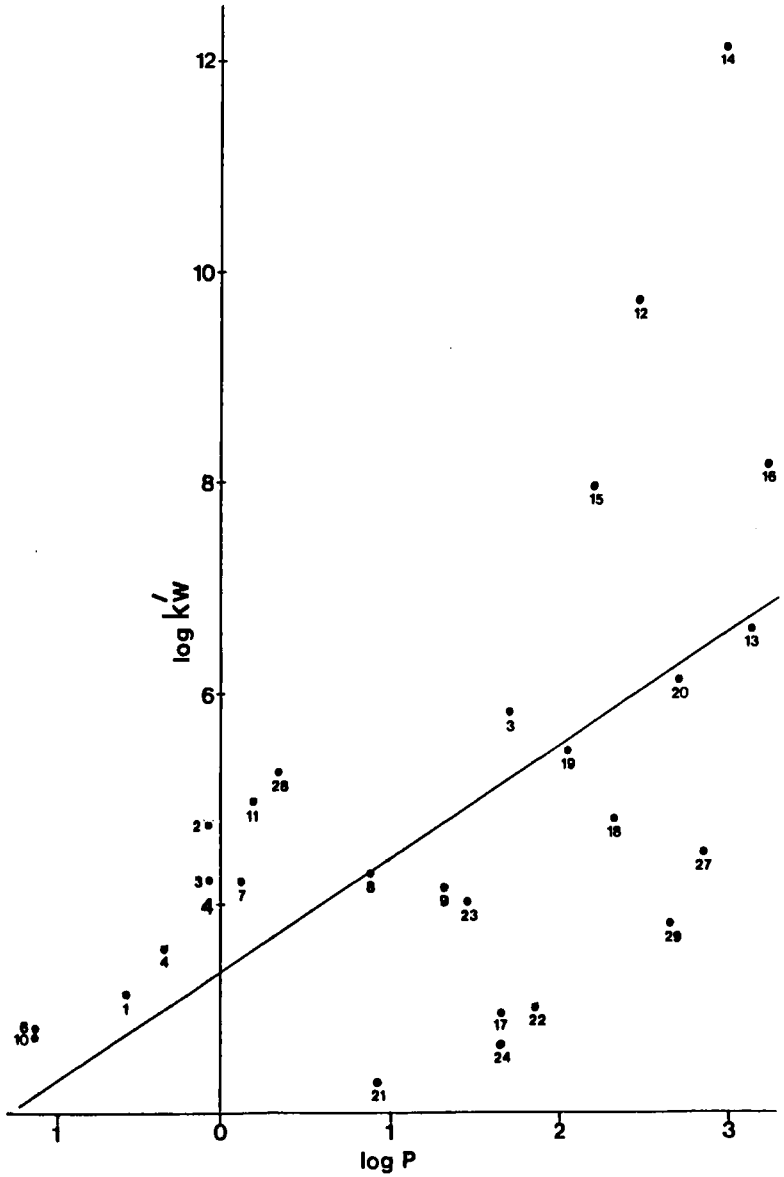


FIGURE 3 Showing the correlation between log k'_w and log P for the 29 compounds studied under non ion-pairing condition. $R^2 = 0.33$; $F_{1,27} = 13.36$.
$\log k'_w = 1.07 \log P + 3.34$

antimalarial drugs. None of these correlations is adequate to be used in any predictive capacity. Under the experimental conditions used namely low pH it is likely that the residual silanol usually held responsible for such poor correlations among basic compounds will be protonated to a large extent. It is likely therefore that the poor correlations are a result of the differences in the electrostatic interactions which are not accounted for by the hydrophobicity parameter, log P.

Ion-pairing situation

Figure 4 shows the variation of capacity factor with pairing ion concentration at a single acetonitrile-water composition for 16 of the 29 compounds studied. All of the compounds studied exhibited the expected maxima. It can be seen that the maximum occurs at a concentration of pairing ion independent of solute studied and in this case at a sodium laurylsulphate concentration of about 9×10^{-2} mol dm^{-3}. Figure 5 shows the corresponding behaviour as the organic modifier content of the mobile phase is changed for a single representative compound, chloroquine. The pattern shown was found to be general for all solutes and is consistent with the interpretation of the nature of the processes occurring during ion pairing (17). As in the case of the non ion

