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Journal of Chromatography A, 1029 (2004) 21–28
JOURNAL OF CHROMATOGRAPHY A
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Saccharose effects on surface association of phenol derivatives with porous graphitic carbon

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Received 25 February 2003; received in revised form 27 November 2003; accepted 5 December 2003

Abstract

In this paper, the effect of saccharose on the association of phenol derivatives on both the porous graphitic carbon (PGC) surface and the C18 stationary phase and for two methanol fractions (v/v) in the mobile phase is described. A novel approach based on an extended Langmuir distribution isotherms was used. The results demonstrated that: (i) the saccharose can be adsorbed on the PGC surface; (ii) the phenol derivatives can be associated with saccharose adsorbed on the PGC surface; and (iii) the saccharose do not interact with the C18 stationary phase. This was confirmed by the thermodynamic data and the Wyman equation parameters.

Keywords: Adsorption; Langmuir distribution isotherm; Saccharose; Phenol; Porous graphitic carbon

1. Introduction

Many papers have examined the retention process in reversed phase system (RPLC). Adsorption-like (solvophobic) and partitioning-like models developed successively by Horvath et al. [1] and Dill [2] constitute the two main mechanistic aspects of solute retention. The distinction between these two models is related to the role of the stationary phase [3]. However, the variation in retention in RPLC when the mobile phase composition varies are mainly dominated by the modification in the solute–mobile phase interaction. For an hydrophobic-organic eluent, it has been demonstrated by Carr et al. [4] that the decreasing retention of weak polar solutes as the volume fraction of organic modifier increased was due to the decrease in the strength of the hydrophobic effect. Recently, the scientific attention has surrounded the use of more polar stationary phase as the porous graphitic carbon (PGC) due to their specific interaction with solutes. The PGC column packing has shown the remarkable ability to separate isomers or closely related compounds [5,6]. Furthermore, PGC has a high chemical and physical stability, enabling repeated use without loss of performance and reproducibility [7,8]. It is an extremely strong adsorbent [8] due to the existence of large dispersion forces between the solute and rigid planar graphite surface. PGC exhibits high retention for polar solutes mainly caused by specific interactions with the π electronic structure of graphite, the retention and selectivity mechanisms are still being investigated [9–11]. More recently, Guillaume's group has investigated the role of lithium perchlorate on the phenol derivative retention mechanism on the PGC surface [10]. It has been demonstrated that it was interesting to take into account the possible adsorption of perchlorate on the PGC surface. In this study, the saccharose effect on the retention process of phenol derivatives (i.e. phenol, hydroquinone, catechol, hydroxyquinone, resorcinol, 1,4-benzoquinone, 4-methoxyphenol, 4-ethoxyphenol, 3-nitrophenol) on both the PGC surface and the C18 stationary phase was investigated using: (i) a new approach based on an extended Langmuir distribution isotherm concept; and (ii) a thermodynamic analysis.

2. Materials

2.1. Apparatus

HPLC was performed with an Hitachi L7100 pump (Merck, Nogent-sur-Marne, France), a Rheodyne (Interchim, Montluçon, France) 7125 injection valve fitted with

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doi:10.1016/j.chroma.2003.12.017

a 20 μl sample loop, and a Hitachi L4500 diode-array detector. The porous graphitic column used was a Shandon (100 mm × 4.6 mm i.d., 7 μm particule size) model Hypercarb S column (Shandon, Eragny/Oise, France). The RP18 column used was a Liochrocart (125 mm × 4 mm) model (Montluçon, France). In the two type of columns, the temperatures were controlled by means of an Interchim TM 701 oven. The mobile phase flow-rate was 0.8 ml/min and the detection wavelength 254 nm.

2.2. Solvents and samples

Methanol was used without further purification (Merck). Water was obtained from an Elgastat water purification system (Odil, Talant, France) fitted with a reverse-osmosis cartridge. Saccharose was supplied by Prolabo (Paris, France). All the phenol derivatives were obtained from Sigma–Aldrich (Saint Quentin, France). Fresh samples were prepared daily at a concentration of 20 mg/l. To examine the concentration dependency of the solute retention, corresponding to the binding capacity of PGC surface, retention measurements were related to varying amounts of injected solute. Solute samples were prepared at different concentrations in the mobile phase: 10–50 μg/ml. Twenty microlitres of each solute were injected in triplicate and retention times measured. The plots of retention factor exhibited a plateau at sample concentrations <35 μg/ml followed by a small decreased at higher solute concentrations. Therefore, each solute was injected at a concentration of 20 μg/ml when the retention was sample concentration independent, i.e. in linear elution conditions.

