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LIPOPHILICITY MEASUREMENTS OF PROTONATED BASIC COMPOUNDS BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

I. RELATIONSHIP BETWEEN CAPACITY FACTORS AND THE METHANOL CONCENTRATION IN METHANOL–WATER ELUENTS

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SUMMARY

The retention behaviour of protonated basic compounds in reversed-phase high-performance liquid chromatography, using methanol–water mixtures as the eluent, is reported. A minimum is found in the relationship between the logarithm of the capacity factor (log k) and the percentage of methanol (x) in the eluent. The deviation from linearity is postulated to be caused by a dual retention mechanism, namely polar interactions between the solute and eluent molecules in water-poor eluents, and hydrophobic expulsion in water-rich ones. The influence of the pH, pK_a and lipophilicity on retention behaviour is also investigated.

INTRODUCTION

The use of alkyl-bonded phases in high-performance liquid chromatography (HPLC) offers a convenient and highly accurate method to evaluate the hydrophobicity of a large variety of compounds[1–5]. However, the mechanisms of retention are based on intermolecular interactions which are still poorly understood.

Solute–eluent interactions play an important rôle in the mechanism of retention. As explained by Sinanoglu[6], these interactions reflect the net free-energy change for the transfer of solute molecules into the eluent, *i.e.*, the sum of (a) the energy required to make a suitable cavity in the eluent, and (b) the free-energy change arising from the interactions of the solute molecules with the surrounding solvent molecules.

In many studies[8–14] the effect on retention behaviour of organic modifiers, *e.g.*, methanol, acetonitrile, has been described by linear relationships between the logarithm of the capacity factor (log k) and the volume per cent of the organic modifier (x), *e.g.*, eqn. 1[15].

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

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where A is a constant for a given organic modifier and a given solute, and $\log k_w$ is the logarithm of the extrapolated capacity factor in 100% water as eluent. However, a number of workers[16–18] have shown that the effect of organic modifiers is not strictly linear. Deviations from linearity have been explained in terms of silanophilic interactions[19–21], conformational changes of the solute[22] and the amount of organic modifier adsorbed on the stationary phase[23]. Schoenmakers *et al.*[24,25] suggested that eqn. 1 is generally invalid, and should be replaced by the quadratic expression:

$$\log k = Ax^2 + Bx + \log k_w \quad (2)$$

In the present study, the behaviour of a series of strongly basic compounds ($pK_a > 7$) has been investigated in order to assess the relationships between capacity factor, mobile phase properties (methanol content, pH) and solute properties (hydrophobicity, pK_a). Since in reversed-phase high-performance liquid chromatography (RP-HPLC) the mobile phase is restricted to pH < 8, strongly basic compounds cannot be eluted in their un-ionized forms. In contrast, Pietrzyk and co-workers[26,27] investigated the effects of pH on the retention of ionizable compounds over a large pH range using a porous polystyrene copolymer stationary phase which is more stable but less efficient than alkyl-bonded stationary phases.

EXPERIMENTAL

Materials

All compounds were of the highest available purity and were obtained either from pharmaceutical companies (sulpiride, sultopride, alizapride, sulmepride, flubepride, metoclopramide, clebopride, spiperone, haloperidol, benperidol, pipamperone, mezilamine and bromocryptine) or from commercial sources (benzylamine, 2,2-diphenylethylamine and *n*-decylamine). Analytical grade methanol and 3-morpholinopropanesulphonic acid were obtained from Merck (Darmstadt, F.R.G.).

Chromatography

A Siemens S101 chromatograph equipped with an Orlita pump Type DMP-AE 10.4 was used. The detector was a Uvikon 740 LC (Kontron), operating at 254 nm; the column (25 cm × 4 mm I.D.) was prepacked with LiChrosorb RP-18, particle size 10 μm (Knauer). A Hewlett-Packard 3390A integrator was used for peak registration and calculation of retention times. Preliminary tests with a deactivated column were not satisfactory; therefore, *n*-decylamine (0.2%, v/v) was used as a masking agent to eliminate silanophilic interactions[28–30].

