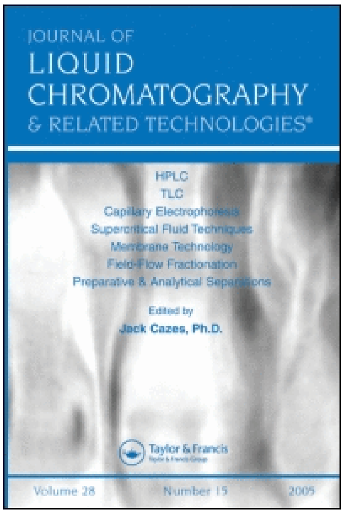

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RELATIONSHIP BETWEEN HYDROPHOBIC PROPERTIES OF AMPHOTERIC SULFONAMIDES AND THEIR RETENTION IN MICELLAR REVERSED PHASE LIQUID CHROMATOGRAPHY

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ABSTRACT

The partition coefficients of 10 selected sulfonamides in the system octanol-water (P_{ow}) and their retention coefficients (k) in RP HPLC with anion micellar mobile phase were determined. The mechanism of retention of the sulphonamides studied was analysed depending on the concentration of sodium dodecylsulphate in the mobile phase and pH of the latter. Relationship between the partition coefficients and retention coefficients was established. It was shown that apart from P_{ow}, a measure of the hydrophobic character of such amphoteric compounds as the sulfonamides studied, could be the values of the retention coefficient determined at pH of the isoelectric point.

INTRODUCTION

Progress in the field of the quantitative structure activity relationship (QSAR) is of great significance for design of drugs, toxicology, and monitoring of the environment. Hansch[1,2] proved a direct relationship between biological activity of compounds and their physical and chemical properties, including their hydrophobic character. A commonly used index describing hydrophobic properties of compounds is the logarithm of the partition coefficient in the two-phase system of solvents octanol-water (log P_{ow}).[3] Unfortunately, the method of its determination cannot be applied for all biologically active systems, is time-consuming, and not always leads to reproducible results. These facts prompted interest in developing a method for determination of hydrophobic character of compounds on the basis of the retention data in chromatographic techniques. In the aspect of QSAR studies a useful method proved to be high performance liquid chromatography with reversed phases (RP-HPLC). The retention data in RP-HPLC as P_{ow}, describe the hydrophobic character of a compound; the logarithms of the retention coefficient and partition coefficient are related through:

$$\log k \ = a\log P_{ow} \qquad (3)$$

where a, b are constants.

Many authors studying QSAR by RP-HPLC have shown a good correlation between biological activity of a compound and the value of the retention coefficient k, as well as, the coefficient of partition P_{ow}.[4-9]. However, the RP-HPLC system is less suitable for the QSAR studies of ionic compounds, and many biologically active compounds have such a character. Hydrophobic properties of both ionic and non-ionic compounds can be studied by the method of micellar liquid chromatography (MLC) using, as a mobile phase, surfactants of a concentration higher than critical micellar concentration (cmc).[10-13] Micelles are often treated as simple chemical models of biomembranes.[14] Both micelles and biomembranes are anisotropic and, in such environments, partition of a given compound depends on the size and shape of its molecule. Therefore, a micellar system can be a good approximation of biomembranes in the studies of hydrophobic interactions. Retention of a compound in MLC depends on the kind of interactions with micelles in the mobile phase and the stationary phase modified with surfactants. Many authors reported successful results obtained when applying MLC in QSAR studies.[15-21]

The study reported in this paper was aimed at determination of partition coefficients of 10 selected sulfonamides in the two-phase system of solvents octanol-water (P_{ow}) and their retention coefficients (k) in the system of anion micellar liquid chromatography in order to find a relationship between these two parameters. Sulfonamides are an important group of drugs of antibacterial activity and are commonly used in medicine and veterinary. This group of compounds has been of considerable interest in the aspect of the QSAR studies.[22-24] Depending on the pH of the environment, they occur either as neutral molecules (HL), or anions (L^-) and cations (H_2L^+). This diverse character of sulfonamide molecules should be reflected both in the process of partition in the two-phase system and in the chromatographic process in the MLC system.

