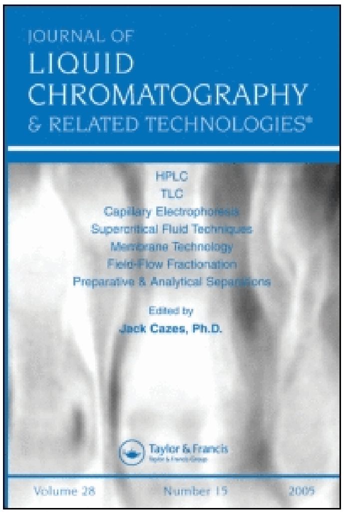

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Determination of 1-Octanol-Water Partition Coefficients for a Group of 1,4-Dihydropyridines by Reversed-Phase High Performance Liquid Chromatography: Study of Micellar Liquid Chromatography for Hydrophobicity Estimation

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DETERMINATION OF 1-OCTANOL-WATER PARTITION COEFFICIENTS FOR A GROUP OF 1,4-DIHYDROPYRIDINES BY REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY: STUDY OF MICELLAR LIQUID CHROMATOGRAPHY FOR HYDROPHOBICITY ESTIMATION

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ABSTRACT

1-Octanol-water partition coefficients (P_{ow}) were determined for a group of 1,4-dihydropyridines by the classical extraction method called "shake-flask" method. Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) was employed to determine the concentration of each solute in the aqueous phase after equilibrium. Micellar Liquid Chromatography (MLC) was used to obtain chromatographic parameters (k', logk', k'_m, logk'_m, P_{mw}, and logP_{mw}) which were tested as hydrophobicity descriptors for the dihydropyridines studied. The correlation of these parameters with logP_{ow} was studied. Sodium Dodecyl Sulphate and Hexadecyltrimethylammonium Bromide modified by n-propanol or n-butanol were used as micellar systems in MLC.

INTRODUCTION

Surfactant solutions are characterized by a monomer concentration (critical micelle concentration, cmc) and a temperature (Krafft's temperature) from which surfactant monomers aggregate to form micelles. Micelles have been employed to modify and to improve some analytical methodologies as spectroscopics, electrochemical, and chromatographic methods.[1-9]

The use of micellar mobile phases in High Performance Liquid Chromatography (HPLC) has originated the Micellar Liquid Chromatography (MLC). The reason why micellar phases increase the affinity of hydrophobic solutes to aqueous media is because surfactants have an aliphatic chain with lipophilic properties making unnecessary the addition of high alcohol percentages for eluting hydrophobic compounds. As a result, MLC became a less toxic technique than HPLC.[10-12]

In Quantitative Structure-Activity Relationships (QSAR) studies, hydrophobicity is a physicochemical parameter employed to evaluate the partition of organic compounds between two media with different polarity characteristics. The standard quantitative scale used to confront hydrophobicities of different organic compounds has been the logarithm of the partition coefficient between octanol and water, $\log P_{ow}$.[13,14] The traditional method to measure $\log P_{ow}$ is the "shake-flask" method, which is tedious, very time consuming, sensitive to solute and solvent impurity, and needs about 100 mg of analyte to replicate determinations and to obtain $\log P_{ow}$ values with confidence.[15,16] Although this hydrophobicity scale is continuous and single, octanol is an isotropic and homogeneous medium, and some factors such as shape and size are not important in the partition of solutes between octanol and water.[17] The resemblance between micelles and biomembranes has been described by some authors, because both have amphiphilic properties and are anisotropic media.[18]

Because of the drawbacks depicted above for octanol-water systems and the likeness in hydrophobic properties of micelles and biomembranes, some scientists have studied the possibilities of MLC for affording new hydrophobicity scales. Mainly, efforts have focused on finding micellar systems with retention data (k' and log k') which correlate well with $\log P_{ow}$. On the other hand, MLC is a useful technique to calculate solute-micelle association constants, K_2, through an equation such as[19]:

$$\frac{V_s}{(V_e - V_m)} = [\frac{v(P_{mw} - 1)}{P_{sw}}]C_M + \frac{1}{P_{sw}} \qquad (1)$$

where V_s, V_m, V_e, and v are the stationary and mobile phase volume, the eluting volume of the solute, and the molar volume of the surfactant, respectively, C_M is the micellized surfactant concentration (total surfactant concentration minus the cmc), and P_{mw} and P_{sw} are the micelle-water and stationary phase-water partition coefficients of the solute, respectively. $K_2= (P_{mw}- 1)v$[20] can be calculated from the ratio slope:intercept of the straight line obtained for the variation between the retention term and the concentration of micellized surfactant in mobile phase. P_{mw} can also be obtained from this ratio if the molar volume of the surfactant is known.