The column void volume was determined by injecting pure bidistilled water with a methanol mobile phase. As the experiments on PGC by Clarot et al. [12], sodium nitrate (Merck) was used as a dead marker. The mobile phase consisted of a 0.7 and 0.8 (v/v) methanol fraction in the methanol–water mixture. The range of saccharose concentration was 0.01–0.1 M.

2.3. Specific operating condition for the Langmuir approach

For each phenol derivative, the equilibration of the column was carried out with 15 different concentrations of solute (0–7 mM) in each mobile phase used to obtain a stable detection. Twenty microlitres of the most concentrated phenol derivative samples were injected three times and the retention time was measured at $T = 30\,^{\circ}\mathrm{C}$.

2.4. Specific operating condition for the thermodynamic study

2.4.1. Temperature study

Compound retention factors were determined over the temperature range 20–50 °C. The chromatographic system was left to equilibrate at each temperature for at least 1 h before each experiment. To study this equilibration, the retention time of 4-ethoxyphenol was measured after 22–24 h. The maximum relative difference between retention times of this compound was never more than 0.7%, meaning that after 1 h the chromatographic system was sufficiently equilibrated for use. All the solutes were injected three times at each temperature and for each saccharose concentration. For each experimental condition, the asymmetry factor of all peaks calculated from measurements made at 50% of the total peak height was in the range $1.00 < \mathrm{As} < 1.10$ showing that the peaks had a Gaussian shape. The solute retention times were also determined at the maxima of the chromatographic peaks.

2.4.2. Saccharose–PGC surface binding studies

In order to eliminate the possible saccharose binding on the PGC surface, the column was allowed to equilibrate for 15 min with pure methanol mobile phase and again 10 min with pure water mobile phase before each change of saccharose concentration. In order to verify if the washing was sufficiently effective to break the possible saccharose–PGC interactions, the compound retention time of nitrophenol was measured when the saccharose concentration in the mobile phase was nil. Then, the chromatographic system was allowed to equilibrate for 1 h with a mobile phase containing 0.1 M saccharose concentration. The column was then washed as described previously, and the nitrophenol retention time was again measured. The maximal relative difference in the retention times of nitrophenol was only 0.03% showing that the washing was sufficient to eliminate the saccharose binding on the PGC surface.

3. Theory

3.1. Langmuir approach

The non-linear chromatography determines the sample adsorption isotherms using the perturbation technique. Indeed, the perturbation technique allowed the determination of adsorption isotherms by measuring the retention times of small sample sizes injected onto a column equilibrated with sample solutions at different concentration levels. The column used for the determination of the isotherm is first equilibrated with a solution containing the sample dissolved in a non-adsorbable solvent. Then a small sample volume containing different (lower or higher) concentrations of the sample is injected onto the column. After the injection, the equilibrium condition is disturbed and a perturbation wave arise which migrate along the column. When such a wave reaches the column outlet, a negative or a positive peak is registered by the detector, depending on whether the concentrations of the sample compounds injected are higher or lower than their equilibrium concentrations at the start of the experiment. The well-known Langmuir theoretical approach relates the total concentration of solute in the

stationary phase (C_s) and that in the mobile phase (C_m) by the following equation [13,14]:

$$C_s = \frac{\alpha K C_m}{1 + K C_m} \quad (1)$$

where α is the column saturation capacity and K the adsorption constant between the solute and the stationary phase. The solute retention factor k was directly proportional to the slope of its adsorption isotherm and can be thus given by the following equation [13,14]:

$$k = \frac{\phi \alpha K}{(1 + K C_m)^2} = \frac{\bar{k}}{(1 + K C_m)^2} \quad (2)$$

where ϕ is the column phase ratio (volume of the stationary phase divided by the volume of the mobile phase) and $\bar{k}$ (equal to $\phi\alpha K$) the solute apparent retention factor (i.e. retention factor when the solute concentration in the mobile phase was nil). For a reversed phase chromatography, Melander and Horvath [15] suggested the expression of ϕ per unit surface area adsorbent (m^2). Davidov et al. [16] divided the mass of material (g) in the column by the column dead volume (cm^3), as is usual in chromatography. The volume in the mobile phase was determined from the weight differences of the column when filled with solvents of different densities (methanol and chloroform) [17]. Since the technique data for the Hypercarb column were available, ϕ can be calculated. According to commercial data and confirmed by Clarot et al. [12], for the porous graphitic column (Shandon), $\phi = 0.29$.

