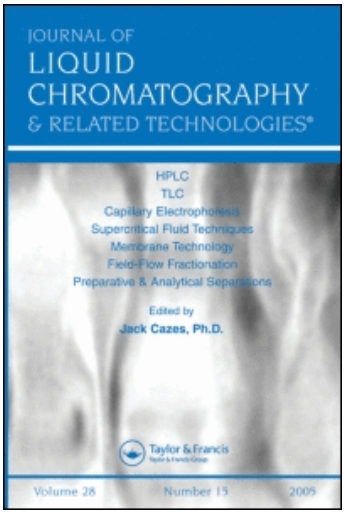

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STUDY OF THE BINDING CONSTANTS OF PHENOLIC ANTIOXIDANTS WITH SURFACTANT/n-PROPANOL/WATER SYSTEMS AT HIGH PERCENTAGES OF ALCOHOL BY RP-HPLC

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STUDY OF THE BINDING CONSTANTS OF PHENOLIC ANTIOXIDANTS WITH SURFACTANT/n-PROPANOL/WATER SYSTEMS AT HIGH PERCENTAGES OF ALCOHOL BY RP-HPLC

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ABSTRACT

In this work, the binding constants for five phenolic antioxidants with aggregates of cationic hexadecyltrimethylammonium bromide (CTAB) and anionic sodium dodecylsulfate (SDS) surfactants in n-Propanol at percentages higher than 20% v/v are calculated. The surfactant concentration needed to obtain aggregates in mobile phase was calculated in the presence of different n-Propanol percentages.

The use of a multiple regression analysis allows one to obtain these constants at any percentage of the alcohol. The results are in very good agreement with the experimental data obtained in these media, taking into account the alcohol concentration in the used equations.

INTRODUCTION

The solute-micelle binding constants are determined for many types of compounds, since in 1981 Armstrong and Nome[1] proposed the three-phase model to describe the behavior of the solute retention in micellar liquid chromatography. Other treatments based on the same model, proposed by Arunyanart and Cline-Love[2] and Foley,[3] have been greatly used to calculate these constants. The binding constant values for many types of compounds and different surfactants, in the absence and in the presence of low percentages of an organic modifier, have been summarized recently.[4] These values are determined, in all cases, in mobile phases with direct micelles in water or direct micelles in water/organic modifier at percentages lower than 20% *v/v*.

Different theoretical equations to describe the influence of organic modifiers upon the retention of solutes in RP-HPLC with micellar mobile phases, have been used in several papers.[5–15]

The mobile phases that contain direct micelles are not always useful. Sometimes, the retention times are very long, especially, when the solutes are very hydrophobic and the efficiency is very low. In these cases, the use of a surfactant in the mobile phase can be a new variable that modified the solute retention and increases the separation possibilities, but it is necessary in the presence of higher percentages of an organic modifier. In the presence of low percentages of these modifiers, the separation and determination of the solutes is not always possible, and an increase in the organic modifier percentage allows one to carry out the separation and enhance the efficiency with respect to a low percentage. These mobile phases formed by a surfactant (cationic CTAB or anionic SDS) and an organic modifier in a percentage higher than 20% *v/v* (Ethanol or n-Propanol), were studied previously by us.

Thus, the separation and determination of metal complexes[16,17] and phenolic antioxidants[18] have been carried out, and the solute-aggregate association constants for metal complexes[19,20] and polycyclic aromatic hydrocarbons[21] have been calculated.

The mobile phases that contain surfactant and different percentages of a short chain alcohol, as methanol, ethanol, or propanol higher than 20 % *v/v,* are isotropic solutions with a phase diagram in which the different aggregates are not well distinguished. In these cases, at low percentages of the modifier, these are direct micelles and at very high percentages reverse micelles are present. In the mixtures with intermediate percentages, bicontinuos structures of surfactant and modifier that are capable to solubilize and interact with different solutes were proposed.[22–27]

Phenolic antioxidants are compounds of great interest due to their use as food additives.[28,29] These compounds can be separated and determined in the presence of mobile phases of surfactant in the presence of *n*-PrOH at high percent-

ages.[18] The retention behavior in RP-HPLC and the binding constants of the antioxidants-aggregates present in these mobile phases, are studied in this work.

EXPERIMENTAL

Apparatus

The chromatographic system involves pump Perkin-Elmer model 250, UV-Vis programmable detector from Applied Biosystems model 785 A, a software Perkin-Elmer Turbochrom 4 as data collection, and an injection valve Rheodyne with an injection volume of 20 μL.

The separation column was Lichrosorb RP-18, 150 x 3.9 mm, particle size 10 μm from Sugelabor (Madrid, Spain).

The conductivity measures were carried out in a Crison 625 conductimeter with a thermostatized cell at 25 ± 1°C.

Reagents

All reagents were of analytical grade. The surfactants hexadecyltrimmethylammonium bromide (CTAB) and sodium dodecylsulphate (SDS) and also n-Propanol (n-PrOH) and the Phosphoric acid (H_3PO_4) from Merck (Darmstadt, Germany) were used as they were received.

The antioxidants: Butylhydroxianisole (BHA), Butylhydroxytoluene (BHT), Propylgallate (PG), and Octylgallate (OG) were obtained from Fluka (Buchs, Switzerland) and Dodecylgallate (DG) from Aldrich (Alcobendas, Madrid, Spain).