Some authors have proposed the partition coefficient between water and micelles as hydrophobicity scale.[18,21] It is noteworthy that this scale is single, continuous, and according to the additivity rules as the partition coefficients between water and octanol. Moreover, the hydrophobic enthalpy constant (P_H) derived from $\log P_{mw}$ determined at different temperatures, has shown good correlations with the transport phenomena through the blood-brain barrier.[22]

Recently, an empirical model has been proposed which describes the dependence between retention in MLC and $\log P_{ow}$[23] and has been applied to neutral compounds whose retention data were collected from literature. This work shows how the logarithm of the retention factor at zero micellar concentration, $\log k'_m$, appears as the parameter which best correlates with $\log P_{ow}$.

k'_m can be calculated from the intercept of the straight line obtained for the variation of the reciprocal of the capacity factor (k') of a solute as a function of the concentration of micellized surfactant in the mobile phase according to Eq. 1, expressed as a function of the capacity factor[10]:

$$\frac{1}{k'} = \frac{1}{k'_m} + \frac{K_2}{k'_m} C_M \qquad (2)$$

In this work, octanol-water partition coefficients have been determined by the shake-flask method for a group of Hantzsch 1,4-dihydropyridines (DHPs), the so-called Hantzsch esters[24], of pharmaceutical interest due to their calcium antagonist activity.[25,26] Calcium channel blockers have been used in cardiovascular research and therapy. Some of these DHPs are useful for treatment of angine pectoris, cardiac arrhythmia, hypertrophic cardiomyopathy, and hypertension.[27]

Micellar chromatographic retention data, by using Sodium Dodecyl Sulphate (SDS) and Hexadecyltrimethylammonium Bromide (CTAB) modified with n-propanol or n-butanol as mobile phases, have been determined in order to calculate K_2, P_{mw}, P_{sw}, and k'_m parameters to evaluate the validity of several hydrophobicity scales (k', k'_m, P_{mw} and their respective logarithms).

EXPERIMENTAL

Apparatus and Materials

A model Unitron 320 OR shaking thermostatic bath, a model Tectron S-473-100 thermostatic bath, and a model Centronic S-577 centrifuge, all from Selecta (Barcelona, Spain) were used. The $\log P_{ow}$ determination was carried out in a chromatograph from Waters (Milford, MA, USA) which consisted of a 510 isocratic pump, a 841 UV-Vis spectrophotometric detector, a 740 integrator and a Rheodyne valve with a 20 mL sample loop. Solutes were detected at their maximum absorption analytical wavelength (ranging from 230 to 360 nm). The column (4.5 mm i.d.; 15 cm long; 5 μm d_p) (Teknokroma, Barcelona, Spain) employed to quantify DHP concentration in aqueous phase by RP-HPLC after equilibrium in $\log P_{ow}$ determination by the shake-flask method was packed with a ODS-2 stationary phase. Micellar retention data were measured in a 1050 Hewlett Packard (Avondale, PA, USA) chromatograph with an isocratic pump, a variable wavelength UV-Vis detector, an autosampler, and a model 3394 integrator. Two Spherisorb C8 columns (4.5 mm i.d.; 15 cm long; 5 μm d_p) (Teknokroma, Barcelona, Spain) were employed in order to introduce only one surfactant in each column. A 0.45 μm filter and filtration system (Millipore, Bedford, MA, USA) were used.

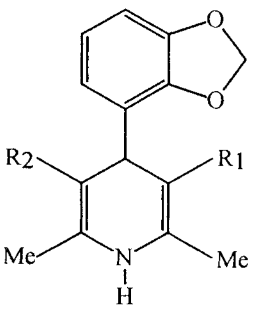
Chemicals

The DHP derivatives studied in this work were synthesized at the Department of Organic Chemistry, University of Alcalá de Henares, by Dr. Alvarez-Builla and co-workers. The structure of DHP derivatives is shown in Figures 1 and 2. The other reagents employed in this work were of analytical grade. n-Propanol, n-butanol, 1-octanol, SDS, CTAB, sodium dihydrogen phosphate, and sodium hydroxide were from Merck (Darmstadt, Germany). Benzophenone and acetophenone were from Carlo Erba Reagenti (Rodano, Milan, Italy).