Then for each saccharose concentration in the bulk solvent, by the plot of the k value versus the solute concentration in the bulk solvent, the constant $\bar{k}$ can be determined using Eq. (2). These initial relations of the Langmuir theory are limited by the fact that the experimental data are evaluated only through the assumption that the saccharose do not modified the solute binding site. However, if the solute bound either on the free PGC surface (no saccharose adsorbed on the PGC surface (adsorption constant K_1, column saturation capacity α_1) or either on saccharose adsorbed on the PGC surface (adsorption constant K_2, column saturation capacity α_2), then the sample concentration C_s in the PGC surface was given by the equation [13,14]:

$$C_s = \frac{\alpha_1 K_1 C_m}{1 + K_1 C_m} + \frac{\alpha_2 K_2 C_m}{1 + K_2 C_m} \quad (3)$$

Then, in this case the solute retention factor directly proportional to the slope of its adsorption isotherm is given by the following equation:

$$k = \phi \left(\frac{\alpha_1 K_1}{(1 + K_1 C_m)^2} + \frac{\alpha_2 K_2}{(1 + K_2 C_m)^2} \right) = \frac{\bar{k}_1}{(1 + K_1 C_m)^2} + \frac{\bar{k}_2}{(1 + K_2 C_m)^2} \quad (4)$$

where $\bar{k}_1$ ($=\phi\alpha_1 K_1$) and $\bar{k}_2$ ($=\phi\alpha_2 K_2$) are the apparent retention factor (retention factor when the solute concentration in the mobile phase was nil), respectively, of the phenol derivative association on the free PGC stationary phase and on the saccharose adsorbed on the PGC surface. Then, using a non-linear regression analysis, by studying the variation of the k values versus the sample concentration in the mobile phase, the apparent retention factors $\bar{k}_1$ and $\bar{k}_2$ can be calculated.

3.2. Thermodynamic study

For these experiments, 20 μM of the phenol derivative were injected throughout the PGC column. The mobile phase consisted of a 0.7 and 0.8 (v/v) methanol fraction in the methanol–water mixture. Much information on the retention mechanism, in a HPLC system, may be gained by examining the temperature dependence of the analyte elution. The Gibbs free energy of the solute molecule transfer ΔG° from the mobile to the PGC surface can be linked to its equilibrium constant K with the following equation [15]:

$$\ln K = \frac{-\Delta G^\circ}{RT} \quad (5)$$

where R is the gas constant and T the column temperature. The solute retention factor (k') can be linked with K by $k' = \phi K$, where ϕ is the column phase ratio. As well, k' could be given by the equation:

$$\ln k' = \frac{-\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln \phi \quad (6)$$

where ΔH° and ΔS° were the enthalpy and entropy of the solute transfer from the mobile to the PGC surface. With an invariant retention mechanism over the temperature range being studied, the enthalpy of transfer ΔH° remained constant and a plot of $\ln k'$ in relation to $1/T$, which is commonly described as a van't Hoff plot, led to a straight line with an enthalpic slope and entropic origin.

A further thermodynamic approach to the analysis of physicochemical data is enthalpy–entropy compensation. This investigation tool has been previously used in chromatographic procedures to analyse and compare the retention mechanism for a group of compounds. This enthalpy–entropy compensation can be described thanks to the following equation [16–20]:

$$\Delta H^\circ = \beta \Delta S^\circ + \Delta G^\circ_\beta \quad (7)$$

where ΔG°_β is the free Gibbs energy of a physicochemical interaction at a compensation temperature β, ΔH° and ΔS° are the corresponding standard enthalpy and entropy, respectively. According to Eq. (7), when enthalpy–entropy compensation is observed for a compound group in a particular chemical transformation, all of the compounds have the same ΔG°_β at temperature β. For example, if enthalpy–entropy compensation is observed on liquid chromatography for a compound group, all the solutes will have the same net retention at the temperature β, although their temperature dependences may differ.