EXPERIMENTAL

Materials and Methods

The chromatographic equipment used in the study consisted of a Hewlett-Packard HP 1050 chromatograph, with a quaternary pump, and UV-Vis detector. The solution was injected into the chromatograph through a Rheodyne valve with a 20 μL loop. A Nucleosil C-18AB column (5 μm, 150 x 4.6 mm) (Alltech Associates, Inc.) was used. The mobile phase flow rate was 1 mL min^{-1}. The detection of sulfonamides was performed in UV at 260 nm.

All the assays were carried out at 40°C. The micellar mobile phase was a aqueous solution of anionic surfactant sodium dodecylsulfate (SDS) (Aldrich). The value of pH of the mobile phase was adjusted using 0.04 mol L^{-1} H_3PO_4 and sodium hydroxide and controlled by pH-meter. The micellar mobile phase was filtered in vacuum through 0.45 μm filters of cellulose acetate and degassed with helium. Standard solutions of the sulfonamides studied (sulfacetamid, sulfaproxyline, sulfadimethoxine, sulfadiazine, sulfamerazine, sulfathiazole, sulfamethazine, sulfamethoxazole, sulfamethizole, sulfachloropyridazine) obtained from Aldrich, of a concentration of 1,0 g L^{-1} were made in methanol. The working solutions of the sulfonamides in concentration of 1 μg mL^{-1} were obtained by dissolving the appropriate standard solution with the currently used mobile phase.

The partition coefficients of sulfonamides were measured using the shake - flask technique at 25.0 ± 0.1°C [25,26]. 1-octanol used was HPLC grade (Aldrich). The organic and aqueous phases were mutually saturated. The sulfonamides studied were dissolved (10^{-4} mol L^{-1}) in aqueous buffer solution (pH 2.0, 3.0, 4.0, 5.1, 5.6, 7.5, 8.5) and the solutions were equilibrated with 1-octanol for 1 h.

Buffer solutions were prepared by mixing the proper volumes of 0,04 mol L^{-1} of H_3PO_4- KH_2PO_4 - Na_2HPO_4. The pH of these solutions was tested by pH-meter.

The phase ratio (V_w/V_o) used was 1:1, 2:3 and 1:2. The samples were centrifuged for phase separation and the concentration of the sulfonamides was determined in the aqueous phase spectrophotometrically (Hewlett Packard 8452A, UV-Vis Spectrophotometer). Each log P_{app} valve is an avarange of six parallel measurements.

Table 1

Values of pK_{a1}, pK_{a2}, and $pK_{a,\,ie.p.}$ of the Sulfonamides Under Study

No.	Sulfonamide	$pK_{a1}{}^{27}$	$pK_{a2}{}^{27}$	$pK_{a,ie.p}$
1	Sulfacetamide	1.8	6.1	3.95
2	Sulfaproxyline	1.7	4.9	3.3
3	Sulfachloropyridazine	1.9	5.1	3.5
4	Sulfadiazine	2.0	6.4	4.2
5	Sulfadimethoxine	1.8	6.2	4.0
6	Sulfamerazine	2.2	7.0	4.6
7	Sulfamethazine	2.4	7.4	4.9
8	Sulfamethizole	2.0	5.4	3.7
9	Sulfamethoxazole	1.7	5.6	3.65
10	Sulfathiazole	2.1	7.1	4.6

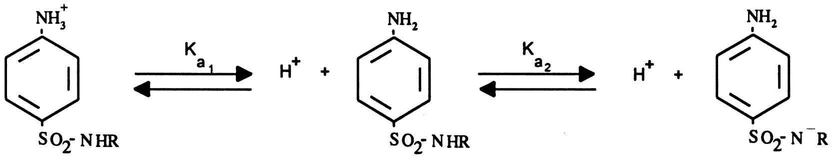
Water to be used in any experiments was taken from Milli-Q apparatus (Millipore, USA).