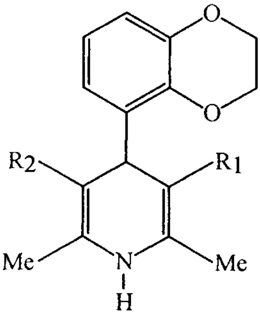
Procedure

$\log P_{ow}$ determination

The "shake-flask" method has been used to determine $\log P_{ow}$ for a group of 20 DHP derivatives. The aqueous and organic phases were a phosphate buffer pH=6.9 (to prevent dissociation of the analyte) and 1-octanol, respectively. Prior to the partition between both phases, they were mutually saturated. Each solute was weighed in centrifuge tubes. At least six centrifuge tubes were used for each DHP



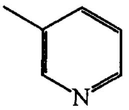
Group 1



Group 2

Group 1	-R_1	-R_2
1	-$COOCH_2$-(1)	-$COOCH_3$
2	-$COOCH_3$	-$COOCH_3$
3	-$COOCH_2CH_2OCH_3$	-$COOCH_2CH_2OCH_3$
5	-$COOCH_2CH_3$	-$COOCH_2CH_3$
6	-$COOCH_2CH_2OCH_3$	-COOIsp
7	-COOIsp	-$COOCH_2CH_3$
12	-$COOCH_2CH_3$	-$COOCH_3$
14	-COOIsp	-COOIsp
17	-COOIsp	-$COOCH_3$

(1)



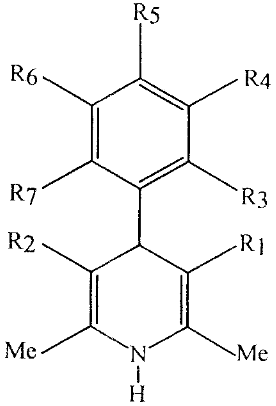
Isp: Isopropyl

Group 2	-R_1	-R_2
4	-$COOCH_2CH_3$	-$COOCH_3$

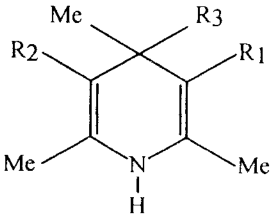
Figure 1. Structures of DHPs studied and the number assigned to each for Groups 1 and 2.

with different analyte amounts (5-15 mg) and 2 mL of octanolic phase and 10 mL of aqueous phase were added in each tube. All the stoppered tubes were introduced in a thermostatic bath at 30°C and shaken at 30 r.p.m. -to avoid emulsification- during 48 hours. Each tube was then allowed to stay for another 48 hours for complete equilibration. Finally, the octanolic layer was removed and the aqueous phase was centrifuged at 2000 r.p.m. for 20 minutes to eliminate any residue of octanolic phase.[16]

The DHP content in the aqueous phase was analyzed by RP-HPLC. The mobile phase was a 70:30 methanol/phosphate buffer (pH 6.9) mixture. The aqueous phases were chromatographed twice. Sample concentration was



Group 3



Group 4

Group 3	$-R_1$	$-R_2$	$-R_3$	$-R_4$	$-R_5$	$-R_6$	$-R_7$
8	$-COOCH_2CH_3$	$-COOCH_3$	-H	$-NO_2$	-H	-H	-H
9	$-COOCH_2CH_3$	$-COOCH_3$	-Cl	-Cl	-H	-H	-H
10	$-COOCH_2CH_3$	$-COOCH_3$	$-OCH_2OCH_3$	-H	-H	-H	-H
11	$-COOCH_2CH_3$	$-COOCH_3$	-H	$-OCH_3$	-H	$-OCH_3$	-H
13	$-COOCH_2CH_3$	$-COOCH_3$	-H	-H	-H	-H	-H
15	$-COOCH_2CH_3$	$-COOCH_3$	-H	$-OCH_3$	$-OCH_3$	-H	-H
16	$-COOCH_2CH_3$	$-COOCH_3$	$-OCH_3$	$-OCH_3$	-H	-H	-H
18	$-COOCH_2CH_3$	$-COOCH_3$	-H	$-OCH_3$	-OH	$-OCH_3$	-H
19	$-COOCH_2CH_3$	$-COOCH_3$	-H	$-OCH_3$	$-OCH_3$	$-OCH_3$	-H
20	$-COOCH_2CH_3$	$-COOCH_2CH_3$	-Cl	-H	-H	-H	-Cl
21	$-COOCH_2CH_3$	$-COOCH_2CH_3$	-Cl	-H	-H	-H	-H
25	-COOIsp	$-COOCH_2CH_2OCH_3$	-H	$-NO_2$	-H	-H	-H

Group 4	$-R_1$	$-R_2$	$-R_3$
22	$-COOCH_3$	$-COOCH_2CH_3$	-H
23	$-COOCH_2CH_2$-(2)	$-COOCH_2CH_3$	-H
24	$-COOCH_2CH_3$	$-COOCH_2CH_3$	-H
26	-CN	-CN	$-CH_3$

Isp: Isopropyl

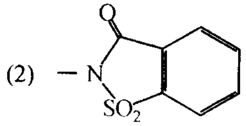


Figure 2. Structures of DHPs studied and the number assigned to each for Groups 3 and 4.

determined by interpolation into a calibration curve with four experimental points constructed according to the internal standard method. Benzophenone and acetophenone were employed as internal standards. In the calibration curve each point was replicated three times and chromatographed twice.

