Adsorption Isotherms of Substituted Benzophenones on a Reverse-Phase Liquid Chromatography System: Effect of the Mobile-Phase Composition

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Linear adsorption isotherms were registered by a frontal analysis method for four substituted benzophenones (BPs) using a liquid chromatography Luna C18(2) column as adsorbent. The isotherm data points were measured at different mobile-phase compositions of methanol−water, varying the methanol volume fraction from 0.4 to 0.6 with a step of 0.05 and keeping the temperature at 30 °C. Henry's constants were obtained from the analysis of experimental data for each mobile-phase composition. The substituent effect on Henry's constant was evaluated, and it was found that the hydrogen-bond acceptor ability of the *p*-substituent, the interactions of the carbonyl group with the polar mobile phase molecules, and the molecular volume are key aspects governing the adsorption process of the studied BPs. In addition, the effect of the mobile-phase composition was analyzed by applying two different models to predict the Henry's constants and compare them to the experimental values. It was found that the Snyder model is the most suitable to describe the variation of the mentioned constant with the volume fraction of methanol in the mobile phase.

Introduction

Benzophenones (BPs) are diphenyl ketones obtained from natural products[1] or by synthetic methods.[2] They exhibit a wide variety of physicochemical and biological applications; therefore, they are frequently employed in industrial and pharmacological processes. For example, 2-hydroxy-4-methoxybenzophenone (2(OH)4(MeO)BP) and derivatives are commonly used in pharmaceutical formulations such as sunscreen lotions due to their capacity to absorb and dissipate UV radiation,[3,4] and for this reason, other BPs like 2,4-dihydroxybenzophenone derivatives are also used in UV protective additives in fabrics.[5] 4-Hydroxybenzophenone (4(OH)BP) is an intermediate for the synthesis of tamoxifen, a drug employed in the treatment of breast cancer,[6] and it is also an intermediate in the synthesis of clomiphene citrate, an ovulation stimulant in human females.[7] In addition, 4-methoxybenzophenone (4(MeO)BP) and many other BPs are involved in the synthesis of industrial polymers.[8,9] Other applications are the design of optical materials,[10] the development of asymmetric catalysts,[11] and enzymatic inhibition.[12] The effects of substituents on their conformations[13,14] and the metal complexing ability in hydroxylic solvents have been studied[15] for a better understanding of the physicochemical properties of BPs. Nevertheless, several characteristics of BPs in solution have not been completely defined yet.

The characterization of adsorption processes of bioactive compounds and the different operative conditions that rule these processes constitute a very important field of research in physicochemical and pharmacological sciences. Moreover, adsorption isotherms are very useful for an adequate description and optimization of separation processes in chromatography because they provide important information on the interaction between the solute and an adsorbent. Experimental isotherms can be measured by many methods (static, dynamic, and numerical) in a high-performance liquid chromatography (HPLC) system.[16] All of these methods have their advantages and limitations, but when accurate measurements for a single column are required, the best method for isotherm determination is frontal analysis chromatography.[17]

In this paper, and with the aim of contributing to clarify the adsorption process of a set of four substituted BPs on a octadecylsilane (ODS) column, the isotherms of 4(OH)BP, 4(MeO)BP, 2,4-dihydroxybenzophenone (2,4(OH)$_2$BP), and 2(OH)4(MeO)BP were determined by means of HPLC in methanol aqueous solutions, with a methanol volume fraction range of 0.4 to 0.6. Furthermore, the influence of the substituent and the mobile phase composition on the corresponding adsorption isotherms was analyzed using different relationships.

Experimental Section

Chemicals and Reagents. Figure 1 shows the chemical structure of the studied compounds. 4(OH)BP and 4(MeO)BP from Fluka, 2,4(OH)$_2$BP from Aldrich, and 2(OH)4(MeO)BP from Sigma were used without further purification. Methanol (MeOH), HPLC grade, from Sigma, was filtered through 0.22 μm nylon filters (Millipore) before use. Milli-Q water was used to prepare the mobile phase. Both solvents were degassed by sonication. All of the solutions were prepared by weight with a precision of ± 0.0001 g.