The ultrapure water used was obtained from a Millipore Milli-Q system (Milford, Mass., USA).

Determination of the CMC of Surfactants in Presence of *n*-PrOH

The determination of the critical micelle concentration (CMC) for direct micelles and critical aggregate concentration (CAC) for the aggregates in the presence of higher quantities of organic modifier, was carried out in solutions with the surfactant concentration below and above the CMC and the appropriate quantity of n-PrOH in order to have the suitable percentage in the solution. The conductivity of these solutions were measured at 25 ± 1°C in a thermostatized flask. The CMC or CAC was determined as the breakpoint of the two straight lines obtained in the conductivity *vs* surfactant concentration curve.

Determination of the Antioxidant Retention Times

The mobile phases were prepared with the cationic (CTAB) and anionic (SDS) surfactant in an appropriate concentration which was ranged from 0.03 M to 0.25 M, and *n*-PrOH percentages were ranged between 20 and 50% *v/v*. In order to obtain an acid pH, a concentration of phosphoric acid 0.01 M was added to the mobile phases, next the phases were filtered through a 0.45μm nylon membrane filter, and finally, placed in an ultrasonic bath for 20 min for degasification before introduction into the chromatographic system. The detection was carried out at the maximum wavelength of each antioxidant as follows: BHA 289 nm; BHT 277 nm; PG 272 nm; OG 272 nm, and DG 271 nm.

The solutions of the antioxidants were prepared by diluting the appropriate quantity in the mobile phase and they were directly injected into the chromatographic system. The injected volume was 20 μL and the mobile phase flow 1 $mL.min^{-1}$. For the different mobile phases, the retention factors expressed as the average of three independent measures were obtained by a function of the surfactant concentration and *n*-PrOH percentage.

RESULTS AND DISCUSSION

Determination of the Surfactants CAC

The variation of the critical micelle concentration was studied in the presence of different percentages of *n*-PrOH. When the percentage of *n*-PrOH is as high as 20% *v/v,* there are no direct micelles in the solution and the system surfactant/alcohol/water corresponds to a bicontinuous structure of water and alcohol separated by the surfactant layer;[22–27] for this reason, the necessary concentration of surfactant to form the structures previously mentioned, will comprise critical aggregate concentration (CAC). At *n*-PrOH percentages below 20% *v/v*, the CAC is the same as CMC.

In all cases studied, the two straight lines obtained have different slopes, although, this difference is higher in the presence of low percentages of the alcohol.

Figure 1 shows the variation of the CAC vs *n*-PrOH percentage ranged between 0 and 70% *v/v*. All the compositions studied show a variation in the slopes of the straight lines with a break point whose values are present in this figure. The formation of the aggregates is well known for the percentages lower than 20% *v/v*, with an increase in the CAC value (CMC in this case) due to the increase in the dissociation degree when increasing n-PrOH percentages. For n-PrOH percentages ranging between 20 and 50% *v/v*, we found a constant CAC

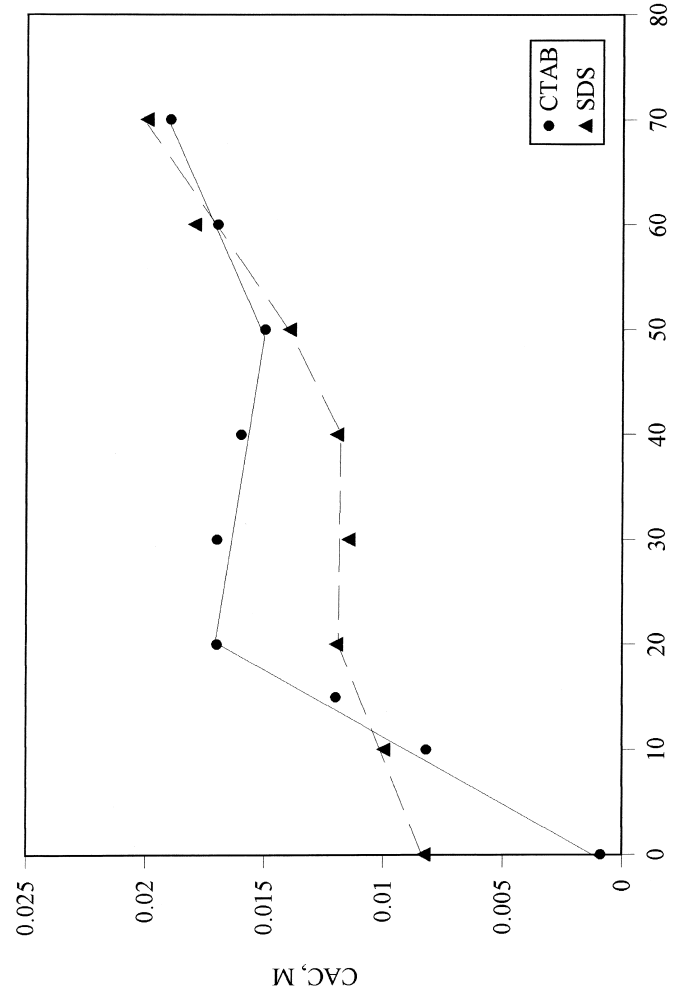

Figure 1 Variation of the critical aggregate concentration of the systems CTAB/*n*-PrOH and SDS/*n*-PrOH in function of the *n*-PrOH percentage.

due to the formation of the bicontinuous structure of surfactant separated by *n*-PrOH monomers.