Table 1 presents the values of dissociation constants (pK_{a_1}, pK_{a_2}) and pK_a at the isoelectric point ($pK_{a_{ie.p.}} = \frac{pK_{a_1} + pK_{a_2}}{2}$) of the ten sulfonamides under study.

RESULTS AND DISCUSSION

The pH Dependence of log P_{app}

Sulfonamides are typical amphoteric compounds and undergo dissociation according to the reaction:



$$H_2A^+ \overset{K_{a_1}}{\longleftrightarrow} H^+ + HA \overset{K_{a_2}}{\longleftrightarrow} H^+ + A^-$$

where K_{a_1}, K_{a_2} are the dissociation constants.

In the two-phase system octanol-water the non-ionised HA molecules of the amphoter undergo partition and the ratio of their concentration is defined as a partition coefficient P_{ow}:

$$P_{ow} = \frac{[HA]_o}{[HA]_w} \tag{2}$$

For molecules undergoing dissociation in the experiment we do not determine the actual partition coefficient P_{ow} but the apparent partition coefficient P_{app}, known also as the partition ratio D. The parameter P_{app} is defined as the ratio of the concentration of neutral HA in the organic phase to the concentration of all forms occurring in the water phase; so in the case of amphoteric sulfonamides we have:

$$P_{app} = \frac{[HA]_o}{[H_2A^+]_w + [HA]_w + [A^-]_w} = \frac{P_{ow}}{K_{a_1}^{-1}[H^+] + 1 + K_{a_2}[H^+]^{-1}} \tag{3}$$

or when expressed in logarithms:

$$\log P_{app} = \log P_{ow} - \log\left[K_{a_1}^{-1}[H^+] + 1 + K_{a_2}[H^+]^{-1}\right] \tag{4}$$

The values of log P_{app} for the sulfonamides studied are given in Table 2. For each compound P_{app} was determined for seven pH values. The results are mean values from 9 independent measurements. From the P_{app} values measured at pH 4.0, 5.1, and 5.6, the corresponding values of P_{ow} were calculated according to equation (4).

The dependencies of P_{ow} on pH values of the environment are displayed in Figures 1 and 2. The shape of these curves can be approximated by parabolic functions with a maximum at pH corresponding to the isoelectric point. Near this point sulfonamides show the highest hydrophobic properties. In this region P_{ow} values are arranged in a plateau, whose value (whose breadth) for amphoteric compounds depends on the difference in the dissociation constants ΔK_a (K_{a_2} - K_{a_1}). For sulfamethazine the plateau is wide as ΔK_a = 4.9, while for sulfachloropyridazine ΔK_a = 3.5 and instead of a plateau there is a peak on the P_{ow}(pH) dependence.

Table 2

The Apparent Partition Coefficient, True Log P_{0w} Values and log $P_{ie.p.}$ of Studied Sulfonamides

	Sulfonamide	Log P_{app} in Studied pH							log P_{ow}	log $P_{i.e.p}$
		2.0	3.0	4.0	5.1	5.6	7.5	8.5	----	---
1	Sulfacetamide	-0.82	-0.44	-0.34	-0.33	-0.38	-0.97	---	-0.33	-0.32
2	Sulfaproxyline	0.69	1.09	1.21	1.05	0.88	-1.16	-1.29	1.37	1.08
3	Sulfachloro-pyridazine	0.42	0.74	0.86	0.75	0.65	-1.09	---	0.07	0.80
4	Sulfadiazine	-0.65	0.33	-0.19	-0.20	-0.24	-0.94	---	-0.18	-0.17
5	Sulfadimethoxine	0.30	0.74	0.96	0.91	0.81	-0.85	---	0.97	0.96
6	Sulfamerazine	-0.65	-0.08	0.05	0.05	0.03	-0.94	-1.24	0.06	0.05
7	Sulfamethazine	-0.50	0.11	0.28	0.30	0.29	0.13	-0.26	0.32	0.30
8	Sulfamethizole	-0.14	0.25	0.46	0.41	0.31	-0.94	000	0.59	0.42
9	Sulfamethoxazole	-0.12	0.64	0.83	0.77	0.71	-0.40	-0.91	0.91	0.82
10	Sulfathiazole	-0.80	-0.18	0.03	0.06	0.06	-0.41	-1.23	0.06	0.06