Instrumentation. The HPLC instrument consisted of a Gilson 322 pump (Gilson Inc., Middleton, WI, USA) and a Gilson 152 UV−vis detector, equipped with a Phenomenex column temperature controller. The system was controlled by UniPoint software v2.10 (Gilson). The analysis was carried out with a reverse-phase Luna (C$_{18}$ (2), 250 mm × 4.6 mm, 5 μm) column from Phenomenex (Torrance, CA, USA). Table 1 shows the

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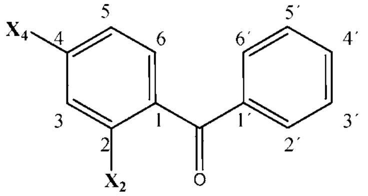

(**1**)	$X_2 = H$	$X_4 = OH$	4(OH)-benzophenone
(**2**)	$X_2 = H$	$X_4 = OCH_3$	4(MeO)-benzophenone
(**3**)	$X_2 = OH$	$X_4 = OH$	2,4(OH)$_2$-benzophenone
(**4**)	$X_2 = OH$	$X_4 = OCH_3$	2(OH)4(MeO)-benzophenone

Figure 1. Structure of the studied BPs.

Table 1. Physicochemical Properties of the Employed Column Supplied by the Manufacturer (Phenomenex)

Luna C18 (2) column (Phenomenex)	
particle size/μm	5
particle shape	spherical
pore size/Å	100
surface area/$m^2 \cdot g^{-1}$	400
carbon load/%	17.5
calculated bonded phase coverage/$\mu mol \cdot m^{-2}$	3.00
end-capping	yes

physicochemical properties of the column used.[18] Each isotherm data point was registered under isocratic elution with a mobile phase of MeOH−water at a fixed ratio with a flow rate of 1.0 $mL \cdot min^{-1}$. The temperature was maintained at 30 °C, and the detector was set at 280 nm.

Procedures. The principle of the frontal analysis method is schematically shown in Figure 2. It consists in quickly replacing the stream of mobile phase with solutions of different concentrations of the studied compounds and recording the breakthrough curves at the column outlet. Commonly, this method can be implemented in two different ways, according to the mobile phase feed concentration profiles, the *staircase* method and the *step series* method.[19] In the staircase method, the feed concentration is increased stepwise, whereas in the step series method, the column is equilibrated with pure mobile phase between successive concentration steps. The adsorbed (q^*) amount can be calculated by the mass conservation of the solute between the time when the new solution enters the column (t_0) and a final time when the plateau concentration is reached. q^* represents the adsorbed amount in the stationary phase at equilibrium with a given concentration C in the mobile phase. The gray area in Figure 2 represents this amount, while the area on the left represents the mass of solute in the mobile phase occupying the column dead volume. The adsorbed amount q^* can be calculated by the following equation[20]

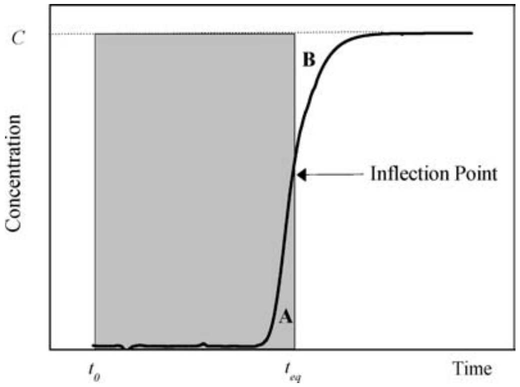

Figure 2. Schematic breakthrough curve for frontal analysis. The solid line represents the analyte concentration at the column outlet. The equivalent time (t_{eq}) is obtained by integration method (equaling area A to B).

$$q^* = \frac{C(V_{eq} - V_0)}{V_a} \qquad (1)$$

In this equation V_{eq} is the elution volume of the equivalent area of the breakthrough curve (corresponding to a t_{eq} time in Figure 2), V_0 represents the column hold-up volume, and V_a is the stationary phase volume. The t_{eq} time is related to the elution volume of the equivalent area and was determined by the integration method, which corresponds to equaling area A to B in Figure 2. The stationary phase volume (V_a) can be calculated by subtracting the geometrical volume of the column tubing with its hold-up volume.[20] The column hold-up volume was estimated from the mean value of the retention time of three repeated injections of potassium dichromate. This value was then corrected with the extra column volumes. For the isotherm data point calculations, it is necessary to previously calibrate the detector to determine the solute concentration C in an accurate way. The calibration was performed by pumping 10 solutions of different and known concentrations of the substituted BP and registering the signal from the detector. This procedure was repeated for each BP and for each mobile phase composition employed. The calibration data were fitted to a second-degree polynomial function.