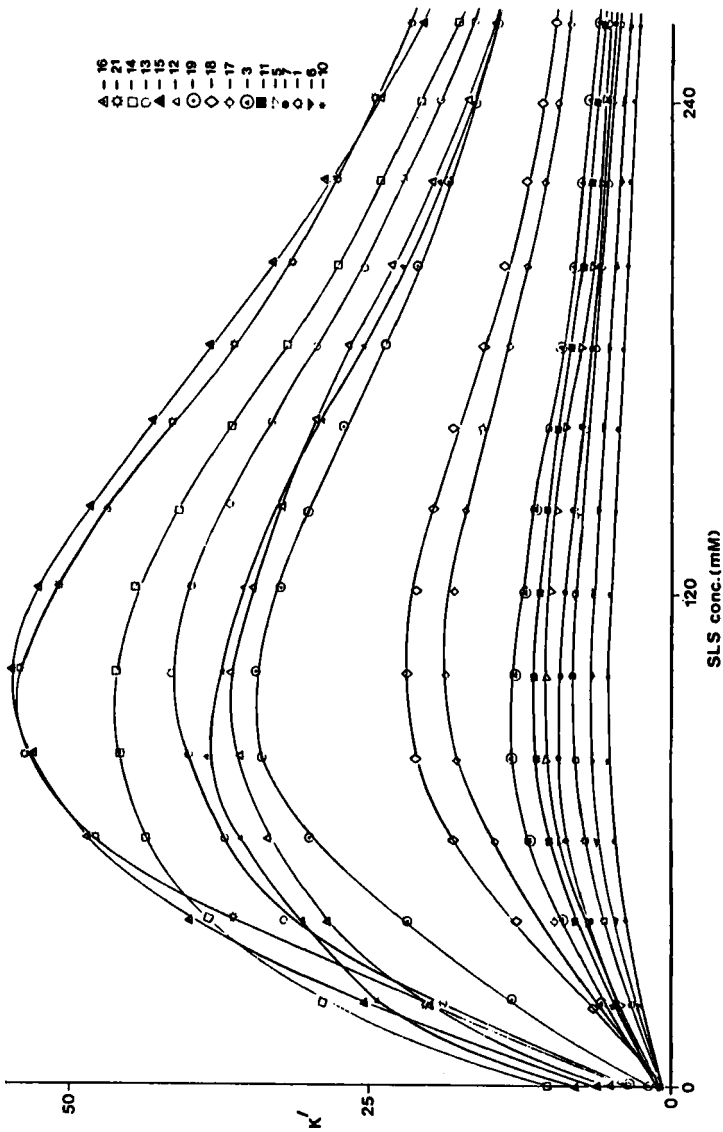


FIGURE 4 Showing the variation of k' with sodium laurylsulphate concentration in an eluent of Acetonitrile:Phosphate buffer, pH 2.5; (50:50), for 16 of the compounds studied.