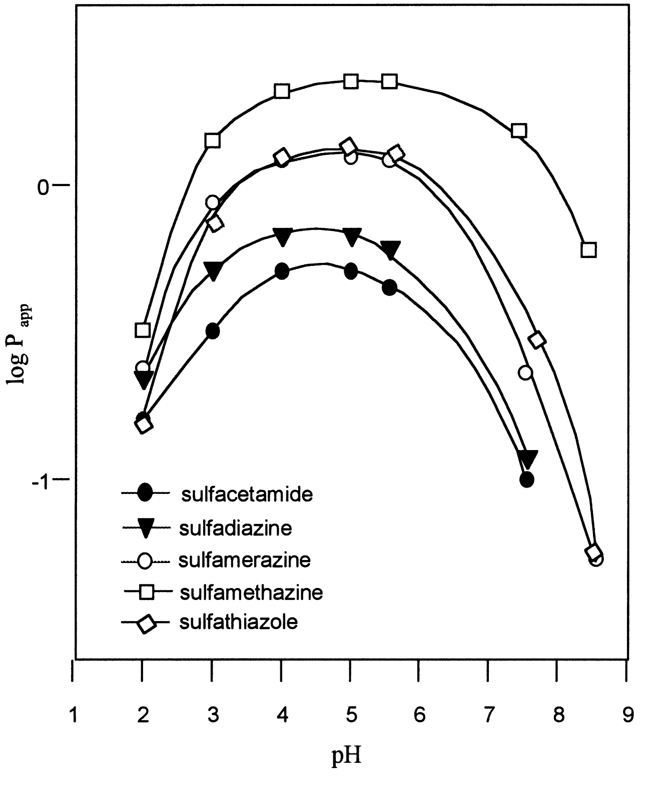


Figure 1. The pH partition profile of the following sulfonamides: sulfacetamide, sulfadiazine, sulfamerazine, sulfamethazine and sulfathiazole.

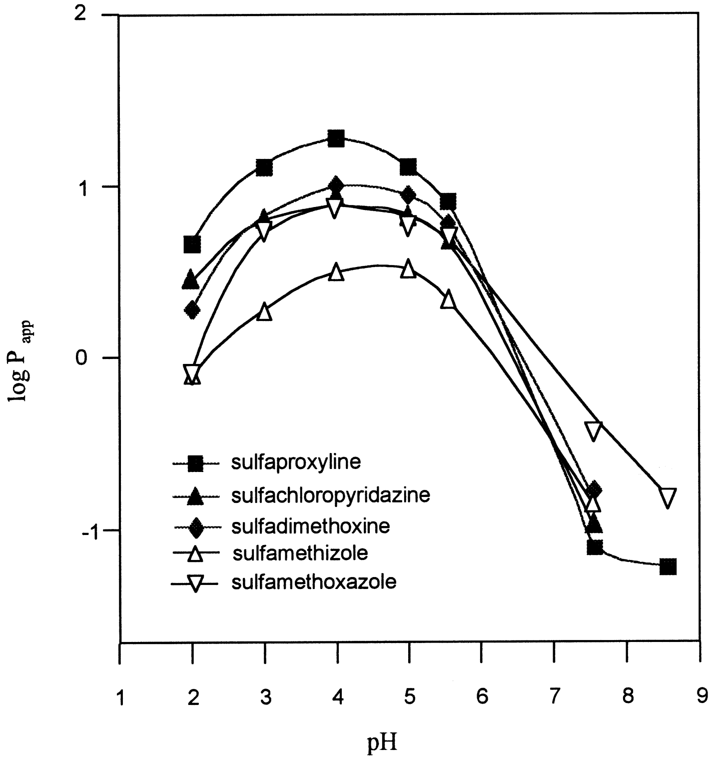


Figure 2. The pH partition profile of the following sulfonamides: sulfaproxyline, sulfachloropyridazine, sulfadimethoxine, sulfamethizole, sulfamethoxazole.