Once the calibration is completed, the corresponding adsorption isotherms were registered using the step series method. Pump A of the HPLC instrument was used to deliver a stream of pure water, while pump B was used to deliver a stream of a BP solution of known concentration dissolved in pure MeOH. The concentration of BP in the mobile phase can be calculated with the flow-rate fractions delivered by the two pumps. After breakthrough, the flow of the solution through the column was continued until the detector signal was constant to ensure equilibrium between the solution and the column. The chromatographic system was then flushed with 50 column volumes of a MeOH−water mobile phase (replacing the stream of pump B with pure MeOH) to remove the solute from the column until a new equilibrium is established between the adsorbent and pure mobile phase.

In each experiment, the solute concentration in the stationary phase was determined from the experimental concentrations of the analyzed BP at the plateaus of the frontal analysis curve and the retention volumes corresponding to the inflection points on the breakthrough curve using eq 1.

Results and Discussion

Effect of the Mobile-Phase Composition. With the frontal analysis methodology, the adsorbed amount of a solute (q^*) and the equilibrium concentration (C) can be calculated. To analyze the obtained data, a great number of theoretical models have been proposed to describe the distribution of a single component between the stationary and the mobile phase. At infinite dilution, under linear chromatography conditions, the molar concentrations of the analyte i in the stationary phase (C_i^S) and the mobile phase (C_i^M) are simply related to the molar fractions x_i^S and x_i^M, respectively, by

$$\left(\frac{x_i^{\mathrm{S}}}{C_i^{\mathrm{S}}}\right) = V_{\mathrm{S,m}} \quad \text{and} \quad \left(\frac{x_i^{\mathrm{M}}}{C_i^{\mathrm{M}}}\right) = V_{\mathrm{M,m}} \tag{2}$$

where $V_{\mathrm{S,m}}$ and $V_{\mathrm{M,m}}$ are the molar volume of the stationary and the mobile phases, respectively.[21] In addition, the retention factor, k' is the ratio of the amounts of solute in the stationary and the mobile phases at infinite dilution

$$k' = \frac{V_{\mathrm{S}}}{V_{\mathrm{M}}} \cdot \left(\frac{C_i^{\mathrm{S}}}{C_i^{\mathrm{M}}}\right)_{\mathrm{eq},\infty} = F \cdot H \tag{3}$$

where F is the phase ratio of the chromatographic system and H is Henry's constant. Under linear chromatography conditions, H represents the ratio of the analyte concentration in the stationary and the mobile phases at equilibrium. If the adsorption isotherm data show a linear relationship between q^* and C, the following equation can be used to describe this equilibrium[16]

$$q^* = H \cdot C = \frac{k'}{F} \cdot C \tag{4}$$

This isotherm is widely used in analytical chromatography, with satisfactory results.[21−24]

The effect of the mobile-phase composition on the adsorption coefficients was analyzed for the four BPs studied in this work. The corresponding adsorption isotherms were registered using five different mobile phase compositions. The volume fractions of the organic modifier were 0.4, 0.45, 0.5, 0.55, and 0.6. The experimental data points are plotted in Figure 3. It can be seen from this figure that, in the investigated concentration range (0 to 0.1) mM, linear relationships between the adsorbed concentration q^* and the mobile phase concentration C at equilibrium of the solute are obtained, and no significant deviations are observed. The H values calculated using eq 4 are listed in Table 2. The least-squares analysis performed on these data gives excellent regression coefficients in all cases, indicating that the linear isotherm is suitable to describe the adsorption process under the experimental conditions adopted in this study.