At percentages of *n*-PrOH higher than 50% *v/v*, the CAC increases again due to new changes in the formed structure. The confirmation by this study of the presence of these aggregates at these *n*-PrOH percentages, enabled us to study the behavior of phenolic antioxidants in reverse phase HPLC with mobile phases that contain them.

Study of the Chromatographic Retention of the Antioxidants in Presence of Surfactant/*n*-PrOH/Water Mobile Phases

The retention behavior of different solutes in RP-HPLC in the presence of mobile phases with surfactants, has been established many years ago and used for many types of compounds. This behavior is described for the mobile phases of surfactant/water or surfactant/short chain alcohol/water, when the percentage of the alcohol is lower than 20% *v/v*. In these cases, the retention of a solute is a function of the micellized surfactant concentration and the lineal correlation between $1/k$ and [surfactant], allowing us to determine the solute-micelle binding constants from different equations, as the Arunyanart-Cline Love:[2]

$$\frac{1}{k} = \frac{k_2}{\phi[L_s]K_1} \qquad (1)$$

where K_2 is the binding constant of a solute to micelles, ϕ is the phase ratio (V_S/V_M), V_S and V_M are the total stationary phase volume and the dead column volume, respectively, $[L_S]$ is the stationary phase concentration, K_1 the binding constant for the solute between the stationary phase and the bulk solvent, and C_M is given by the total surfactant concentration minus the CMC. This equation does not consider the presence of small quantities of the alcohol, it supposes that the micelles are not modified and they have the same behavior as that in the absence of the modifier; that is to say, the system behaves as direct micelles.

Equation (1) was applied, in many occasions, to determine the binding constants, K_2, for a great number of solutes in Micellar Liquid Chromatography, MLC. However, as it has already been mentioned, in the deduction of the Eq. (1) by Arunyanart and Cline-Love, it does not keep in mind the influence of the organic modifier in the retention.

In a previous work,[21] we wrote the equilibrium model[2] with certain modifications in such a way, that takes into account the interaction of the solutes with the stationary phase modified by the presence of growing quantities of the alcohol, FEOH. The complete equilibria are:

$$S_W + FES \overset{K_1}{\rightleftharpoons} S_{ES}$$

$$S_W + D \overset{K_2}{\rightleftharpoons} S_M$$

$$S_W + FEOH \overset{K_3}{\rightleftharpoons} S_{EOH}$$

$$ROH \_ FES \overset{K_4}{\rightleftharpoons} FEOH$$

The equation obtained in this case:

$$\frac{1}{k} = \frac{1}{\phi\,[FES]\,K_1 + \phi K_3 K_4\,[FES][ROH]} + \frac{K_S}{\phi\,[FES]\,K_1 + \phi K_3 K_4\,[FES][ROH]}[D] \quad (2)$$

where [*D*] and [*ROH*] are the total concentration of surfactant and alcohol. The Eq. (2) has been tested for the phenolic antioxidants and Figures 2 and 3 show the experimental relation between 1/*k* and the surfactant concentration for the percentages of n-PrOH in the mobile phase ranging between 20 and 50% *v/v* for the five antioxidants in CTAB and SDS, respectively.

In these figures, it can be observed that there is the same retention behavior as in the presence of direct micelles (in absence of n-PrOH or in presence of percentages lower than 20% *v/v*). Thus, the Eq. (2) can be used in phases of surfactant/n-PrOH/water in which the aggregates are bicontinuous structures instead of direct micelles. From Eq. (2), we can determine the binding constants solute-aggregate in the same form as that from the Arunyanart-Cline Love's equation, from the relation *1/k versus* [surfactant] as the ratio slope:intercept.

Tables 1 and 2 show the regression parameters of the straight lines (intercept, slope, and correlation coefficient) and the binding constants of the antioxidants with the aggregates, obtained by Eq. (2), in mobile phases of CTAB and SDS, respectively, in the presence of n-PrOH. In these tables, it can be observed that there is very good correlation between the values of 1/*k* and the surfactant concentration.

The binding constants decrease as the *n*-PrOH percentage increases for all antioxidants and for the gallates (PG, OG and DG). The values of the constant augment when the hydrophobicity of the antioxidant structure increases. Thus, the binding constants are higher when the antioxidant hydrocarbon chain is longer. The values that are in parenthesis are those that have a relative error higher than 10%.

Several papers were published describing the modelization of the retention in MLC with low percentages of an organic modifier, generally a short chain alcohol.[5,6,8,10,11] In these papers, the equations use as a retention parameter the ln *k*