Retention Mechanism of Sulfonamides in Anionic Micellar Liquid Chromatography

Retention of sulfonamides was studied using anionic micellar mobile phase (sodium dodecylsulphate - SDS). In the case of amphoteric compounds, retention is determined first of all, by the concentration of the micellar mobile phase and its pH. Figure 3 presents a dependence of the retention coefficient on concentration of SDS in the micellar mobile phase at a constant pH. With increasing SDS concentration retention of all sulfonamides decreases and interestingly, the greatest retention changes are observed for highly hydrophobic sulfonamides. Figures. 4 and 5 show changes in the retention coefficient k on pH of the micellar mobile phase for SDS concentrations of 0.02 mol L^{-1} and 0.08 mol L^{-1}. Retention of compounds in MLC depends on the kind of interactions between the compound molecules and micelles, as well as, stationary phase

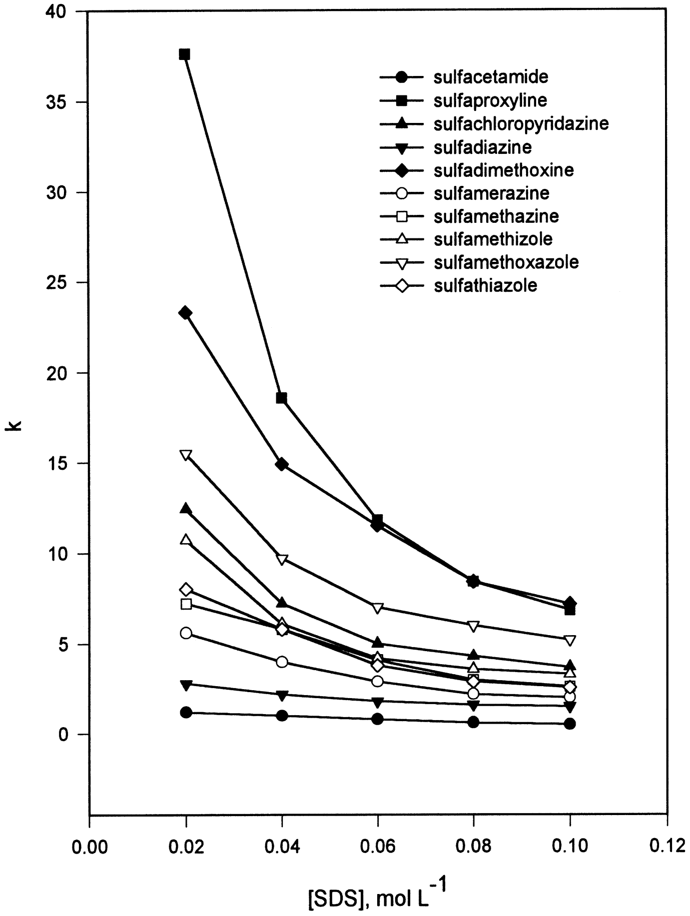


Figure 3. The effect of the SDS micelle concentration on the retention behaviour of sulfonamides at the pH = 3.0 of the mobile phase.

modified with surfactant. In the case of charged compounds, the interactions are hydrophobic and electrostatic. For amphoteric sulphonamides there are:

Hydrophobic and electrostatic interactions of the positively charged

H_2L^- molecules with the stationary phase modified with negatively charged molecules of surfactant,