Buffering agent and ion-pair formation

Most of the strongly basic compounds have a $pK_a > 7$ implying that they are almost completely protonated under the described pH conditions. Protonated molecules tend to partition as an ion pair depending on the nature of the available anions in the mobile phase. Thus, phosphate which is frequently used as a buffer, forms ion pairs with protonated molecules. To avoid ion-pair formation a zwitterionic buffer (0.02 *M* 3-morpholinopropanesulphonate, MPS) was used in our study; it is known to have a large buffering capacity and poor ion-pair formation ability[31].

Mobile phase

The mobile phase was made up volumetrically from various combinations of methanol and 3-morpholinopropanesulphonate buffer (0.02 *M*) and *n*-decylamine (0.2%, v/v) in the range $10 \leqslant x \leqslant 80$. The pH of the mobile phase was adjusted to the desired value by addition of HCl to the aqueous solution of MPS and *n*-decylamine, *i.e.*, the measured pH corresponds to the pH in water (without methanol). All solutions were purified by filtration using a Millipore-Q system. Retention times were measured at ambient temperature, the flow-rate was 1.5 ml/min and the column dead time, t_0, was determined using methanol as the non-retained compound. The capacity factor, k, is defined as

$$k = (t_R - t_0)/t_0 \tag{3}$$

where t_R is the retention time of the solute.

RESULTS AND DISCUSSION

Effect of methanol concentration

As is well known, the capacity factor is specially sensitive to changes in the surface tension of the eluent and to the surface area of the solute. Thus, the solvophobic theory[32,33] predicts that the capacity factor increases with the surface tension over almost the entire range of mobile phase composition.

In the present study, minima were observed in the capacity factor as a function of mobile phase composition for partially or completely protonated polar solutes (see later, Figs. 1 and 2). At high methanol percentages, a decrease in its concentration results in a decrease in the capacity factor until a minimum is reached. Further decrease in the methanol concentration results in an increase in capacity factor. This may be due to the fact that at high methanol concentration the hydrophobic expulsion is generally attenuated, the hydrogen bonds no longer forming an isotropic network throughout the solvent as is the case in pure water. In other words, the surface tension is minimized and the energy required to form a cavity is negligible; the driving force for retention will arise from polar interactions of the solute molecules with surrounding solvent molecules. Furthermore, at very high methanol concentrations (low dielectric constant), the ionisation of solutes decreases, thus increasing their retention time. In contrast, when the methanol concentration is decreased, the surface tension and dielectric constant increase and the hydrophobic expulsion becomes the predominant retention mechanism. In summary, deviations from linearity observed in the present study are postulated to be due to a dual retention mechanism, namely polar interactions in a water-poor eluent, and hydrophobic interactions in a water-rich one.

Effects of pH

For ionizable compounds, the effect of pH on retention behaviour has often been described[34–39], the capacity factor being a sigmoidal function of pH. In general, the ionized form is eluted faster than the neutral one. In the present paper, we are concerned with the effect of pH on the minimum in the curve of capacity factor *versus* per cent methanol. The results obtained with fifteen basic compounds, mostly neuropharmacological agents, are gathered in Tables I–IV, and are illustrated for a typ-

ical solute, namely sulpiride, in Fig. 1. The curves have the general shape of a parabola, and very good fits were obtained ($r^2 > 0.95$).

For a given solute, the methanol concentration at which a minimum in the capacity factor is observed depends on the proportion of the protonated species. At low pH, the proportion of protonated species increases, and as a consequence the magnitude of the ionic interaction between the solute and solvent molecules will increase. This displaces the minimum to lower methanol concentrations (Tables I–IV and Fig. 1).