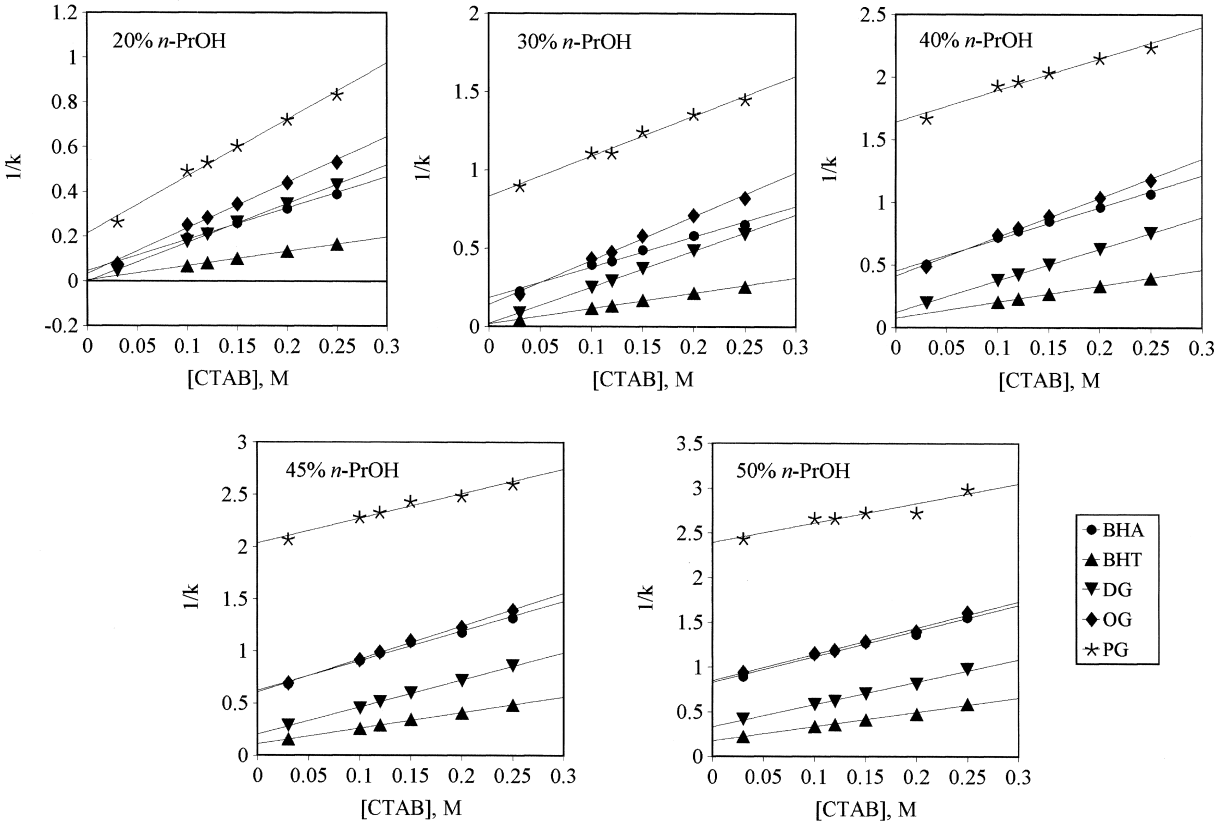

Figure 2. Variation of the inverse of retention factor (1/*k*) with the CTAB concentration for different percentages of *n*-PrOH between 20 and 50% *v/v*.