The variation of Henry's constants obtained for the BPs with the volume fraction of the organic modifier (MeOH) in the mobile phase is depicted in Figure 4. It can be seen from this figure that an increase in the volume fraction of MeOH produces a decrease in H values. To describe the effect of the mobile-phase composition on the adsorption process in liquid chromatography, two well-accepted physicochemical models are frequently used. Both models postulate the formation of an adsorbed monolayer; the surface is completely covered by adsorbed solute molecules. The Snyder model[25,26] assumes a homogeneous adsorbent surface and relates the retention volume (or the capacity factor k') with the concentration of organic modifier in the mobile phase, φ, by the following logarithmic equation

$$\log k' = \log k'_{\mathrm{W}} - m \cdot \varphi \tag{5}$$

where k'_{W} is the capacity factor extrapolated to pure water as the mobile phase. The other model was formulated by Soczewinski,[27,28] proposes discrete adsorption sites, and relates k' and φ in the following way where A has a constant magnitude. Parameters m and n, which represent the slopes of eqs 5 and 6, are constant for a given solute−eluent combination. Taking into account that eqs 5 and 6 relate the capacity factor with φ, and that k' is related to the H constant by eq 4, many authors have expressed this constant as a function of φ as follows[22,29]

$$\ln H = \ln f_1 - f_2 \cdot \varphi \tag{7}$$

and

$$\ln H = -f_2 \cdot \ln f_1 - f_2 \cdot \ln \varphi \tag{8}$$

In these equations f_1 and f_2 have their own physical significance. However, they are used in this work as empirical parameters to fit experimental data and model the system. The H values listed in Table 2 were related to φ according to eqs 7 and 8, and the Snyder and Soczewinski model parameters were determined, respectively. Figures 5 and 6 illustrate the graphical representation of eqs 7 and 8 for the analyzed BPs, and the parameter values obtained for both models are reported in Table 3.

To evaluate the efficiency of each model, the obtained parameter values were used to calculate Henry's constants of the four employed BPs using eqs 7 and 8. The calculated H constants were then compared to the ones determined experimentally. The comparison is depicted in Figure 7 for the Snyder model and in Figure 8 for the Soczewinski model. Both models seem to fit the experimental results fairly well. However, when the standard deviation between the experimental and the calculated H values is observed (Table 3), the Snyder model is slightly better to predict adsorption isotherms. It should be noticed that the standard deviation obtained for 2,4(OH)$_2$BP is very good for both cases. On the basis of these results, the Snyder model can be considered the most appropriate to describe the effect of the mobile phase composition on H constants for the BPs studied. For this reason, this model can be very useful to predict H values for BPs and for the design of chromatographic experiments with these compounds.

Substituent Effect. The substituent effect on the adsorption isotherms of 4(OH)BP, 4(MeO)BP, 2,4(OH)$_2$BP and 2(OH)4-(MeO)BP were also analyzed. Figure 3 shows the experimental adsorption isotherms fitted with the linear isotherm model. The H constants obtained by linear regression are all listed in Table 2. With this result, the following order for the strength of the adsorption process can be established:

$$H4(\mathrm{OH})\mathrm{BP} < H2,4(\mathrm{OH})_2\mathrm{BP} < H4(\mathrm{MeO})\mathrm{BP} < H2(\mathrm{OH})4(\mathrm{MeO})\mathrm{BP}$$

To evaluate this result, it is convenient to separate the analyzed BPs in two groups, with and without the OH in position 2, that is to say, 2,4(OH)$_2$BP and 2(OH)4(MeO)BP in one group and 4(OH)BP and 4(MeO)BP in another group. Considering the numerical values of H for the last group of BPs, it may be observed that 4(MeO)BP exhibits a stronger adsorption than 4(OH)BP on the C18 column used in the experiments. These BPs differ only in the substituent group in position 4; one possesses the methoxy, and the other one possesses the hydroxyl group. Carr et al. arrived at the conclusion that in reverse-phase chromatographic systems like the one employed in the present study (C18 column and hydroalcoholic mobile phase), the adsorption of organic compounds with polar substituents takes