Micellar liquid chromatography

Retention of a group of 26 DHPs was determined in a MLC system. Micellar mobile phases (with a surfactant concentration ranging from 0.031 M to 0.120 M) were prepared by dissolving the appropriate amount of surfactant and n-butanol (B) or n-propanol (P) in 0.01 M phosphate buffer (pH=6.9) followed by filtration. Stock solutions of test solutes were prepared in methanol and their concentration adjusted to permit their detection by injection of a 20 μL sample. As molar volumes of CTAB and SDS, values of 0.364 and 0.246 $L\times mol^{-1}$ were taken respectively. It was assumed that these values did not vary appreciably under the experimental conditions employed in this work.[11] The column and the mobile phase were water jacketed and thermostated at 25 ± 1°C with a circulating bath.

Data manipulation

The error in determining solute-micelle association constants was ascertained from the statistical parameters of the least-squares fitting and from error propagation.[28] The non-parametric test used in this work to compare association constants and squared correlation coefficient values was the Wilcoxon matched pairs test. It was carried out by using the SOLO Statistical System.[29]

RESULTS AND DISCUSSION

Octanol-Water Partition Coefficients

Table 1 groups $\log P_{ow}$ values corresponding to 20 of the DHPs studied. For some DHPs, $\log P_{ow}$ could not be determined due to the difficulty in measuring their concentration in the aqueous phase after equilibrium. The lower and upper values for $\log P_{ow}$ were 2.43 and 4.31 but most of the DHPs have $\log P_{ow}$ values ranging from 3 to 4. Table 1 also groups the confidence interval for a 95% of certainty for $\log P_{ow}$ values obtained. The highest confidence interval corresponds to DHP 7 which has isopropyl (Isp) and ethyl (Et) groups which have been reported as hard contributors to hydrophobicity.[30] The high hydrophobic character of this DHP can explain the dispersion obtained for $\log P_{ow}$ values as a result of the difficulty to measure solute concentration in the aqueous phase.

Table 1

Log P_{OW} Values Determined by the "Shake-Flask" Method for 20 DHPs

DHP	Log P_{OW}	DHP	Log P_{OW}
1	3.21 ± 0.04	14	----
2	3.06 ± 0.01	15	3.20 ± 0.01
3	2.53 ± 0.02	16	3.18 ± 0.02
4	3.17 ± 0.03	17	3.75 ± 0.04
5	3.92 ± 0.02	18	2.43 ± 0.01
6	3.54 ± 0.04	19	2.99 ± 0.01
7	4.31 ± 0.13	20	----
8	4.09 ± 0.03	21	----
9	----	22	3.27 ± 0.03
10	3.38 ± 0.01	23	----
11	3.68 ± 0.02	24	----
12	3.47 ± 0.02	25	4.22 ± 0.07
13	3.74 ± 0.03	26	2.47 ± 0.01

On the other hand, if $\log P_{ow}$ values for DHPs 2, 5, and 7 are compared, a higher contribution to hydrophobicity can be observed when the length of the alkyl chain of ester groups in 3 and 5 positions increases. The comparison of structures and $\log P_{ow}$ values for DHPs 11, 15, and 16 allows one to observe that the meta-meta substitution over phenyl ring (DHP 11, $\log P_{ow}=3.68$) has a higher contribution to DHP hydrophobicity than ortho-meta (DHP 16, $\log P_{ow}=3.18$) or meta-para (DHP 15, $\log P_{ow}=3.20$) substitutions. Finally, Linear Free Energy Relationships[31] (LFER) are corroborated, making it possible to estimate the experimental $\log P_{ow}$ value for DHP 12 (3.47) as ($\log P_{ow}$DHP-2 + $\log P_{ow}$DHP-5)/2, which results in 3.49.