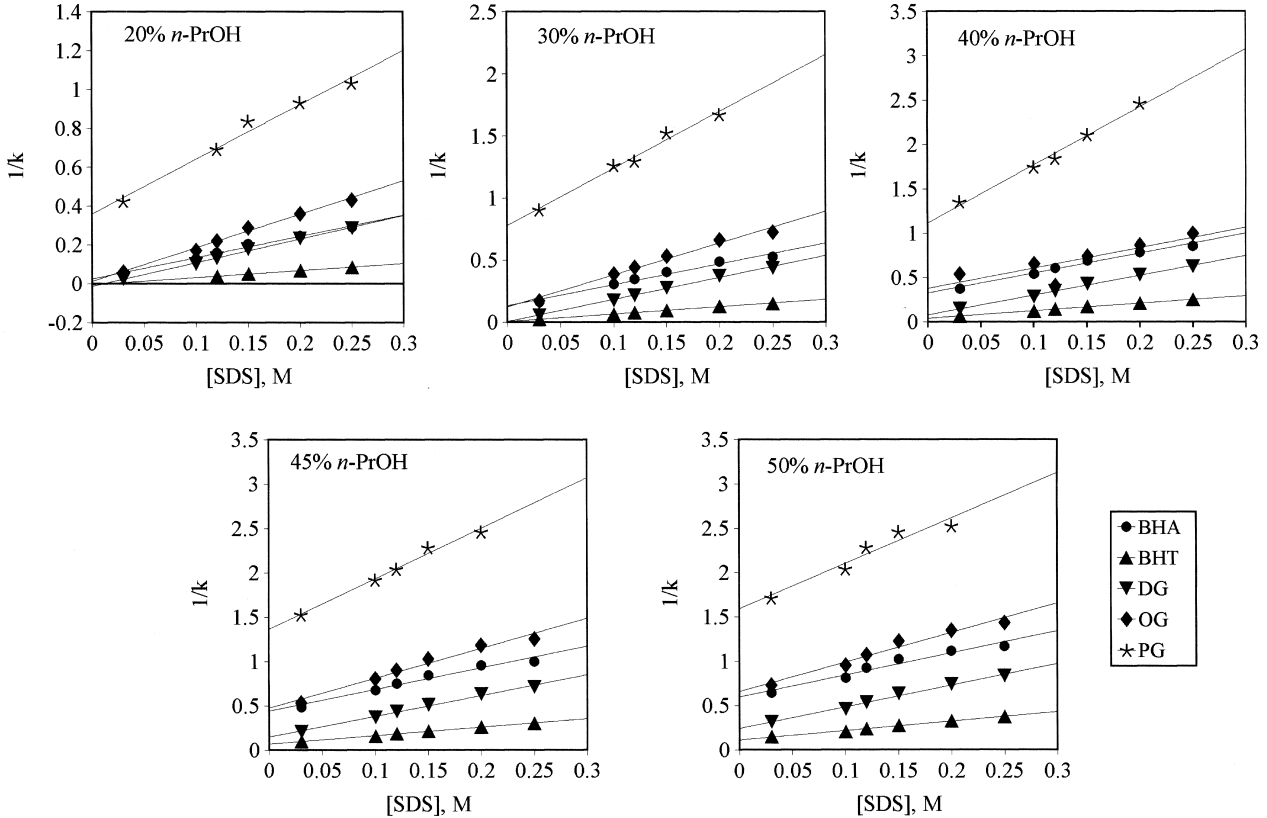

Figure 3. Variation of the inverse of retention factor ($1/k$) with the SDS concentration for different percentages of *n*-PrOH between 20 and 50% *v/v*.

Table 1. Regression Parameters and Binding Constants of the Antioxidants with CTAB in Presence of Different n-PrOH Percentages Obtained from Equation (2)

	CTAB/*n*-PrOH/Water				
Antioxidant	% *n*-PrOH	Intercept	Slope	*r*	K_s (M^{-1})
BHA	20	0.065 ± 0.002	1.30 ± 0.01	0.9998	20.0 ± 0.9
	30	0.214 ± 0.016	1.79 ± 0.09	0.9962	(8 ± 1)
	40	0.50 ± 0.01	2.29 ± 0.06	0.9990	4.6 ± 0.2
	45	0.67 ± 0.03	2.6 ± 0.2	0.9939	(3.9 ± 0.4)
	50	0.86 ± 0.01	2.79 ± 0.06	0.9995	3.2 ± 0.1
BHT	20	0.0042 ± 0.0005	0.647 ± 0.003	0.9999	(153 ± 18)
	30	0.017 ± 0.004	0.98 ± 0.02	0.9988	(57 ± 14)
	40	0.071 ± 0.003	1.31 ± 0.02	0.9995	18 ± 1
	45	0.113 ± 0.005	1.50 ± 0.03	0.9990	13.2 ± 0.9
	50	0.174 ± 0.005	1.64 ± 0.03	0.9993	9.4 ± 0.5
PG	20	0.258 ± 0.005	2.31 ± 0.03	0.9997	8.9 ± 0.3
	30	0.84 ± 0.02	2.5 ± 0.1	0.9959	3.0 ± 0.2
	40	1.72 ± 0.01	2.12 ± 0.08	0.9981	1.23 ± 0.05
	45	2.08 ± 0.01	2.09 ± 0.05	0.9993	1.00 ± 0.03
	50	2.361 ± 0.005	2.50 ± 0.03	0.9998	1.06 ± 0.02
OG	20	0.061 ± 0.003	1.89 ± 0.02	0.9998	31± 2
	30	0.14 ± 0.02	2.8 ± 0.1	0.9971	(20 ± 3)
	40	0.443 ± 0.005	2.95 ± 0.03	0.9999	6.6 ± 0.1
	45	0.61 ± 0.01	3.15 ± 0.06	0.9992	5.2 ± 0.2
	50	0.84 ± 0.01	3.04 ± 0.09	0.9988	3.6 ± 0.1
DG	20	− 0.001 ± 0.003	1.74 ± 0.02	0.9997	—
	30	0.020 ± 0.005	2.31 ± 0.03	0.9996	(116 ± 33)
	40	0.122 ± 0.002	2.54 ± 0.01	0.9999	20.8 ± 0.4
	45	0.206 ± 0.007	2.60 ± 0.04	0.9995	12.6 ± 0.6
	50	0.348 ± 0.005	2.32 ± 0.04	0.9995	6.7 ± 0.2

or the inverse one, in functions of the micellized surfactant and alcohol concentrations, as well as combinations of these magnitudes like their product, their square, etc. In order to find an equation that adjusts the data for the five antioxidants in the presence of the mobile phases employed, we have tested the equations that are given in the literature, finding that the best adjustment by means of a multiple regression analysis completes the equation:

$$\frac{1}{k} = a + b[D] + c[ROH] \qquad (3)$$

Tables 3 and 4 show the obtained values for the parameters *a*, *b,* and *c* with the confidence interval (α =0.05), and the agreement percentage for the five

Table 2. Regression Parameters and Binding Constants of the Antioxidants with SDS in the Presence of Different *n*-PrOH Percentages Obtained from Equation (2)