Effects of solute pK_a and hydrophobicity

At a fixed pH value, the ionization constant (pK_a) and the hydrophobicity of solutes determine to a great extent their retention behaviour (Fig. 2). Our results

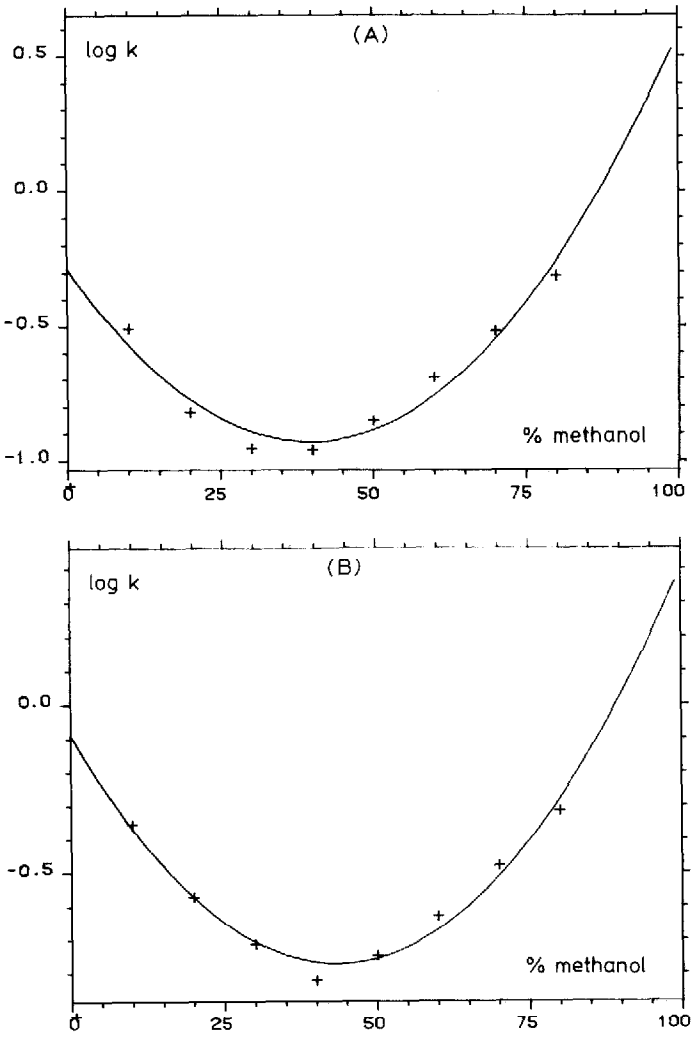

Fig. 1.