Combining Eqs. (6) and (7), the following equation is obtained [16–20]:

$$\ln k' = \frac{-\Delta H^\circ}{R}\left(\frac{1}{T} - \frac{1}{\beta}\right) - \frac{\Delta G^\circ_\beta}{R\beta} + \ln \phi \quad (8)$$

Eq. (8) shows that if a plot of $\ln k'_T$ versus ΔH° evaluated at a constant temperature T, is a linear function, a compensation temperature β can be therefore evaluated from the slope [20]. As Ranatunga et al. have been recently demonstrated, the similarity of the values for the compensation temperature β showed only that the relative contributions of enthalpy and entropy to the overall free energy are the same in the two processes [21]. If, however, the compensation temperature are different for two processes, then one can conclude that the mechanism for the two processes must be different.

4. Result and discussion

4.1. Saccharose concentration effect (x) on the retention mechanism on the PGC surface and the C18 stationary phase (with 0.7 (v/v) methanol fraction in the mobile phase)

For each phenol derivative concentration and saccharose concentration in the mobile phase (0.7 (v/v) methanol fraction), the most concentrated sample was injected on both the PGC and the C18 stationary phase and its retention time (k) were determined (i.e. Langmuir theory). The variation coefficient of the k values were always <0.3% whatever the stationary phase used, indicating a high reproducibility and a good stability for the two chromatographic systems (i.e. PGC and C18 stationary phase). For a given stationary phase, the variation of the k values versus the phenol derivative concentration in the bulk solvent was similar for all phenol derivatives. An example of plot for methoxyphenol for both the PGC surface and the C18 stationary phase is given in Fig. 1. Using a non-linear regression and for each saccharose concentration, the non-linear coefficients of Eq. (2) were determined for all phenol derivatives. Table 1 gives the values obtained for methoxyphenol for all the saccharose concentrations in the mobile phase and for the two stationary phases (i.e. PGC and C18). On a C18 stationary phase, the r^2 values of Eq. (2) were always >0.989 showing that the use of the simplified relation of Langmuir theory (Eq. (2)) was sufficient to described accurately the association mechanism of phenol derivative with the C18 stationary phase (Table 1). It corresponded to the case in which the saccharose addition do not modified the C18 stationary phase-phenol derivative association (i.e. phenol derivatives bound only on the free C18 stationary phase). The apparent retention factor values, $\bar{k}_{C18}$, obtained for all the phenol derivatives when the saccharose concentration was equal to 0.01 M were given in Table 2. On the PGC surface, values of non-linear coefficient of Eq. (2) confirmed that the simplified Langmuir equation was not sufficient accurate to fit the experimental data (Table 1, $r^2_{PGC\,0.7}$). Then, for the PGC surface, using a non-linear regression, the $\bar{k}_{1,PGC}$ and $\bar{k}_{2,PGC}$ values (i.e. the apparent retention factor, respectively, of the phenol derivative association on the free PGC surface and on the saccharose adsorbed on the PGC stationary phase) were determined from Eq. (4) at all the saccharose concentrations in the bulk solvent and for all the phenol derivatives. The non-linear coefficient results proved that the two-order Langmuir model described accurately the binding mechanism of

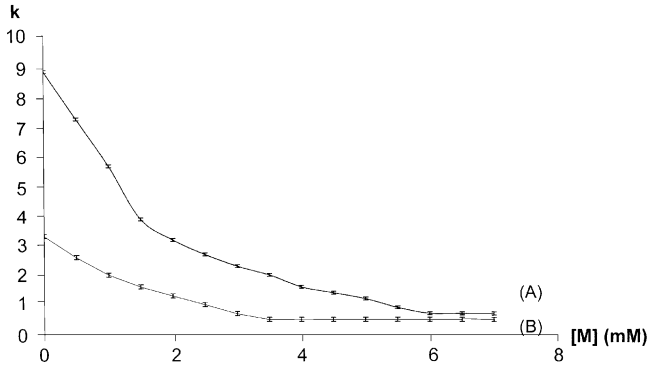

Fig. 1. Plot of the k values vs. the methoxyphenol concentration ([M]) in the bulk solvent at a column temperature equal to 20 °C and for $x = 0.01$ M for the PGC surface (A) and the C18 stationary phase (B).