Solute-Micelle Association Constants

Solute-micelle association constants were calculated for the group of 26 DHPs by equation 1. Values obtained for K_2 are presented in Tables 2 and 3, together with the relative errors obtained in their determination. P_{mw} and P_{sw} coefficients were calculated respectively from the slope:intercept ratio (P_{mw})and from the intercept (P_{sw})of the straight line of variation of the retention term as a function of C_M (Equation 1) (data not shown). The mobile phases were composed by SDS or CTAB in presence of an organic modifier (n-propanol or n-butanol). Each alcohol was added at three different percentages ranging from 3% to 10%.

Table 2

Solute-Micelle Association Constants and Errors for the DHPs by Using SDS Mobile Phases Modified With N-Propanol (P) or n-Butanol (B)

DHP	SDS 3%P	SDS 5%P	SDS 10%P	SDS 3%B	SDS 5%B	SDS 7.5%B
1	**241** (36%)	**149** (23%)	**56** (15%)	**120** (14%)	**52** (12%)	**33** (9%)
2	**206** (18%)	**145** (11%)	**52** (13%)	**85** (9%)	**52** (10%)	**30** (9%)
3	**135** (15%)	**103** (10%)	**35** (10%)	**71** (11%)	**40** (10%)	**20** (8%)
4	**202** (18%)	**150** (18%)	**56** (14%)	**93** (9%)	**52** (10%)	**32** (8%)
5	**217** (26%)	**221** (19%)	**92** (16%)	**112** (8%)	**61** (12%)	**45** (6%)
6	**167** (16%)	**192** (25%)	**69** (14%)	**121** (13%)	**55** (12%)	**38** (7%)
7	**235** (19%)	**209** (67%)	**106** (15%)	**127** (11%)	**64** (15%)	**48** (5%)
8	**212** (25%)	**278** (33%)	**105** (18%)	**112** (7%)	**64** (15%)	**46** (6%)
9	**294** (26%)	**598** (51%)	**167** (22%)	**106** (10%)	**73** (16%)	**58** (7%)
10	**218** (18%)	**155** (8%)	**67** (14%)	**99** (8%)	**55** (11%)	**37** (7%)
11	**173** (13%)	**174** (15%)	**70** (14%)	**104** (10%)	**55** (11%)	**37** (6%)
12	**271** (21%)	**163** (12%)	**67** (15%)	**106** (11%)	**58** (11%)	**40** (8%)
13	**220** (26%)	**318** (13%)	**87** (15%)	**109** (8%)	**61** (11%)	**45** (6%)
14	**202** (20%)	**298** (33%)	**113** (17%)	**121** (11%)	**65** (16%)	**47** (7%)
15	**193** (16%)	**171** (16%)	**66** (13%)	**92** (8%)	**59** (11%)	**36** (7%)
16	**173** (11%)	**165** (14%)	**65** (13%)	**94** (8%)	**57** (10%)	**36** (7%)
17	**195** (16%)	**224** (19%)	**93** (16%)	**121** (9%)	**62** (11%)	**46** (6%)
18	**159** (14%)	**138** (11%)	**40** (11%)	**73** (10%)	**51** (8%)	**24** (7%)
19	**181** (16%)	**146** (15%)	**46** (12%)	**84** (10%)	**49** (11%)	**25** (7%)
20	**206** (15%)	**337** (27%)	**133** (15%)	**155** (12%)	**79** (15%)	**56** (5%)
21	**269**(5%)	**331** (37%)	**138** (21%)	**121** (10%)	**72** (16%)	**56** (7%)
22	**157** (15%)	**159** (21%)	**59** (14%)	**90** (9%)	**57** (11%)	**35** (10%)

(continued)

Table 2 (continued)

Solute-Micelle Association Constants and Errors for the DHPs by Using SDS Mobile Phases Modified With N-Propanol (P) or n-Butanol (B)

DHP	SDS 3%P	SDS 5%P	SDS 10%P	SDS 3%B	SDS 5%B	SDS 7.5%B
23	**198** (19%)	**219** (23%)	**59** (14%)	**200** (24%)	**73** (11%)	**41** (10%)
24	**172** (13%)	**174** (11%)	**75** (16%)	**114** (11%)	**61** (11%)	**44** (7%)
25	**193** (16%)	**240** (24%)	**100** (18%)	**119** (10%)	**62** (15%)	**43** (6%)
26	**79** (18%)	**86** (18%)	**32** (13%)	**50** (8%)	**38** (10%)	**23** (11%)