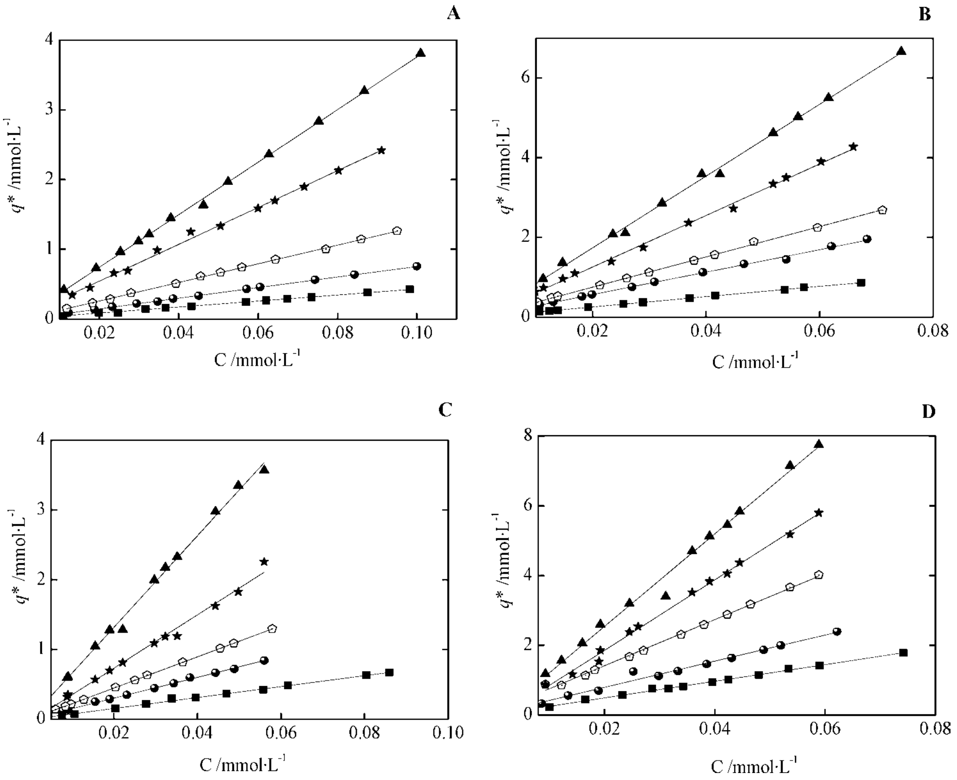

Figure 3. Adsorption isotherms of (A) 4(OH)BP, (B) 4(MeO)BP, (C) 2,4$(OH)_2$BP, and (D) 2(OH)4(MeO)BP at different methanol volume fractions in the mobile phase. Symbols: ■, 0.60 MeOH; ●, 0.55 MeOH; ⬠, 0.50; ★, 0.45 MeOH; ▲, 0.40 MeOH.

Table 2. Values of the Henry Constants (*H*) for the Studied BPs Obtained from the Adsorption Equilibrium Data with the Corresponding Standard Errors in Parentheses[a]

BP	φ	*H*	R^2
4(OH)BP	0.40	37.66 (0.36)	0.9989
	0.45	26.39 (0.49)	0.9962
	0.50	13.36 (0.11)	0.9992
	0.55	7.61 (0.04)	0.9996
	0.60	4.28 (0.07)	0.9973
4(MeO)BP	0.40	89.95 (1.31)	0.9976
	0.45	64.61 (1.01)	0.9973
	0.50	37.99 (0.32)	0.9992
	0.55	28.12 (0.43)	0.9974
	0.60	12.87 (0.07)	0.9966
2,4$(OH)_2$BP	0.40	65.65 (1.33)	0.9954
	0.45	38.20 (1.16)	0.9899
	0.50	22.38 (0.06)	0.9992
	0.55	14.97 (0.17)	0.9986
	0.60	7.83 (0.12)	0.9972
2(OH)4(OMe)BP	0.40	132.61 (3.90)	0.9905
	0.45	100.97 (1.90)	0.9961
	0.50	66.05 (1.13)	0.9968
	0.55	37.53 (1.43)	0.9843
	0.60	23.88 (0.50)	0.9951

[a] φ represents the volume fraction of methanol in the mobile phase, and R^2 the square regression coefficients of the least square linear fit.