Table 1
Non-linear coefficient of Eq. (2) for the C18 stationary phase and non-linear coefficient of Eqs. (2) and (4) for the PGC surface

[M]	r^2_{C18}	$r^2_{PGC\,0.7}$ (Eq. (2))	$r^2_{PGC\,0.7}$ (Eq. (4))	$r^2_{PGC\,0.8}$ (Eq. (2))
0.010	0.989	0.725	0.987	0.992
0.015	0.997	0.722	0.999	0.996
0.020	0.989	0.727	0.989	0.996
0.030	0.991	0.715	0.999	0.994
0.040	0.999	0.721	0.996	0.993
0.050	0.992	0.718	0.992	0.994
0.060	0.995	0.719	0.997	0.994
0.070	0.998	0.721	0.991	0.997
0.080	0.997	0.715	0.992	0.998
0.100	0.996	0.720	0.989	0.996

Table 2
The $\bar{k}_{C18}$ values determined from Eq. (2) for all the phenol derivatives at $x = 0.01$ M

Solute molecule	$\bar{k}_{C18}$
Benzoquinone	2.52
Catechol	2.87
Ethoxyphenol	6.66
Hydroquinone	2.99
Hydroxyquinone	2.71
Methoxyphenol	3.34
Nitrophenol	8.52
Phenol	3.12
Resorcinol	2.65

Standard deviation <0.06.

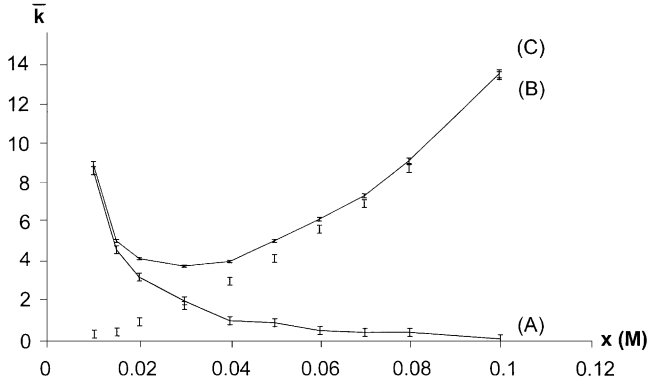

Fig. 2. Plots of the $\bar{k}_1$ (A), $\bar{k}_2$ (B) and $\bar{k}$ ($=\bar{k}_1 + \bar{k}_2$) (C) values vs. the saccharose concentration in the bulk solvent (x) for the PGC surface.

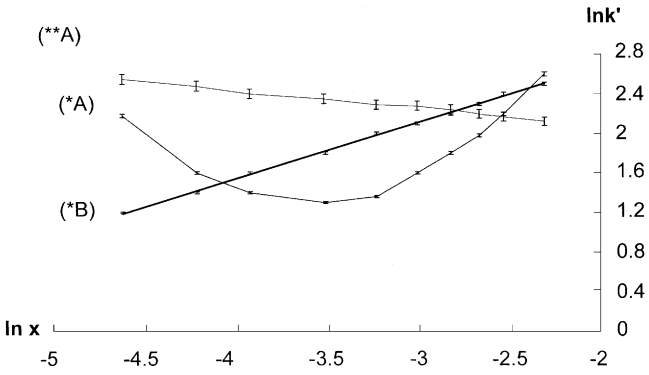

Fig. 3. The plot of $\ln k'$ vs. $\ln x$ at $T = 30\,^{\circ}\mathrm{C}$ for nitrophenol and a 0.7 (v/v) (*) and 0.8 (v/v) (**) methanol fraction in the mobile phase on the PGC surface (A) and the C18 stationary phase (B).