Hydrophobic interactions of neutral HA molecules,

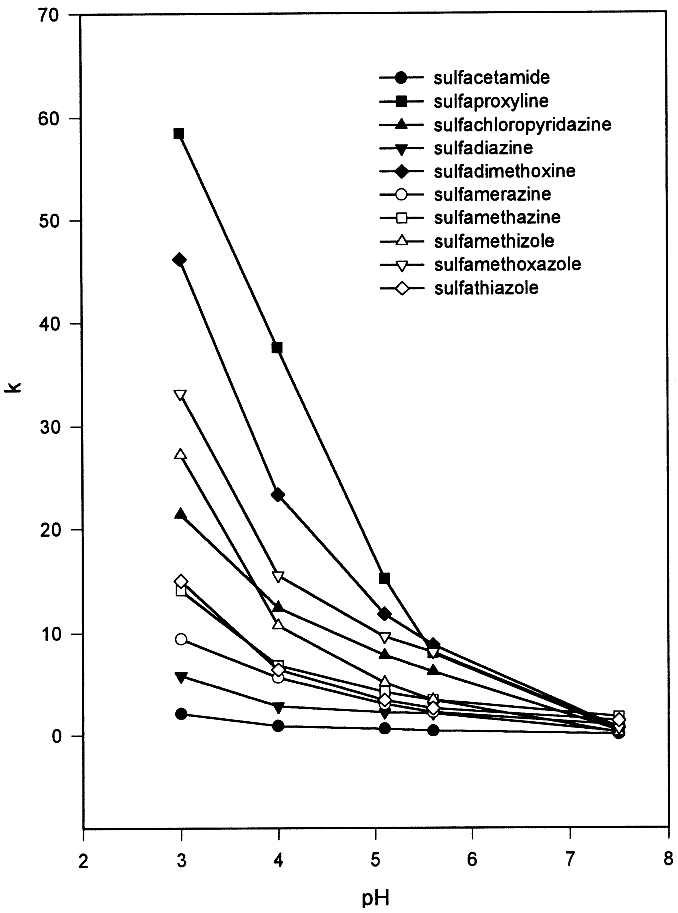


Figure 4. The effect of the pH the mobile phase on the retention behaviour of sulfonamides at 0.02 mol L^{-1} SDS concentration in the mobile phase.

Hydrophobic interactions and electrostatic repulsion of the negatively charged A^- with the stationary phase modified with negatively charged surfactant molecules.

As follows from the curves shown in Figures 4 and 5, an important parameter is the pH of the mobile phase in which molecules of H_2A^+ and HA occur, as in this pH region, the differences in hydrophobic properties of compounds are the most pronounced.

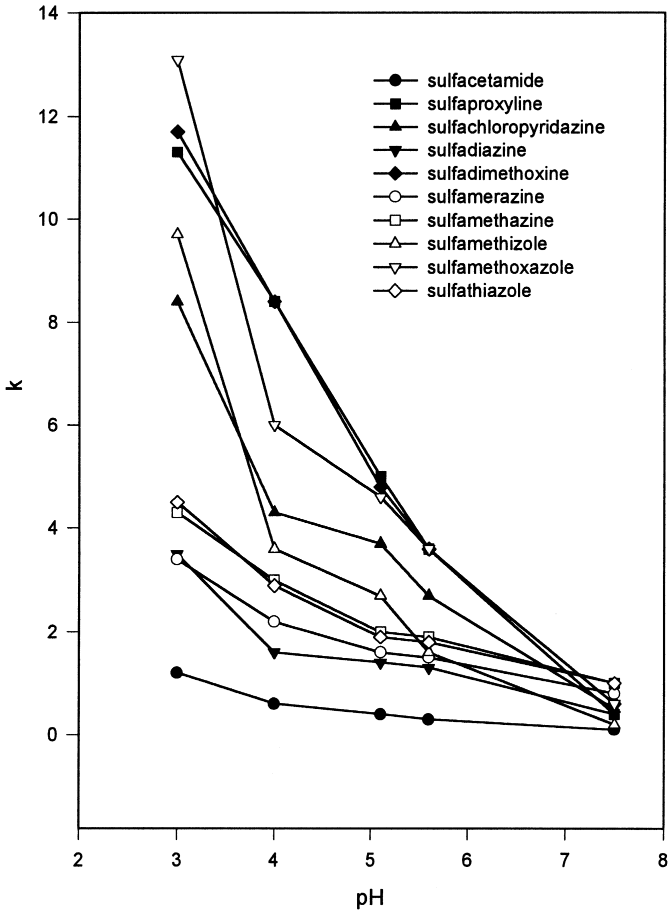


Figure 5. The effect of the pH the mobile phase on the retention behaviour of sulfonamides at 0.08 mol L^{-1} SDS concentration in the mobile phase.