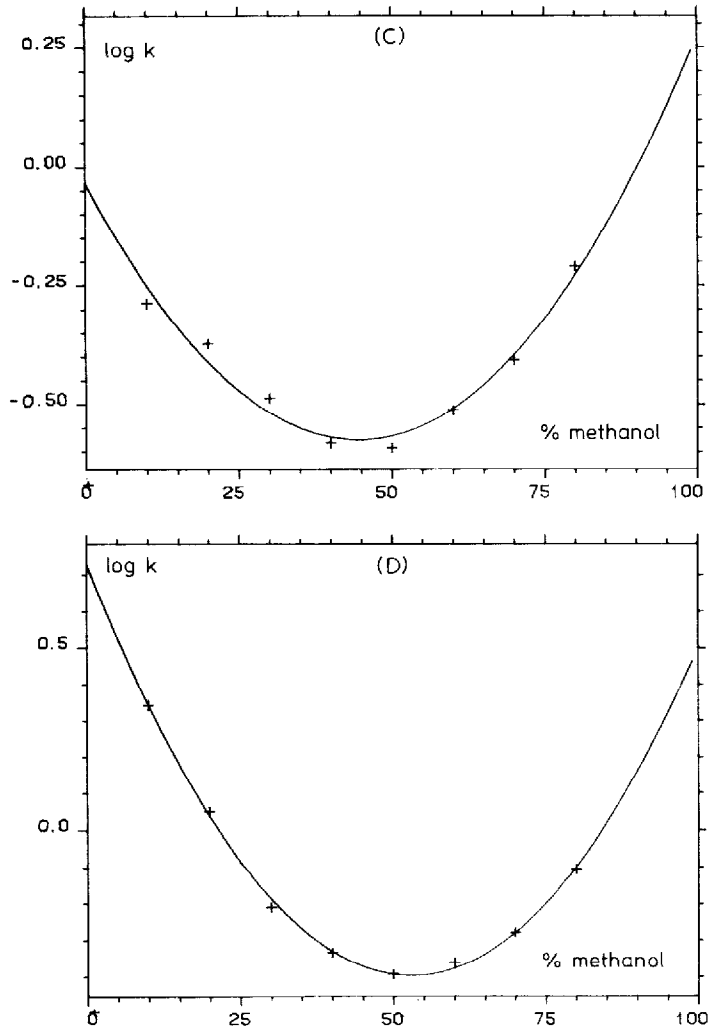

Fig. 1. Retention behaviour of sulpiride at different pH values in RP-HPLC. A minimum is observed in the curve relating log k and methanol content, the displacement of which is dependent on the proportion of protonated species. The curves have the general shape of a parabola ($r^2 > 0.95$). pH values: 6.0 (A), 6.5 (B), 7.0 (C) and 7.5 (D).

show a linear relationship between the methanol concentration at which a minimum is observed and the lipophilic index of the solutes[40]. As shown in Table V, the minimum (as defined by the calculated parabola) moves to higher methanol concentrations and even vanishes for the most lipophilic compound (bromocryptine). No minimum is observed for the most hydrophilic compound (benzylamine). Excluding these two compounds, the relationship between methanol content at minimum position and the lipophilic index (log k_w) is given by eqn. 4

$$\text{minimum position} = 14.16\ (\pm\ 2.69)\ \log k_w + 44.84\ (\pm\ 4.00) \quad (4)$$

$$r = 0.961,\ s = 4.18,\ n = 13,\ F = 134$$

TABLE I

RP-HPLC CAPACITY FACTORS OF BASIC COMPOUNDS AT pH 6.00

Underlined log k values are observed minima; a broken line indicates that the minimum was not reached. log k_x corresponds to the capacity factor determined at x% methanol in the methanol–water eluent.

Compound	*log* k_{80}	*log* k_{70}	*log* k_{60}	*log* k_{50}	*log* k_{40}	*log* k_{30}	*log* k_{20}	*log* k_{10}
Benzylamine	−0.264	−0.383	−0.504	−0.641	−0.801	−0.919	−1.011	−1.145
Sulpiride	−0.312	−0.518	−0.691	−0.852	−0.959	−0.953	−0.820	−0.507
Sulmepride	−0.298	−0.507	−0.701	−0.848	−0.919	−0.907	−0.810	−0.482
Sultopride	−0.100	−0.340	−0.491	−0.608	−0.636	−0.511	−0.399	−0.273
2,2-DPEA*	−0.092	−0.209	−0.222	−0.169	−0.010	0.157	0.291	0.474
Metoclopramide	0.101	0.056	−0.152	−0.215	−0.198	−0.060	0.179	0.515
Alizapride	−0.135	−0.381	−0.507	−0.544	−0.441	−0.079	0.243	0.784
Flubepride	−0.176	−0.355	−0.400	−0.292	−0.032	0.384	0.657	–
Pipamperone	−0.085	−0.134	−0.118	0.030	0.322	0.684	0.936	–
Mezilamine	0.333	0.185	0.294	0.532	0.794	1.007	–	–
Haloperidol	−0.065	−0.011	0.085	0.318	0.689	1.028	–	–
Spiperone	−0.101	−0.149	−0.082	0.158	0.605	1.005	–	–
Clebopride	−0.102	−0.156	−0.101	0.273	0.671	1.035	–	–
Benperidol	−0.113	−0.176	−0.121	0.224	0.801	1.092	–	–
Bromocryptine	0.384	0.904	1.524	−**	–	–	–	–

* 2,2-Diphenylethylamine.
** Not measurable due to excessive retention time.