phenol derivatives with the PGC surface (Table 1). This showed that: (i) the saccharose interacted on the PGC surface; and (ii) that phenol derivative can bound on the saccharose adsorbed on the PGC surface. Several authors have already shown the capacity of saccharose to bind on weak polar surface by van der Walls interactions [22,23]. More recently, Clarot et al. have been demonstrated that cyclodextrin can interacted by polar interactions on the PGC surface [12]. The $\bar{k}_{1,\mathrm{PGC}}$ and $\bar{k}_{2,\mathrm{PGC}}$ values were plotted against the saccharose concentration (x) in the bulk solvent. Fig. 2 presents the plot obtained for methoxyphenol on the PGC surface. Similar plot was obtained for the other phenol derivatives. At low saccharose concentration in the bulk solvent ($x < x_c = 0.03\,\mathrm{M}$), the $\bar{k}_{1,\mathrm{PGC}}$ values decreased whereas the $\bar{k}_{2,\mathrm{PGC}}$ values increased with x, but the apparent retention factor corresponding to the phenol derivative association on the free PGC surface ($\bar{k}_{1,\mathrm{PGC}}$) was always higher than the one obtained for the phenol derivative bound to the saccharose adsorbed on the PGC surface (for example, at a saccharose concentration equal to 0.02 M and for the methoxyphenol compound, $\bar{k}_{1,\mathrm{PGC}} < \bar{k}_{2,\mathrm{PGC}}$, Fig. 2). This demonstrated that under x_c, the phenol derivative association on the saccharose adsorbed on the PGC surface was negligible in relation to the phenol derivative bound to the free PGC stationary phase. To confirmed these results, the plot $\ln k'$ values versus $\ln x$ in the bulk solvent was drawn for all the phenol derivatives. Fig. 3 gives the curve obtained for nitrophenol at 30 °C. The dependence of $\ln k'$ on $\ln x$ was similar for all phenol derivatives on PGC. In accordance with the previous results obtained with the Langmuir theory, the plots showed a greater curvature at a critical x_c value around 0.03 M. At lower saccharose concentration ($x < x_c$), the affinity decrease was due to a maximal competition effect between the saccharose and the solute molecules to bind on the PGC surface. The saccharose contribution which normally increased the surface tension of bulk solvent was largely counterbalanced by the saccharose specific polar retention, i.e. direct saccharose competition phenomena with phenol solutes to bind on the PGC (effects (i) and (ii)). At high saccharose concentration ($x > x_c$), the $\bar{k}_{2,\mathrm{PGC}}$ values were always higher than the $\bar{k}_{1,\mathrm{PGC}}$ values demonstrating well that phenol derivative bound on the saccharose adsorbed on the PGC surface rather than on the free PGC stationary phase. The values of the retention factor k' were also determined by a linear chromatographic approach (i.e. see thermodynamic approach). In order to compare the results obtained on the PGC surface and the one on the C18 stationary phase, the plot $\ln k'$ versus $\ln x$ was drawn also for the C18 stationary phase (Fig. 3). In accordance with the Langmuir theory, on a C18 stationary phase, the dependence of $\ln k'$ versus the saccharose concentration was linear. On a C18, the solute retention increased when x increased was related to the salting-out effect of the saccharose. The increase of surface tension concomitant to the enhancement in the cavitation energy determined a facilitation in the interaction between the phenol derivative and the stationary phase [23].

In order to gain further insight into these dual mechanisms, a thermodynamic study of the solute transfer between the bulk solvent and the PGC surface was undertaken. The calculated thermodynamic data can be expressed by [10]:

$$\Delta H^{\circ} = H^{\circ}_{\mathrm{PGC}} - H^{\circ}_{\mathrm{m}} \tag{9}$$

$$\Delta S^{\circ} = S^{\circ}_{\mathrm{PGC}} - S^{\circ}_{\mathrm{m}} \tag{10}$$

where H°_{PGC}, H°_{m}, S°_{PGC} and S°_{m} are, respectively, the molar enthalpy and entropy of the solute associated with the PGC surface and the bulk solvent. The plots ΔH° and ΔS° versus $\ln x$ were drawn for all solutes and two different plot profiles were observed (Figs. 4 and 5). For the methoxyphenol, ethoxyphenol, nitrophenol, the plots could be divided into two domains confirming the existence of a double retention mechanism on the PGC surface. At the beginning, for a saccharose concentration under the critical value ($x_c = 0.03$), the effect of saccharose on the surface tension was negligible compared with the competition for the association with the PGC surface between the saccharose and the phenol derivative molecules. The increase of ΔH° and ΔS°