MLC Retention – Octanol/Water Partition Relationships

The possibility of using the retention data for assessment of their physical and chemical properties, including hydrophobic character, is an interesting aspect of quantitative structure retention relationship (QSRR) studies. The retention data in MLC were correlated with the partition coefficient in water/octanol system in different ways.[15,19,20] For ionic compounds the dominant relationship was:

$$\log k = a \log P_{app} + b \qquad (5)$$

Table 3

Statistical Analysis of the Linear Regressions for Sulfonamides*

	Equation	pH	C_{SDS}	a	b	r^2
1	$\log k_{pH} = a\log P_{app} + b$	3.0	0.02	0.730	1.031	0.8127
2		4.0	0.02	0.81	0.56	0.8816
3		5.1	0.02	0.81	0.34	0.9036
4		5.6	0.02	0.84	0.26	0.8610
5		3.0	0.08	0.546	0.621	0.7608
6		4.0	0.08	0.60	0.25	0.8682
7		5.1	0.08	0.64	0.11	0.8480
8		5.6	0.08	0.65	0.06	0.7926
9	$\log k_{ie.p.} = a\log P_{ie.p} + b$	---	0.02	0.9922	0.4977	0.9141
10		---	0.08	0.751	0.233	0.9119
11	$\log k_{ie.p.} = a\, P_{ow} + b$	---	0.02	0.888	0.504	0.9273
12		---	0.08	0.663	0.242	0.9004

---

* a - slope, b - intercept, r^2 - squared correlation coefficient, pH - of mobile phase and aqueous phase in partition systems, C_{SDS} - concentration of SDS in mobile phase.

In such a case the retention coefficient k and the apparent partition coefficient should be determined at the same pH values of the mobile phase and water solution in the partition system. For amphoteric compounds such as sulfonamides we propose a correlation of the retention coefficient with the partition coefficient in the system octanol/water at pH corresponding to the isoelectric point $pK_{a_{ie.p.}}$ The values of $pK_{a_{ie.p.}}$ for the sulfonamides studied are given in Table 1. The values of the partition coefficient at the isoelectric point were read out from the dependencies presented in Figures 1 and 2, while the retention coefficients at the isoelectric point ($k_{ie.p.}$) from the curves given in Figures. 4 and 5. The values of log $k_{ie.p.}$ and $P_{ie.p}$. can be related by an equation analogous to equation (5):

$$\log k_{ie.p.} = a\log P_{ie.p.} + b \qquad (6)$$

However, a measure of hydrophobic character of compounds is the coefficient of partition of the neutral form of the compound defined by equation (1), P_{ow}.

The coefficient P_{ow} was correlated with the retention data for ionic compounds introducing the terms describing the content of the neutral molecules at a given pH of the mobile phase.[19,20] In this paper we propose a correlation of the