Tables 2 and 3 show that for SDS, K_2 of DHPs can be calculated in all of the experimental conditions used in this work. However, solute-micelle association constants with CTAB micelles were obtained for all DHPs only in media modified by 10% n-propanol or 5% or 10 % n-butanol. The reason for this result could be the large slopes obtained for the variation of Vs/(Ve-Vm) vs. C_M when CTAB is used as surfactant. Furthermore, for those DHPs for which P_{sw} is high the intercept obtained is low. Small changes in the slope would result in relatively large changes in the intercept and even in negative intercepts.[19] This causes either the unfeasibility of calculation of the association constant or obtaining these constants with very high associated errors. In those cases, the addition of an alcohol to the mobile phase enables the calculation of the association constants for many solutes. In fact, increasing the percentage and the eluent strength of the alcohol (the length of the chain of the alcohol) increases the number of solutes whose association constants can be calculated and considerably decreases the error in the determination of these constants (see Table 3).

The effect of alcohol percentage in the mobile phase on the values obtained for K_2 can be observed at Tables 2 and 3. For SDS, for which the calculation of the association constants was possible for all solutes, Table 2 shows that these constants generally decrease when increasing the alcohol percentage and this is true for n-propanol as well as for n-butanol. The decrease in K_2 values, when increasing the organic modifier percentage, has already been depicted and it has been attributed to the competition of the alcohol and the solute for the micelle. These differences have been tested from a statistical viewpoint by using a non-parametric Wilcoxon test for matched pairs. The results showed that an increase of organic modifier percentage affords statistical differences for SDS in all cases except when mobile phases modified by 3% and 5% n-propanol are considered.

Table 3

Solute-Micelle Association Constants and Errors for the DHPs by Using CTAB Mobile Phases Modified with n-Propanol (P) or n-Butanol (B)

DHP	CTAB 5%P	CTAB 7.5%P	CTAB 10%P	CTAB 3%B	CTAB 5%	CTAB 10%B
1	—	—	**305** (95%)	**79** (22%)	**153** (41%)	**13** (5%)
2	**658** (60%)	**1160** (445%)	**133** (34%)	**104** (11%)	**63** (11 %)	**14** (6%)
3	**253** (40%)	**135** (72%)	**49** (21%)	**35** (18%)	**37** (14%)	**9** (4%)
4	**1600** (250%)	**1826** (771%)	**196** (40%)	**113** (6%)	**63** (4%)	**15** (4%)
5	—	**1059** (264%)	**178** (28%)	—	**85** (4%)	**17** (3%)
6	—	**594** (158%)	**116** (24%)	**2464** (105%)	**74** (4%)	**16** (4%)
7	—	—	**174** (34%)	—	**93** (7%)	**23** (5%)
8	—	—	**347** (51%)	—	**94** (5%)	**20** (7%)
9	**7398** (367%)	—	**522** (42%)	—	**91** (7%)	**23** (3%)
10	—	**6933** (2665%)	**279** (24%)	**162** (15%)	**74** (7%)	**17** (2%)
11	—	—	**426** (36%)	**405** (79%)	**76** (6%)	**17** (2%)
12	—	**1063** (476%)	**269** (32%)	**445** (95%)	**69** (4%)	**17** (3%)
13	**1411** (96%)	**11015** (2735%)	**394** (29%)	—	**87** (5%)	**21** (6%)
14	—	—	**497** (119%)	—	**100** (6%)	**24** (11%)
15	—	**695** (275%)	**231** (18%)	**78** (19%)	**66** (5%)	**17** (4%)
16	—	**751** (295%)	**254** (20%)	**103** (3%)	**67** (4%)	**16** (2%)
17	—	**662** (167%)	**214** (30%)	—	**88** (6%)	**20** (8%)
18	**196** (46%)	**195** (41%)	**45** (22%)	**84** (25%)	**35** (6%)	**20** (24%)
19	**1498** (234%)	**267** (112%)	**127** (13%)	**53** (24%)	**55** (3%)	**11** (4%)
20	—	—	**203** (30%)	—	**78** (19%)	**27** (6%)
21	—	**3732** (989%)	**264** (30%)	—	**91** (5%)	**23** (6%)
22	**386** (53%)	**357** (111%)	**154** (15%)	**178** (41%)	**56** (1%)	**15** (4%)
23	**1501** (220%)	—	**544** (76%)	**229** (76%)	**100** (5%)	**18** (6%)
24	**797** (80%)	**1047** (238%)	**172** (29%)	**1291** (632%)	**74** (3%)	**18** (3%)
25	—	—	**181** (36%)	—	**97** (2%)	**21** (6%)
26	**80** (10%)	**157** (49%)	**86** (11%)	**42** (18%)	**52** (3%)	**16** (1%)

In fact, for these mobile phases an increase in K_2 can be observed in some instances when the percentage of n-propanol is increased from 3% to 5%. However, these exceptions are generally associated to high errors in the determination of association constants.