TABLE II

RP-HPLC CAPACITY FACTORS OF BASIC COMPOUNDS AT pH 6.50

Compound	*log* k_{80}	*log* k_{70}	*log* k_{60}	*log* k_{50}	*log* k_{40}	*log* k_{30}	*log* k_{20}	*log* k_{10}
Benzylamine	−0.191	−0.354	−0.488	−0.601	−0.784	−0.889	−0.894	−0.932
Sulpiride	−0.314	−0.480	−0.629	−0.747	−0.820	−0.712	−0.572	−0.355
Sulmepride	−0.929	−0.457	−0.611	−0.682	−0.742	−0.635	−0.517	−0.335
Sultopride	−0.090	−0.292	−0.398	−0.452	−0.456	−0.374	−0.041	0.279
2,2-DPEA	−0.070	−0.170	−0.156	−0.058	0.128	0.308	0.413	0.610
Metoclopramide	0.100	0.069	−0.110	−0.135	−0.093	0.065	0.263	0.548
Alizapride	−0.131	−0.327	−0.281	−0.013	0.443	0.795	1.262	–
Flubepride	−0.151	−0.261	−0.218	0.037	0.357	0.775	1.134	–
Pipamperone	−0.076	−0.073	0.011	0.244	0.577	0.962	1.154	–
Mezilamine	0.340	0.310	0.445	0.753	1.064	1.38	–	–
Haloperidol	−0.062	−0.016	0.153	0.512	0.849	1.217	–	–
Spiperone	−0.110	−0.097	0.047	0.420	0.826	1.239	–	–
Clebopride	−0.097	0.048	0.184	0.544	0.935	1.373	–	–
Benperidol	−0.110	−0.085	0.123	0.531	0.971	1.456	–	–
Bromocryptine	0.424	1.001	1.670	–	–	–	–	–

TABLE III

RP-HPLC CAPACITY FACTORS OF BASIC COMPOUNDS AT pH 7.00

Compound	*log* k_{80}	*log* k_{70}	*log* k_{60}	*log* k_{50}	*log* k_{40}	*log* k_{30}	*log* k_{20}	*log* k_{10}
Benzylamine	−0.116	−0.302	−0.408	−0.470	−0.593	−0.628	−0.712	−0.685
Sulpiride	−0.210	−0.407	−0.512	−0.592	−0.582	−0.487	−0.372	−0.287
Sulmepride	−0.167	−0.359	−0.465	−0.504	−0.463	−0.395	−0.326	−0.241
Sultopride	−0.016	−0.171	−0.266	−0.295	−0.253	−0.054	0.140	0.404
2,2-DPEA	−0.048	−0.070	−0.010	0.162	0.337	0.498	0.623	0.816
Metoclopramide	0.170	0.081	−0.018	−0.040	0.095	0.254	0.422	0.697
Alizapride	−0.083	−0.156	−0.092	0.178	0.617	1.046	–	–
Flubepride	−0.091	−0.021	0.218	0.506	0.822	1.160	–	–
Pipamperone	0.017	0.089	0.289	0.559	0.883	1.148	–	–
Mezilamine	0.479	0.572	0.783	1.224	1.435	–	–	–
Haloperidol	−0.006	0.099	0.338	0.706	1.144	–	–	–
Spiperone	−0.049	0.070	0.312	0.699	1.185	–	–	–
Clebopride	0.036	0.177	0.492	0.878	1.300	–	–	–
Benperidol	−0.017	0.116	0.428	0.877	1.436	–	–	–
Bromocryptine	0.530	1.105	1.782	–	–	–	–	–

where the uncertainties are 95% confidence limits. To allow comparison with an un-ionized base, Fig. 3 shows the capacity-factor variations for aniline at pH = 7.5. Here, the hydrophobic expulsion predominates over the entire range of eluent composition, and a linear relationship is observed, corresponding to the vast majority of observations reported in the literature.