	SDS/*n*-PrOH/Water				
Antioxidant	% *n*-PrOH	Intercept	Slope	*r*	$K_s\,(M^{-1})$
BHA	20	0.027± 0.005	1.08 ± 0.03	0.9991	(40 ± 9)
	30	0.113 ± 0.008	1.90 ± 0.06	0.9986	17 ± 2
	40	0.30 ± 0.02	2.5 ± 0.1	0.9962	8.2 ± 0.6
	45	0.40 ± 0.02	2.8 ± 0.1	0.9973	7.0 ± 0.6
	50	0.555 ± 0.005	3.09 ± 0.04	0.9999	5.6 ± 0.1
BHT	20	− 0.003 ± 0.003	0.36 ± 0.02	0.9981	—
	30	0.006 ± 0.002	0.59 ± 0.01	0.9989	(92 ± 33)
	40	0.040 ± 0.002	0.84 ± 0.01	0.9993	21 ± 2
	45	0.072 ± 0.003	0.94 ± 0.02	0.9994	13.1 ± 0.8
	0	0.121 ± 0.004	1.03 ± 0.03	0.9990	8.5 ± 0.5
PG	20	0.35 ± 0.02	2.8 ± 0.1	0.9979	(8.1 ± 0.9)
	30	0.750 ± 0.003	5.15 ± 0.03	0.9999	6.86 ± 0.07
	40	1.13 ± 0.04	6.5 ± 0.3	0.9980	5.8 ± 0.5
	45	1.37 ± 0.01	5.5 ± 0.1	0.9996	4.0 ± 0.1
	50	1.53 ± 0.01	6.2 ± 0.1	0.9999	4.0 ± 0.1
OG	20	0.009 ± 0.008	1.72 ± 0.05	0.9986	(181 ± 164)
	30	0.092 ± 0.009	2.89 ± 0.07	0.9991	(31 ± 4)
	40	0.45 ± 0.01	2.08 ± 0.09	0.9991	4.6 ± 0.3
	45	0.43 ± 0.02	3.9 ± 0.2	0.9977	9.0 ± 0.8
	50	0.61 ± 0.03	4.1 ± 0.2	0.9982	6.7 ± 0.7
DG	20	− 0.009 ± 0.005	1.20 ± 0.03	0.9991	—
	30	0.008 ± 0.005	1.73 ± 0.04	0.9993	(202 ± 138)
	40	0.090 ± 0.008	2.19 ± 0.05	0.9992	25 ± 2
	45	0.134 ± 0.008	2.51 ± 0.06	0.9991	19 ± 2
	50	0.24 ± 0.01	2.5 ± 0.1	0.9984	10 ± 1

antioxidants in CTAB and SDS, respectively. The results found are in very good agreement with the proposed model. The lower adjustment is 96.7 for BHT in SDS, and the higher 99.6 for PG in CTAB. Generally, the adjustment for all antioxidants is better in CTAB than in SDS. Figures 4 and 5 show the relation between the experimental and calculated values for $1/k$ in CTAB and SDS, respectively. The obtained relation is very good and the adjustment of the values to the theoretical equation in consequence is correct.

From the equations obtained by multiple regression, we can calculate the binding constants of the antioxidant with aggregates of CTAB and SDS in the

Table 3. Adjusted Equations for $1/k$ in Function of CTAB Concentration and *n*-Propanol Percentage Obtained for Multiple Regression with a Confidence Level of 95% for the Five Antioxidants, $1/k = a + b\,[\text{CTAB}] + c\,(\%\text{PrOH})$

	a	*b*	*c*	% Agreement
BHA	−0.8050 ± 0.0842	1.9837 ± 0.2907	0.0257 ± 0.0019	97.27
	$1/k = -0.8050 + 1.9837\,[\text{CTAB}] + 0.0257\,(\%\text{PrOH})$			
	a	*b*	*c*	% Agreement
BHT	−0.3541 ± 0.0374	1.2778 ± 0.1149	0.0111± 0.0008	97.97
	$1/k- = -0.3541 + 1.2778\,[\text{CTAB}] + 0.0111\,(\%\text{PrOH})$			
	a	*b*	*c*	% Agreement
DG	−0.4298 ± 0.0520	2.4139 ± 0.1705	0.0149 ± 0.0011	98.25
	$1/k = -0.4288 +\,[\text{CTAB}] + 0.0149\,(\%\text{PrOH})$			
	a	*b*	*c*	% Agreement
OG	−0.7195 ± 0.0920	2.8005 ± 0.2973	0.0313 ± 0.0020	98.17
	$1/k = -0.7195 + 2.8005\,[\text{CTAB}] + 0.0313\,(\%\text{PrOH})$			
	a	*b*	*c*	% Agreement
PG	−1.2499 ± 0.0804	2.4299 ± 0.2776	0.0723 ± 0.0018	99.61
	$1/k = -1.2499 + 2.4299\,[\text{CTAB}] + 0.0723\,(\%\text{PrOH})$			

presence of any percentage of n-PrOH. In this way, Eq. (3) can be written as a function of a new parameter $a' = a + c\,[ROH]$:

$$\frac{1}{k} = a' + b[D] \qquad (4)$$

and thus, the binding constants can be calculated for any alcohol concentration by $K_2 = b/a'$. The binding constants obtained from the data of Tables 3 and 4 for the five antioxidants with the aggregates of CTAB/n-PrOH and SDS/*n*-PrOH are introduced in Table 5.

The binding constant values for the lower percentages of *n*-PrOH studied, can not be calculated for BHT and DG at 20 and 30% *v/v* and for BHA and OG at 20% *v/v* . In these cases, the retention of the antioxidant is very long for its high hydrophobicity and the high polarity of the mobile phase. In the case of PG, the binding constants can be calculated for all the *n*-PrOH percentages studied.