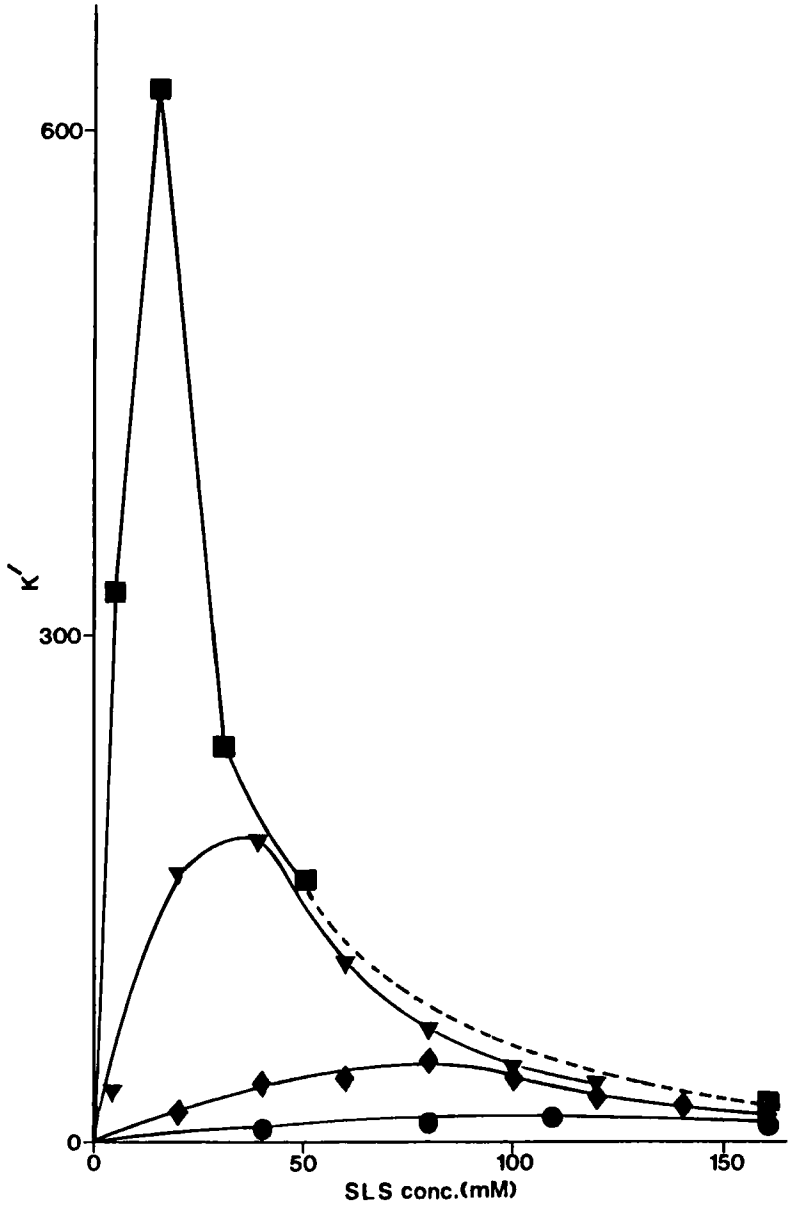


FIGURE 5 Showing the variation of k' for chloroquine with change in the pairing ion concentration added to the mobile phase at four different acetonitrile concentrations (■ 30%, ▲ 40%, ♦ 50%, ● 60%).

pairing behaviour the capacity factor increases as the organic modifier concentration decreases but also the concentration of pairing ion at which the maximum occurs decreases. This is as a result of the adsorption isotherm type changing and supports the contention that the capacity factor at the maximum represents a value which is independent of electrostatic interactions between solute and solvent.

In contrast to the non ion pairing results it was found that the log k'max varied linearly with the solvent organic modifier concentration. Table 4 shows the characteristics of the regression lines obtained of log k'max against acetonitrile concentration. The intercept is shown as log $k'_{w}max$. In this case the slope of these lines is very much more constant ranging from 0.043 to 0.057. This apparent uniformity is interpreted to be a result of the elimination of differences in electrostatic interactions between different solutes and the solvent so that the variation of retention with solvent strength is a result of van der Waals' interactions which are more similar among the set of compounds.

Figure 6 shows the correlation obtained between log $k'_{w}max$ and log P for the set of 11 cycloguanil analogues together with the regression equation of this line. It is seen that the correlation is considerably

TABLE 4

Showing the slope and intercept (log $k'_{w}max$) of the best line for the plot of log k'max versus acetonitrile concentration, and the log P for the compounds.

COMPOUND SERIAL NO	log P	log $k'_{w}max$	SLOPE	SD ($\times 10^{-3}$)	R
1	-0.550	3.272	0.047	3.907	0.986
2	-0.083	3.305	0.047	6.846	0.959
3	1.440	3.987	0.055	8.051	0.959
4	-0.340	3.258	0.046	7.740	0.947
5	-0.073	3.409	0.048	8.159	8.946
6	-1.094	3.349	0.049	2.969	0.993
7	0.122	3.476	0.049	2.613	0.994
8	0.890	3.839	0.055	2.762	0.995
9	1.320	3.981	0.057	2.716	0.995
10	-1.094	2.948	0.043	2.553	0.993
11	0.220	3.553	0.049	3.289	0.991
12	2.736	4.496	0.055	3.553	0.991
13	3.122	4.504	0.054	1.296	0.999
14	2.945	4.657	0.057	3.165	0.994
15	2.210	4.230	0.050	1.774	0.997
16	3.234	4.792	0.057	2.705	0.996
17	1.658	4.082	0.055	2.621	0.995
18	2.330	4.390	0.059	1.897	0.998
19	2.039	4.224	0.053	3.445	0.991
20	2.704	4.340	0.051	5.253	0.979
21	0.934	3.540	0.052	6.811	0.967
22	1.867	4.284	0.059	7.863	0.965
23	1.462	3.878	0.054	6.536	0.972
24	1.658	3.945	0.054	7.880	0.959
25	0.274	3.165	0.047	7.853	0.947
26	1.135	3.660	0.053	5.692	0.977
27	2.850	4.118	0.049	6.455	0.968
28	0.350	3.281	0.045	1.944	0.998
29	2.645	4.460	0.058	1.322	0.999