TABLE IV

RP-HPLC CAPACITY FACTORS OF BASIC COMPOUNDS AT pH 7.50

Compound	*log* k_{80}	*log* k_{70}	*log* k_{60}	*log* k_{50}	*log* k_{40}	*log* k_{30}	*log* k_{20}	*log* k_{10}
Benzylamine	−0.025	−0.204	−0.309	−0.380	−0.437	−0.495	−0.517	−0.470
Sulpiride	−0.105	−0.278	−0.361	−0.393	−0.333	−0.209	0.050	0.343
Sulmepride	−0.060	−0.185	−0.263	−0.289	−0.198	−0.050	0.104	0.327
Sultopride	0.072	−0.031	−0.083	−0.079	0.033	0.212	0.390	0.586
2,2-DPEA	0.020	0.079	0.153	0.303	0.512	0.747	0.853	–
Metoclopramide	0.244	0.159	0.140	0.162	0.283	0.469	0.658	0.864
Alizapride	−0.009	0.061	0.220	0.519	0.998	–	–	–
Flubepride	−0.030	0.139	0.354	0.668	1.108	–	–	–
Pipamperone	0.134	0.255	0.436	0.704	1.067	–	–	–
Mezilamine	0.543	0.723	0.939	1.262	–	–	–	–
Haloperidol	0.090	0.266	0.516	0.890	1.386	–	–	–
Spiperone	0.053	0.241	0.513	0.911	1.444	–	–	–
Clebopride	0.163	0.404	0.703	1.111	–	–	–	–
Benperidol	0.092	0.330	0.654	1.074	1.662	–	–	–
Bromocryptine	0.536	1.134	1.825	–	–	–	–	–

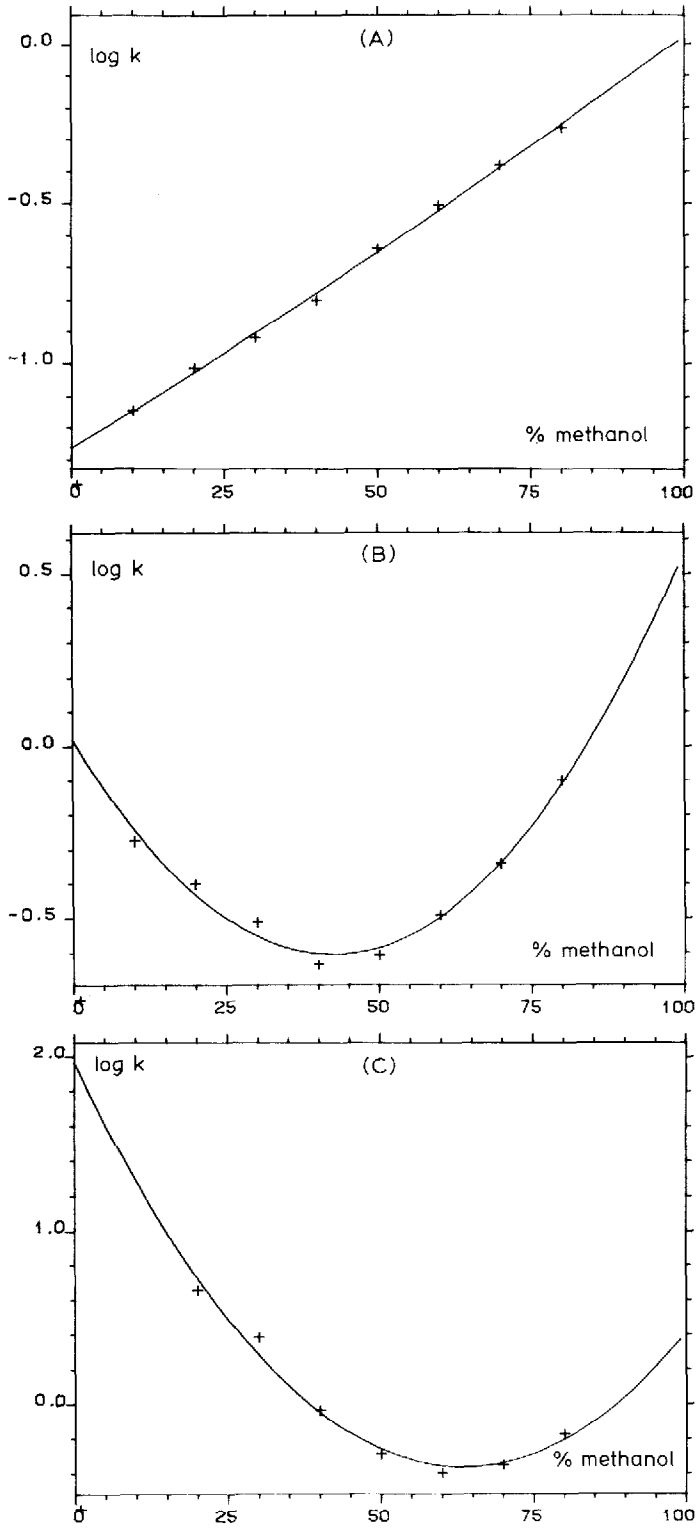
(A)
log k
0.0
-0.5
-1.0
% methanol
0
25
50
75
100
(B)
log k
0.5
0.0
-0.5
% methanol
0
25
50
75
100
(C)
log k
2.0
1.0
0.0
% methanol
0
25
50
75
100