Table 4. Adjusted Equations for $1/k$ as a Function of SDS Concentration and *n*-Propanol Percentage Obtained for Multiple Regression with a Confidence Level of 95% for the Five Antioxidants, $1/k = a + b$ [SDS] + c (%PrOH)

	a	*b*	*c*	% Agreement
BHA	−0.6957 ± 0.0823	2.1034 ± 0.2695	0.0265 ± 0.0018	97.74
	$1/k = -0.6957 + 2.1034$ [SDS] + 0.0265 (%PrOH)			
	a	*b*	*c*	% Agreement
BHT	−0.2477 ± 0.0331	0.7902 ± 0.0932	0.0076± 0.0007	96.74
	$1/k = -0.2477 + 0.7902$ [SDS] + 0.0076 (%PrOH)			
	a	*b*	*c*	% Agreement
DG	−0.4994 ± 0.0616	2.1355 ± 0.1894	0.0153 ± 0.0013	97.53
	$1/k = -0.4994 + 2.1355$ [SDS] + 0.0153 (%PrOH)			
	a	*b*	*c*	% Agreement
OG	−0.7920 ± 0.1063	2.8170 ± 0.3595	0.0299 ± 0.0023	97.35
	$1/k = -0.7920 + 2.8170$ [SDS] + 0.0299 (%PrOH)			
	a	*b*	*c*	% Agreement
PG	−0.7990 ± 0.1677	5.0333 ± 0.7727	0.0495 ± 0.0039	97.98
	$1/k = -0.7990 + 5.0333$ [SDS] + 0.0495 (%PrOH)			

The binding constant values decrease when the *n*-PrOH percentage increases, and the values obtained by this method are very similar to those obtained by Eq. (2), although, the associate error is greater than 10%. In this form, the values of the binding constants can be determined by multiple regression for any *n*-PrOH percentage.

CONCLUSIONS

The mobile phases that contain a surfactant in presence of intermediate percentages of *n*-PrOH (20 to 50% *v/v*), have a behavior similar to micellar mobile phases. However, these mobile phases are very suitable in RP-HPLC because they reduce the retention times and enhance the efficiency.

For high percentages of *n*-PrOH, the relationship among the inverse of the retention factor and the surfactant concentration is linear; that is, it presents the

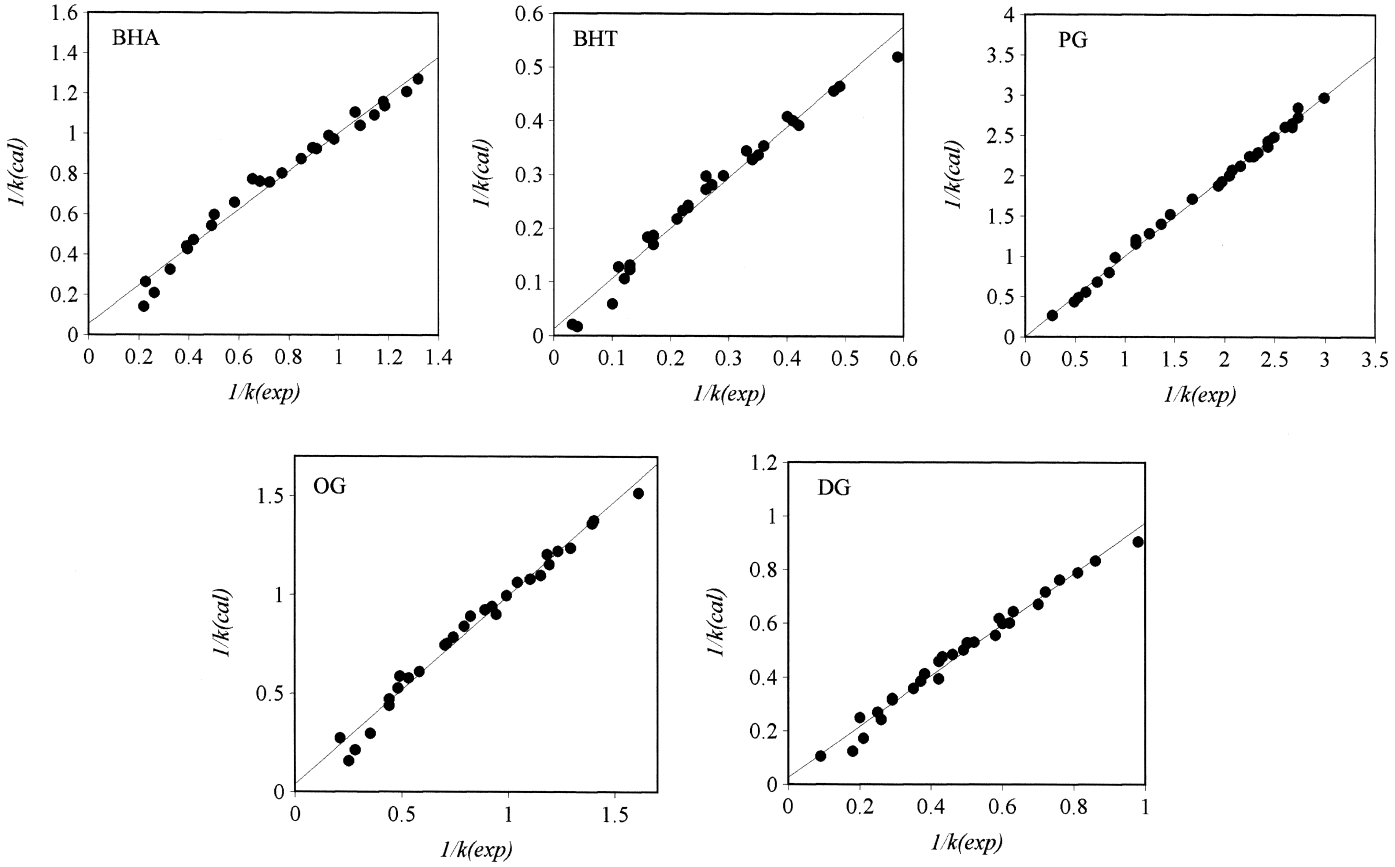

Figure 4. Relation between the experimental and calculated $1/k$ by the multiple regression for the mobile phases of CTAB.