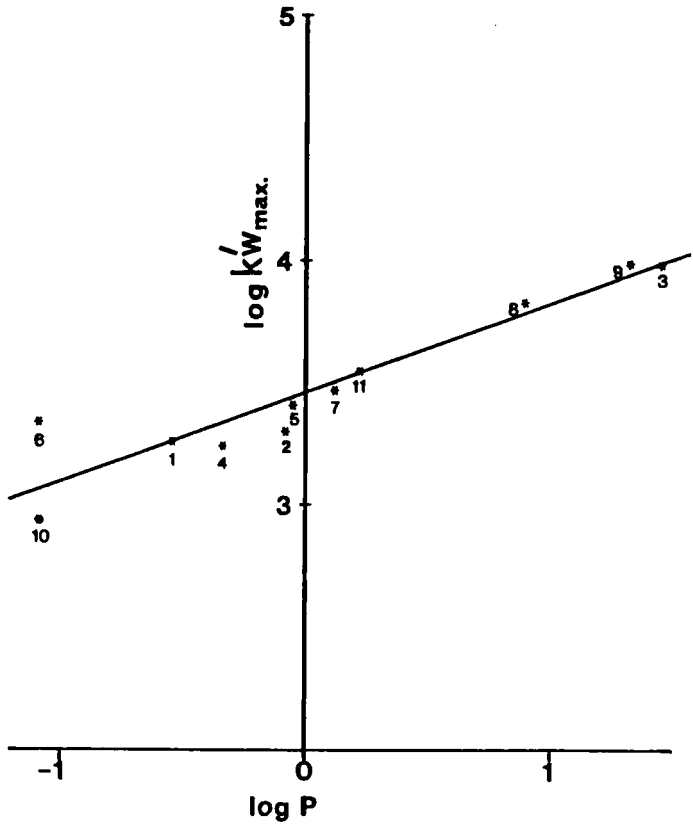


FIGURE 6 Showing the correlation between log k'$_w$max and log P for the 11 cycloguanil analogues under ion-pairing condition. $R^2 = 0.88$; $F_{1,9} = 69.29$.
log k'$_w$max = 0.35log P + 3.46

improved. Figure 7 shows the corresponding results for the set of 15 4-chlorophenylbiguanide compounds used and Figure 8 the correlation for all 29 compounds tested as a single set. In all cases it is obvious that the correlation is better when log k'$_w$max is used

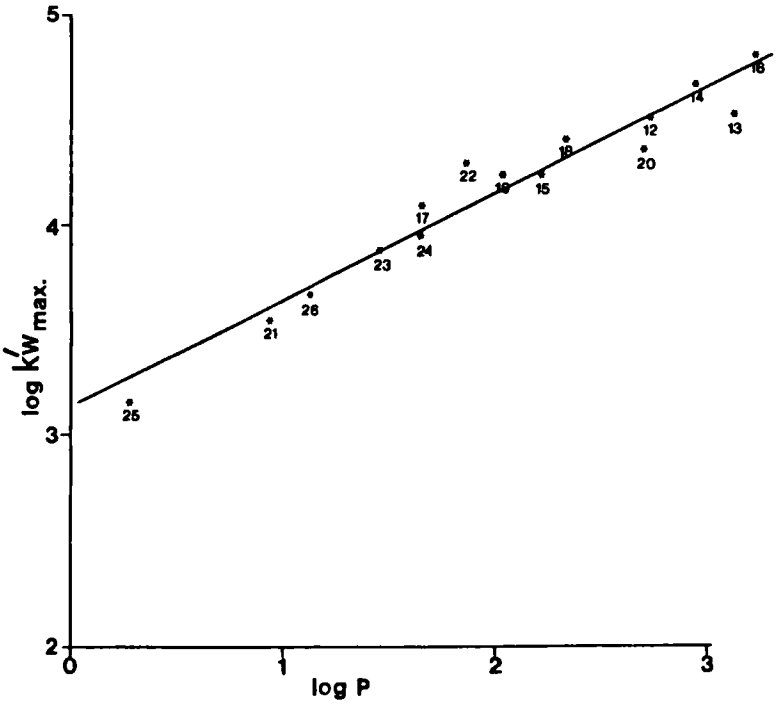


FIGURE 7 Showing the correlation between log k'_wmax and log P for the 4-chlorophenylbiguanide analogues under ion-pairing condition. R^2 = 0.94; $F_{1,13}$ = 219.06.
log k'_wmax = 0.50log P + 3.13

as the chromatographic parameter. In the case of the cycloguanil derivatives (Figure 6) points 6 and 10 refer to <u>para</u> and <u>meta</u> isomers of the same compound and no satisfactory distinction based on the hydrophobic fragmental constant approach was found possible. Omission of compound 6 from the set resulted in a correlation of 0.982 for this set.