In the case of CTAB, the effect of the alcohol percentage can only be studied for all solutes by considering mobile phases modified with 5% and 10% n-butanol and the decrease in the association constants values with the n-butanol percentage appeared as statistically significant.

Table 4

r^2 Values for k′-LogP$_{OW}$ and Log k′-LogP$_{OW}$ Correlations for SDS Mobile Phases

[SDS]	SDS-3% n-Propanol		SDS-5% n-Propanol		SDS-10%n-Propanol	
	k′	Log k′	k′	Log k′	k′	Log k′
0.031	0.954	0.936	0.938	0.940	0.946	0.922
0.045	0.951	0.941	0.950	0.946	0.947	0.909
0.060	0.952	0.938	0.952	0.938	0.941	0.908
0.072	0.950	0.939	0.952	0.938	0.940	0.903
0.090	0.950	0.938	0.938	0.933	0.938	0.898
$r^2_{average}$	**0.951**	**0.938**	**0.946**	**0.939**	**0.942**	**0.908**

[SDS]	SDS-3% n-Butanol		SDS-5% n-Butanol		SDS-7.5% n-Butanol	
	k′	Log k′	k′	Log k′	k′	Log k′
0.031	0.951	0.944	0.947	0.917	0.932	0.851
0.045	0.953	0.939	0.946	0.913	0.927	0.878
0.060	0.953	0.938	0.940	0.905	0.927	0.878
0.072	0.952	0.937	0.942	0.904	0.927	0.877
0.090	0.951	0.935	0.040	0.901	0.925	0.869
$r^2_{average}$	**0.952**	**0.939**	**0.943**	**0.908**	**0.927**	**0.876**

The influence of the nature of the alcohol on K_2 values can be studied if the values of these constants corresponding to mobile phases of SDS modified with 3% and 5% alcohol and mobile phases of CTAB modified with 10% alcohol are considered. A decrease is generally observed in K_2 values when n-butanol is used with respect to n-propanol (significant differences from a statistical viewpoint). This behavior is related again to the above-mentioned longer hydrophobic tail of n-butanol.

The effect of the nature of the surfactant can be studied by comparing the association constants values obtained for the DHPs with SDS and CTAB when identical mobile phases were used. Mobile phases modified by a 10 % n-propanol or 5% n-butanol can be compared. K_2 values for CTAB were generally higher or similar than for SDS and the Wilcoxon test reported differences statistically significant between these two groups of constants. This behavior does not agree with that previously observed for K_2 values obtained for the same solutes with SDS and CTAB modified by a 5% n-butanol when an octadecylsilica column was used at a different temperature (30°C). In this case, K_2 values were higher for SDS than for CTAB.[32]

Table 5

r^2 Values for k′-LogP$_{OW}$ and Log k′-LogP$_{OW}$ Correlations for CTAB Mobile Phases

[CTAB]	CTAB-5% n-Propanol		CTAB-7.5% n-Propanol		CTAB-10% n-Propanol	
	k′	Log k′	k′	Log k′	k′	Log k′
0.035	0.873	0.834	0.935	0.934	0.931	0.922
0.050	0.878	0.862	0.934	0.935	0.940	0.936
0.067	0.869	0.862	0.888	0.925	0.928	0.934
0.080	0.873	0.862	0.908	0.928	0.913	0.918
0.100	0.866	0.861	0.918	0.926	0.885	0.902
r^2_{average}	**0.872**	**0.856**	**0.916**	**0.930**	**0.919**	**0.922**

[CTAB]	CTAB-3% n-Butanol		CTAB-5% n-Butanol		CTAB-10% n-Butanol	
	k′	Log k′	k′	Log k′	k′	Log k′
0.035	—	—	0.912	0.902	0.902	0.872
0.050	0.946	0.946	0.930	0.932	0.902	0.881
0.067	0.936	0.943	0.929	0.927	0.872	0.845
0.080	0.932	0.943	0.928	0.928	0.907	0.896
0.100	0.928	0.936	0.923	0.925	0.901	0.884
0.120	0.865	0.863	—	—	—	—
r^2_{average}	**0.921**	**0.926**	**0.924**	**0.923**	**0.897**	**0.876**