Fig. 2.

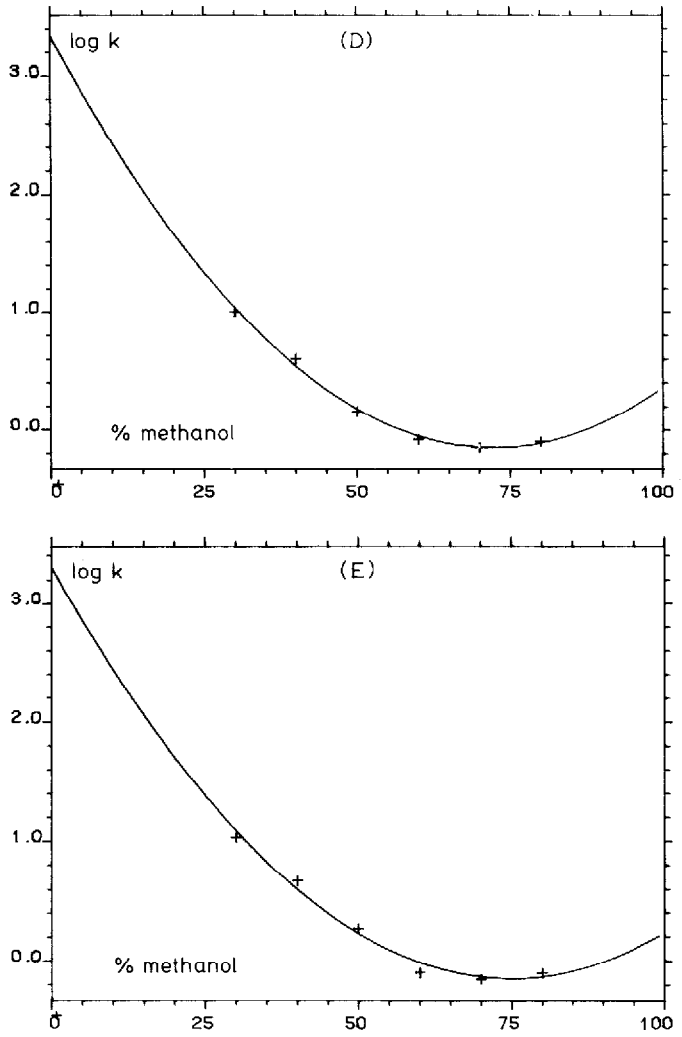

Fig. 2. Retention behaviour of compounds of different lipophilicity as a function of the eluent composition at fixed pH (6.0). Compounds: benzylamine (A), sultopride (B), flubepride (Cl), spiperone (D) and clebopride (E).