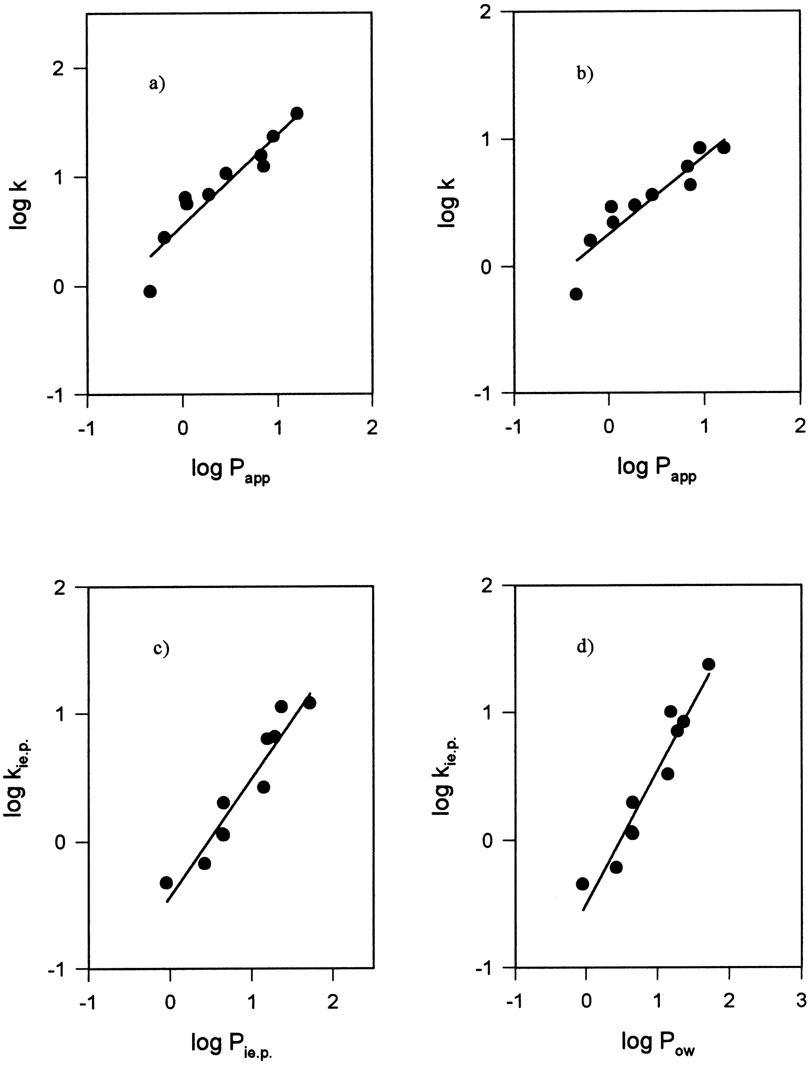


Figure 6. Plots showing the relationship between log k with the octanol - water partition coefficient (log P): a) log k = f(log P_{app}) at pH = 4.0 and C_{SDS} = 0.02 mol L^{-1}, b) log k = f(log P_{app}) at pH = 4.0 and C_{SDS} = 0.08 mol L^{-1}, c) log $k_{ie.p.}$ = f(log $P_{ie.p.}$) at C_{SDS} = 0.02 mol L^{-1}, d) log $k_{ie.p.}$ = f(log P_{ow}) at C_{SDS} = 0.02 mol L^{-1}.

partition coefficient P_{ow} with the retention coefficient k at pH corresponding to the isoelectric point, i.e.:

$$\log k_{ie.p.} = a \log P_{ow} + b \tag{7}$$

The statistical results of linear regression performed using the equations 5, 6, 7 are shown in Table 3, while Figure 6 presents exemplary curves log k = f(log P).

The data displayed in Table 3 and Figure 6 allow the following conclusions:

For amphoteric compounds such as the sulfonamides studied, we obtain a good linear relationship between the logarithm of the retention coefficient k in micellar liquid chromatography and the logarithm of the partition coefficient in the system octanol/water.

Linear relationships have been obtained between log k_{pH} – log $P_{app\ pH}$, log $k_{ie.p.}$ – log $P_{ie.p.}$ and log $k_{ie.p.}$ – log P_{ow}, and the correlation coefficients increase in this order.

High values of the correlation coefficients for the correlations between log $k_{ie.p.}$ – log $P_{ie.p.}$ and log $k_{ie.p.}$ – log P_{ow} indicate that apart from P_{ow}, a measure of the hydrophobic properties of compounds can be the value of the retention coefficient k, determined by the MLC method at the pH value of the mobile phase corresponding to the isoelectric point of a given compound; higher values of the correlation coefficients have been obtained for lower concentrations of the micellar mobile phase.

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