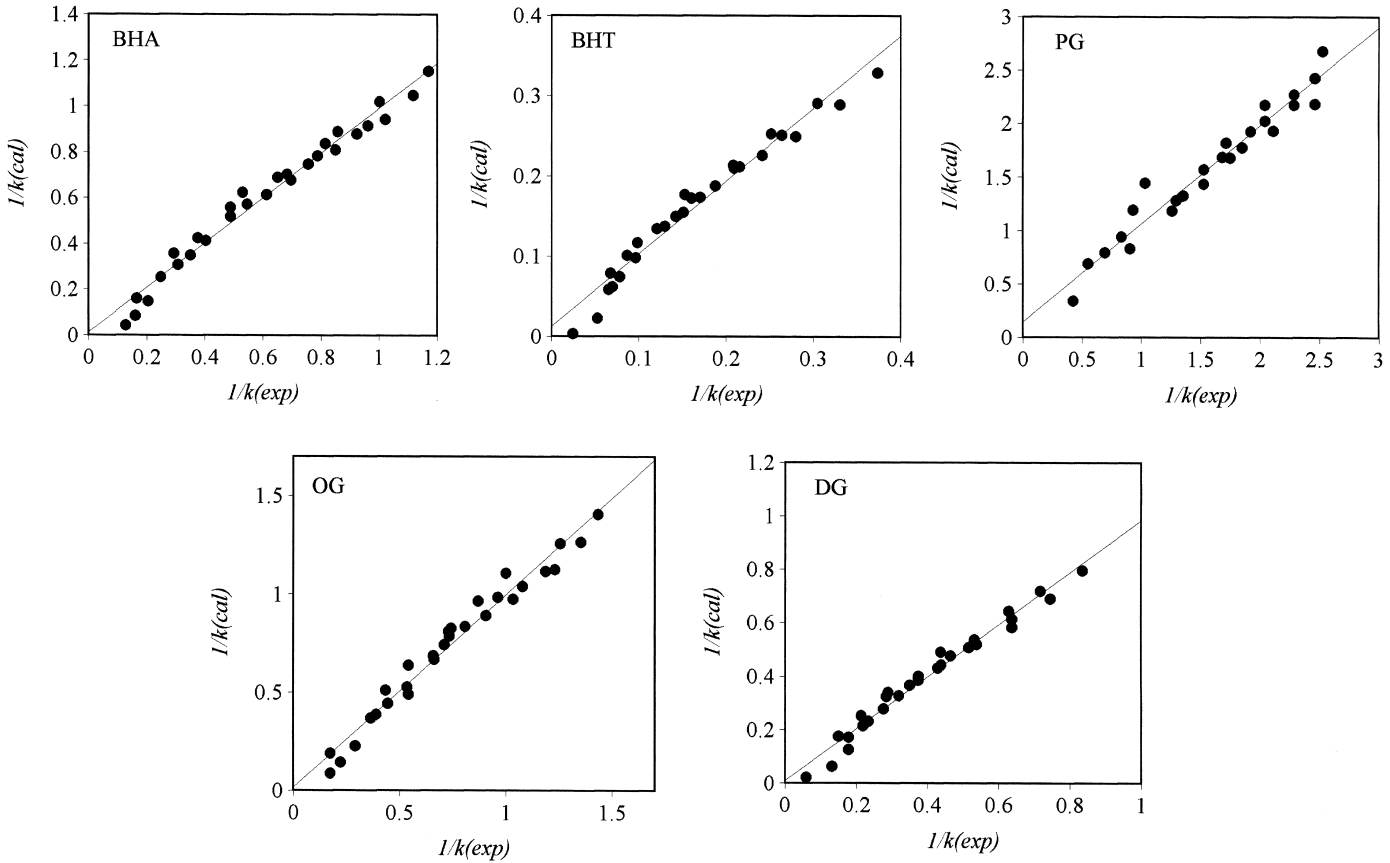

Figure 5. Relation between the experimental and calculated $1/k$ by the multiple regression for the mobile phases of SDS.

Table 5. Binding Constant Values (M^{-1}) Obtained from the Multiple Regression for the Antioxidants with SDS and CTAB at Different Percentages of *n*-PrOH

Antioxidant	% *n*-PrOH	SDS	CTAB
BHA	20	—	—
	30	21.18	15.67
	40	5.77	5.17
	45	4.23	3.87
	50	3.34	3.10
BHT	20	—	—
	30	—	—
	40	14.04	14.21
	45	8.38	8.79
	50	5.97	6.36
PG	20	26.35	12.39
	30	7.34	2.64
	40	4.26	1.48
	45	3.52	1.21
	50	3.00	1.03
OG	20	—	—
	30	26.83	12.76
	40	6.97	5.26
	45	5.09	4.06
	50	4.01	3.31
DG	20	—	—
	30	—	—
	40	18.97	14.76
	45	11.29	9.70
	50	8.04	7.23

same behavior as that in the presence of direct micelles. So, it is possible to calculate the solute-aggregate association constants.

Finally, by means of a multiple regression analysis that considers the presence of the alcohol, it is possible to obtain and to predict the binding constants at any alcohol percentage.

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