$$\log k' = A - n \log \varphi \tag{6}$$

place with the polar group residing at the interface between the mobile and the stationary phases.[30] In addition, by means of linear solvation energy relationships (LSER) these authors have determined that in HPLC systems the hydrogen-bond basicity ($\sum\beta_2^H$) and the molecular volume (V_x) of the solutes are the main factors governing the retention using MeOH−water mobile phases.[31] Solutes with hydrogen-bond acceptor ability (greater $\sum\beta_2^H$) are significantly less retained, and increasing the solute size leads to increased retention. It is evident that both OH and MeO groups have an oxygen atom able to accept hydrogen bonds (HB) from solvent molecules. However, a compound with an OH group exhibits a slightly higher value of $\sum\beta_2^H$ than the same compound with a MeO group. For example, the $\sum\beta_2^H$ value for phenol (hydroxybenzene) is 0.30 and for anisole (methoxybenzene) is 0.29.[32] Although this difference is small, the magnitude of the fitting coefficient for $\sum\beta_2^H$ in the LSER is great,[31] so small changes in the solute $\sum\beta_2^H$ values lead to important changes in the retention of the solute. The stronger adsorption of 4(MeO)BP with respect to 4(OH)BP can be explained in view of this argument.

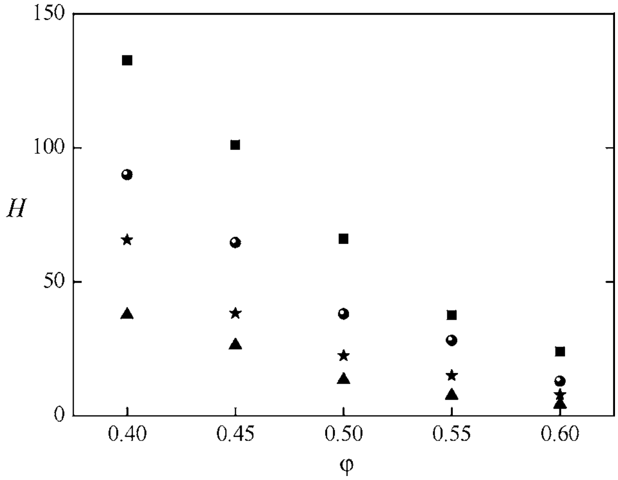

Figure 4. Variation of the Henry constants with the methanol volume fraction φ in the mobile phase. Symbols: ▲, 4(OH)BP; ★, 2,4$(OH)_2$BP; ●, 4(MeO)BP; ■, 2(OH)4(MeO)BP.

On the other hand, 2,4$(OH)_2$BP and 2(OH)4(MeO)BP present the same trend, the *H* constant of the BP substituted with the *p*-methoxy group is higher than the *p*-hydroxylated BP. Moreover, with the same substituent in position 4, the numerical value of *H* is higher for the *o*-hydroxylated-BP than the respective

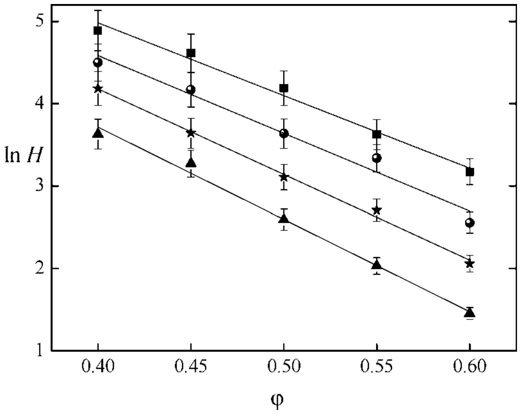

Figure 5. Graphical representation of the Snyder model (eq 7) for the variation of *H* with φ in the mobile phase. Error bars of 5 %. Symbols: ▲, 4(OH)BP; ★, 2,4(OH)$_2$BP; ●, 4(MeO)BP; ■, 2(OH)4(MeO)BP.