Estimation of Hydrophobicity of DHPs using Alternative Scales

The classical hydrophobicity scales (k' and logk') and other less used, but that have recently afforded good results (k'$_m$, logk'$_m$, P$_{mw}$, and logP$_{mw}$), were considered in this work. Tables 4 and 5 group the squared linear correlation coefficients (r^2) for the plots k'-logP$_{ow}$ and logk'-logP$_{ow}$ at different surfactant concentrations and the average values for each kind of mobile phase. These correlations were obtained by using k' values corresponding to the 20 DHPs for which the logP$_{ow}$ could be determined.

k' seems to be a better hydrophobicity index than logk' when SDS is used as surfactant. r^2 values corresponding to correlations k'-logP$_{ow}$ and logk'-logP$_{ow}$ appeared as statistically different except for the mobile phase in which SDS is modified by 5% n-propanol. When CTAB is considered, the differences between r^2 for k'-logP$_{ow}$ and logk'-logP$_{ow}$ correlations were not statistically significant except for the mobile phases modified with 5% n-propanol and 10% n-butanol for which k' correlated better than logk' with logP$_{ow}$. This result is typical in MLC[33] where three partition equilibria exist for the solutes which originates a curvature in the logk'-

Table 6

r^2 Values for k'_m - $LogP_{OW}$ and $Logk'_m$ - $LogP_{OW}$ Correlations for Different Mobile Phases

Mobile Phase	k'_m	$Logk'_m$
SDS-3%P, n[a]=20	0.898	0.876
SDS-5%P, n[a]=20	0.878	0.946
SDS-10%P, n[a]=20	0.919	0.956
SDS-3%B, n[a] =20	0.924	0.936
SDS-5%B, n[a]=19	0.942	0.940
SDS-7.5%B, n[a]=20	0.922	0.901
CTAB-5%P, n[a]=8	0.612	0.669
CTAB-7.5%P, n[a]=13	0.458	0.673
CTAB-10%P, n[a]=20	0.552	0.673
CTAB-3%B, n[a]=13	0.526	0.748
CTAB-5%B, n[a]=19	0.924	0.954
CTAB-10%B, n[a]=19	0.889	0.859

[a] Number of solutes employed.

$logP_{OW}$ correlation. This curvature was explained by the solubility limit theory developed by Hinze and Weber[34] based on the predominance either of the water-stationary phase partitioning or the micelle-stationary phase partitioning depending on the hydrophobicity of the solute ($logP_{OW}$ value). This k'-$logP_{OW}$ correlation is not significantly influenced by the nature of the alcohol and seems to be poorer when increasing the alcohol percentage only for n-butanol.

If r^2 average values are considered, a low percentage of organic modifier (3% n- propanol or 3% n-butanol) and SDS as surfactant appear as the best conditions to estimate hydrophobicity by using the k' vs. $logP_{OW}$ correlation.

Table 6 groups the r^2 values for the correlations of the parameters k'_m and $logk'_m$,with $logP_{OW}$. These r^2 values are only comparable to those obtained for corrrelations between k' and logk' with $logP_{OW}$ when SDS is used as surfactant; for CTAB the r^2 values are very low. In order to know which parameter (k'_m or $logk'_m$) best correlates with $logP_{OW}$ for SDS, the Wilcoxon test was carried out, and the results showed that the differences between the r^2 values for both correlations were not statistically significant. The poorest correlations (the lowest r^2 values) were obtained for the variation of P_{mw}, and $logP_{mw}$ as a function of $logP_{OW}$.

The best hydrophobicity scale appears to be the correlation k'-logP$_{ow}$ for a mobile phase 0.031 M SDS-3% n-propanol and the equation obtained is the following:

$$k' = 48.3 \log P_{ow} - 98.49 \qquad [3]$$

From this equation, logP$_{ow}$ values for the group of 20 DHPs can be recalculated from the values obtained for their capacity factors in this mobile phase. With these calculated logP$_{ow}$ values and the experimental ones, the difference between these two values can be obtained for each of these DHPs. This difference was generally minor than 4 %, although greater values were obtained for DHPs 3 (7.1 %), 7 (5.8 %), and 22 (8.8 %), being the average for the 20 DHPs 2.7 %. Equation 3 also enables the estimation of logP$_{ow}$ for the six DHPs for which the capacity factor was measured in MLC but the logP$_{ow}$ was not experimentally measured by the shake-flask method. The logP$_{ow}$ values obtained for these DHPs were the following: 4.4 for DHP 9, 5.4 for DHP 14, 4.7 for DHP 20, 4.4 for DHP 21, 3.0 for DHP 23, and 3.4 for DHP 24. The indirect estimation of these logP$_{ow}$ values by Micellar Electrokinetic Chromatography[35] gave values differing from 7 to 20 % from those calculated in this work by MLC.

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