CONCLUSION

The present study shows that in RP-LC the relationship between capacity factor (log k) and the methanol content (x) depends on the nature of the solute.

(a) For neutral and/or non-polar compounds, the relationship is generally linear in accordance with eqn. 1 in the range $10 \leqslant x \leqslant 80$ where hydrophobic expulsion is the predominant retention mechanism. At higher methanol concentrations ($x > 80$) some of the structural properties of bulk water begin to disappear and the liquid structure of pure methanol begins to predominate[41], *i.e.*, methanol exerts its own solvophobic effects. At low methanol concentrations ($x < 10$) the monomeric al-

TABLE V

RELATIONSHIP BETWEEN THE POSITION OF THE PARABOLA MINIMUM AND SOLUTE HYDROPHOBICITY AT pH 6.00

Compound	*Minimum position* (% methanol)*	*log k_w***
Benzylamine	(<0)	−1.250
Sulpiride	39.55	−0.447
Sulmepride	39.30	−0.360
Sultopride	42.78	−0.156
2,2-DPEA	57.35	0.625
Metoclopramide	50.25	0.786
Alizapride	54.87	1.126
Flubepride	63.39	1.321
Pipamperone	71.88	1.571
Mezilamine	72.99	1.737
Haloperidol	79.67	1.971
Spiperone	72.08	2.090
Clebopride	74.73	2.182
Benperidol	74.29	2.396
Bromocryptine	(>100)	4.927

* Calculated from the parabolic eqn. 2.

** Obtained by linear extrapolation to 100% water as eluent[40].

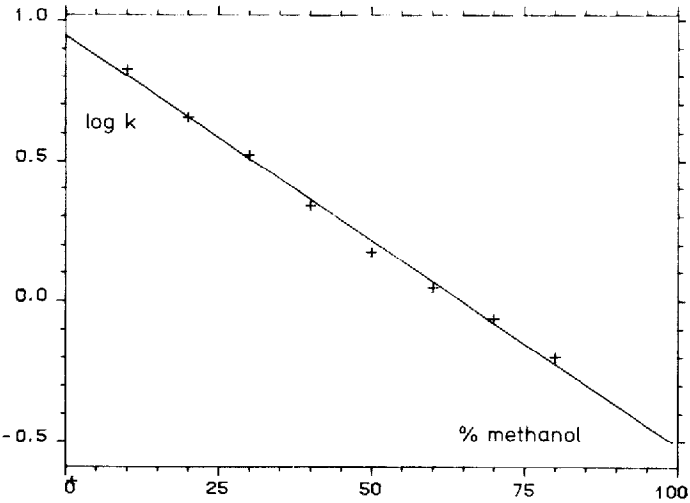

Fig. 3. Retention behaviour of aniline at pH = 7.5. A linear relationship between log k and methanol content is observed, implying that the hydrophobic expulsion predominates over the entire range of eluent composition.

kyl-bonded chains (brush-like bonded chains[42]) are not completely solvated by methanol. The intra- and intermolecular dispersion interactions involving these chains will be intact, resulting in a change in the properties of the stationary phase and in the mechanism of retention as compared to the case at higher x values.

(b) For partially or completely ionized polar compounds the relationship appears to be nearly parabolic (eqn. 2) for the compounds investigated here. The deviation from linearity is postulated to be caused by a dual retention mechanism. In water-poor eluents, polar interactions play the major rôle in the retention mechanism, while in water-rich eluents hydrophobic expulsion is predominant. The part of the parabola corresponding to water-rich eluents can be regarded as linear, permitting the linear extrapolation of capacity factors to log k_w. The position of the parabola minimum in RP-LC depends on the solute hydrophobicity and the proportion of the ionized species. A detailed discussion of the extrapolation of capacity factors at different methanol–water compositions to 100% water as eluent will be presented in the following paper[40].

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