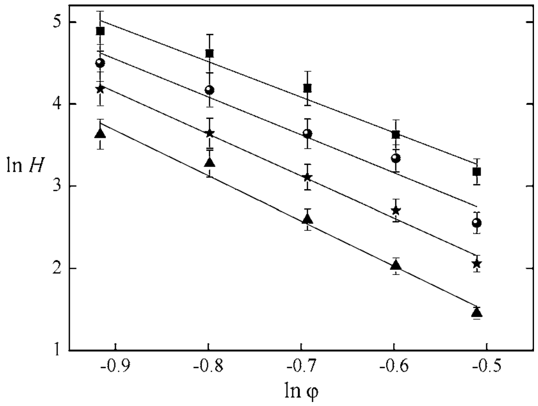

Figure 6. Graphical representation of the Soczewinski model (eq 8) for the variation of *H* with φ in the mobile phase. Error bars of 5 %. Symbols: ▲, 4(OH)BP; ★, 2,4(OH)$_2$BP; ●, 4(MeO)BP; ■, 2(OH)4(MeO)BP.

value of the BP without the OH in position 2. In previous work, Blanco et al. determined that the most stable conformation for 2(OH)BP (X_2=OH and X_4=H in Figure 1) presents an intramolecular HB between the OH group and the carbonyl group.[33] The variation of the Henry's adsorption constants may be explained in terms of this structural feature of the *o*-hydroxylated BPs. If the adsorption process of the analyzed BPs takes place with the *p*-substituent residing at the interface between the mobile and the stationary phase, the carbonyl group interacts directly with the mobile phase. The intramolecular HB mentioned above decreases the polarization of the carbonyl group and decreases HB acceptor ability. As a result, the interactions of the molecule with the polar mobile phase decrease because of an impediment of the mobile-phase molecules (H_2O and MeOH) to form intermolecular HB with the carbonyl group of the BPs.

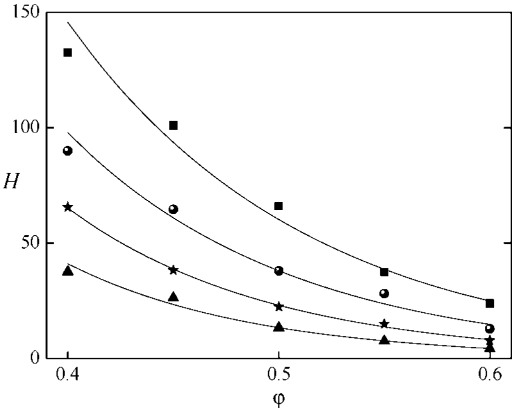

Figure 7. Comparison of experimental (points) and predicted (line) *H* constants using the Snyder model. Symbols: ▲, 4(OH)BP; ●, 4(MeO)BP; ★, 2,4(OH)$_2$BP; and ■, 2(OH)4(MeO)BP.

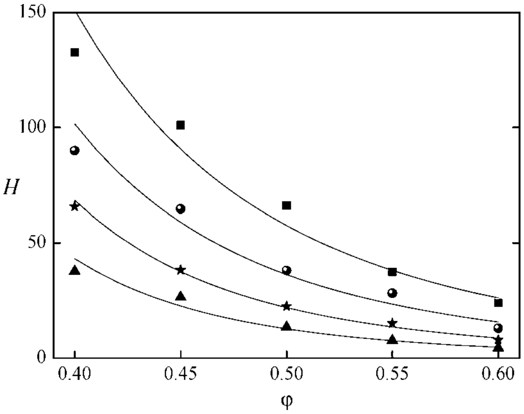

Figure 8. Comparison of experimental (points) and predicted (line) *H* constants using the Soczewinski model. Symbols: ▲, 4(OH)BP; ●, 4(MeO)BP; ★, 2,4(OH)$_2$BP; and ■, 2(OH)4(MeO)BP.

In addition, solute size can be estimated by McGowan's molecular volume V_x[34] which can be calculated by the addition of the atomic volumes of all of the atoms in the molecule and subtraction, from the total, of 6.56 $\text{mL}\cdot\text{mol}^{-1}$ for each bond regardless of whether it is a single, a double, or a triple bond.[35] The calculated values of V_x for the analyzed BPs are 156.8 $\text{mL}\cdot\text{mol}^{-1}$ for 4(OH)BP, 162.8 $\text{mL}\cdot\text{mol}^{-1}$ for 2,4(OH)$_2$BP, 171.2 $\text{mL}\cdot\text{mol}^{-1}$ for 4(MeO)BP, and 177.2 $\text{mL}\cdot\text{mol}^{-1}$ for 2(OH)4(MeO)BP. It can be seen that the *H* constants of the BPs increase as the V_x parameter increases. This fact indicates that the molecular volume also affects the adsorption of the BPs; the greater the V_x value, the greater the adsorption constants.