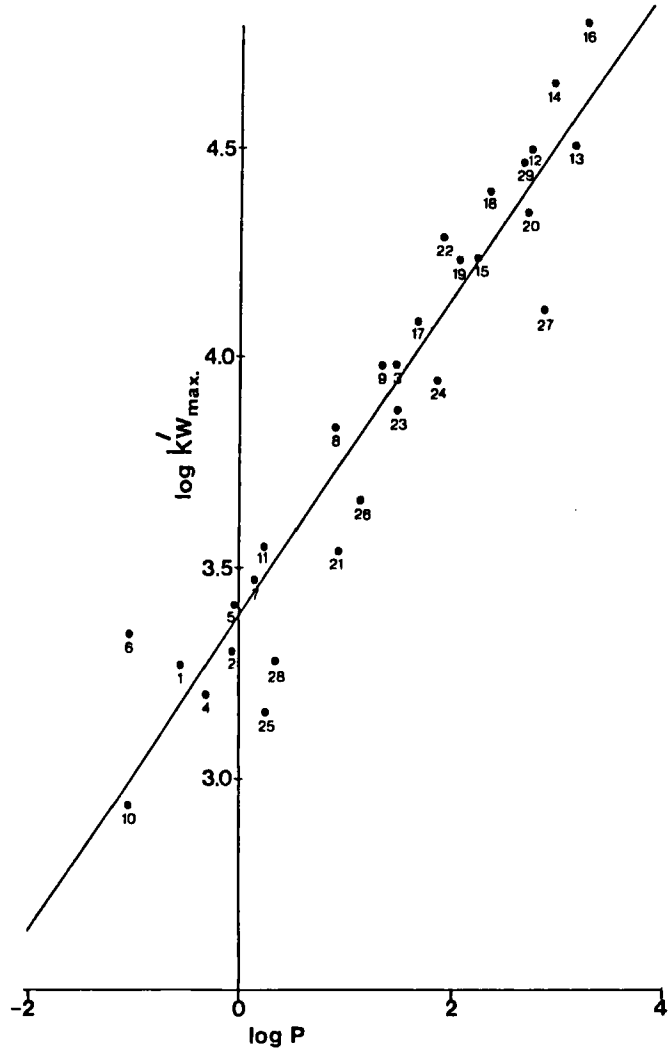


FIGURE 8 Showing the correlation between log k'$_w$max and log P for all the 29 compounds under ion-pairing condition.
R^2 = 0.90; $F_{1,27}$ = 262.77
log k'$_w$max = 0.37log P + 3.40

CONCLUSION

The present results confirm that poor correlations are obtained between the chromatographic capacity factor and the hydrophobic property of a given solute in the case of basic compounds even when the capacity factor is normalised to a solvent of pure water. They also indicate that this is not wholly attributable to the effect of residual silanol groups since under the acidic conditions of chromatography used, these would at least be partially protonated and therefore unlikely to interact with positively charged base cations. Under these conditions the present measurements showed that extremely poor correlations are still obtained.

The strategy of minimising differences in electrostatic interaction between solute and solvent by the mechanism of oppositely charged pairing ion is supported by the present work. This allows correlation to be made to a much higher statistical level between the retention under ion pairing conditions and the hydrophobic properties of the solutes. In addition, it provides an alternative to the use of multiparameter correlation which would require additional physical data such as dissociation constants that have been quoted as variable (18). While this is only of relevance when used under ion pairing conditions it is in this context that it may prove useful in obtaining

more rapid analytical method development. The present work has involved making large number of measurements to establish the k'max at a given organic modifier concentration. It is obvious from the results obtained that, for a given solvent composition, the concentration at which the maximum occurs is dependent only upon the pairing ion and solvent composition. Thus the variation of k' with pairing ion concentration need not be studied for all compounds in a given set.

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