The variation of the adsorption constants obtained for the analyzed BPs can be explained in terms of a combined effect between the interactions of the *p*-substituent and the carbonyl group with the polar mobile-phase molecules and the molecular

Table 3. Fitting Parameters of the Equations Corresponding to the Snyder and the Soczewinski Model Describing the Dependences of the Henry's Constants on the Volume Fraction of MeOH in the Mobile Phase φ[a]

	Snyder model				Soczewinski model			
BP	$f_1\cdot10^{-3}$	f_2	σ	R^2	f_1	f_2	σ	R^2
4(OH)BP	3.601	11.186	2.58	0.9908	1.260	5.492	3.88	0.9778
4(MeO)BP	4.272	9.441	5.72	0.9658	0.919	4.617	8.23	0.9432
2,4(OH)$_2$BP	4.144	10.379	0.95	0.9948	1.093	5.109	2.00	0.9885
2(OH)4(MeO)BP	4.997	8.837	9.34	0.9827	0.784	4.326	13.27	0.9624

[a] R^2 are the square regression coefficients of the least square linear fit, and σ is the standard deviation. σ is calculated as $\sigma = [\sum_{i=1}^{n}(H^{\text{calc}} - H^{\text{exp}})^2/(n - p)]^{1/2}$ where H^{calc} are Henry's constants predicted by the model, H^{exp} are the experimental Henry's constants, *n* the number of data points, and *p* the number of fitted parameters.

volume of the BPs. The presence of a substituent in position 4 in BPs with higher HB acceptor ability will disfavor the adsorption process enhancing the interaction with the mobile phase, and the presence of the OH group in position 2 will favor the adsorption process due to the formation of the intramolecular HB bond with the carbonyl group, preventing intermolecular HB formation with the mobile phase. For the analyzed BPs, the first effect seems to be more important than the presence of the *o*-hydroxyl group, taking into account that the difference between the *H* values of 4(OH)BP and 4(MeO)BP is greater than the difference between the *H* values of 4(OH)BP and 2,4(OH)$_2$BP. Moreover, the increase of Henry's constants with the McGowan's molecular volume is almost linear. These observations support the results obtained by Carr et al.[30−32]

Conclusion

Adsorption isotherms of 4(OH)BP, 4(MeO)BP, 2,4(OH)$_2$BP, and 2(OH)4(MeO)BP on a Luna C18 column were measured by frontal analysis experiments at different mobile-phase compositions with various methanol volume fractions. Linear relationships between the adsorbed amount of solute (q^*) and the mobile-phase concentration C of the solute were obtained in the investigated concentration range ($\leq$ 0.1 mM) for all of the registered isotherms. The linearity observed allowed us to determine Henry's constant for each BP and each mobile phase composition. The results indicate that Henry's constants change notably with the mobile-phase composition. The Snyder and the Soczewinski models were used to analyze this variation. The parameters for these models were determined, and *H* constants were then calculated with these parameters and compared with the ones observed experimentally. Both models predict the dependence of *H* with the MeOH volume fraction of the mobile phase fairly well, although the Snyder model exhibits better values of standard deviation. This model, under the MeOH volume fraction range used, is the most suitable for the description of the mobile phase effect on *H* constant for the analyzed BPs. In addition, the determined *H* value of 2(OH)4(MeO)BP was the highest, followed by 4(MeO)BP and 2,4(OH)$_2$BP, while 4(OH)BP presents the smallest values. This variation may be explained in terms of the HB acceptor ability of the *p*-substituent. Higher HB acceptor ability leads to smaller *H* values, and the presence of the *o*-hydroxyl group forming an intramolecular HB with the carbonyl group in the BP increases the *H* values with respect to those BPs without the OH group. The first effect is more significant on the variation of *H* than the latter one. Moreover, an increase in McGowan's molecular volume leads to an almost linear increase in the *H* constant.

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