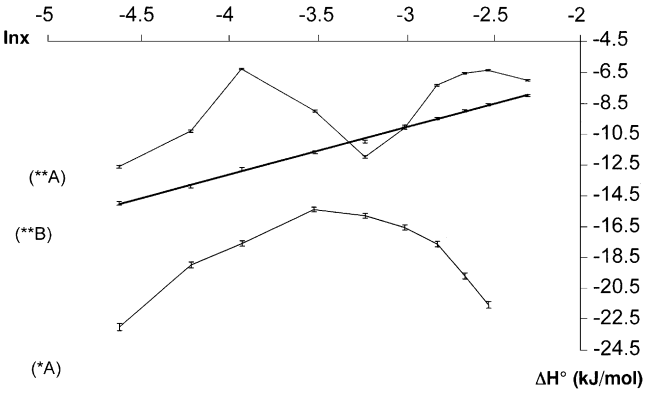

Fig. 4. ΔH° (kJ/mol) vs. $\ln x$ for nitrophenol (*) and hydroquinone (**) compound for a 0.7 (v/v) methanol fraction in the mobile phase on the PGC surface (A) and the C18 stationary phase (B).

values was principally due to this dual effect. If the saccharose concentration increased, the surface tension of: (a) the bulk solvent; and (b) the PGC surface increased. Thus, over x_c, when the saccharose concentration in the mobile phase increased, the effect (b) was dominant and the solute molar enthalpy and entropy associated with the PGC surface, i.e. H°_{PGC} and S°_{PGC} decreased strongly, leading to a decrease in the solute transfer thermodynamic data ΔH° and ΔS° (Eqs. (9) and (10)) (Figs. 4 and 5). For the 1,4-benzoquinone, hydroxyquinone and hydroquinone, the plots presented two break delimited three distinct areas (Figs. 4 and 5). At the beginning ($x < x_c$) as previously, the competition between the molecules and the saccharose for association on the PGC surface led to an increase in the thermodynamic data. Then, effect (b) was preponderant to a second critical value ($x'_c = 0.06$), H°_{PGC} and S°_{PGC} decreased leading a decrease of ΔH° and ΔS°. Over x'_c, the effect (a) was dominant, H°_m and S°_m decreased and thus ΔH° and ΔS° values increased (Eqs. (9) and (10)) (Figs. 4 and 5). If an RP18 stationary phase is used instead of the PGC, the effect (b) will always be hidden whatever the compound. Consequently, the interaction mechanism of these phenol derivative molecules is dependent of saccharose concentration in the mobile phase. The existence of saccharose adsorption on the PGC surface and the possible association of phenol with the adsorbed saccharose is then confirmed. In order to confirm the explanation given on the solute retention on both the PGC and the C18 stationary phase, an enthalpy–entropy compensation was also investigated for both the PGC surface and the C18 stationary phase and for each phenol derivative at 30 °C. Contrary to the C18 stationary phase where linear plot was observed in the entire saccharose concentration range, on the PGC surface, two plots $\ln k'$ versus ΔH° can be distinguished (i.e. for the region I ($x < x_c$) and for the region II ($x > x_c$) (Fig. 6) confirming a specific solute retention mechanism on the PGC surface. The regression linear for the two stationary phases were:

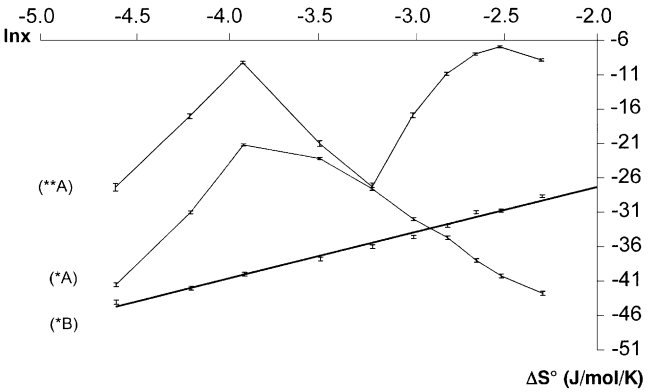

Fig. 5. ΔS° (J/(mol K)) vs. $\ln x$ for nitrophenol (*) and hydroquinone (**) for a 0.7 (v/v) methanol fraction in the mobile phase on the PGC surface (A) and the C18 stationary phase (B).

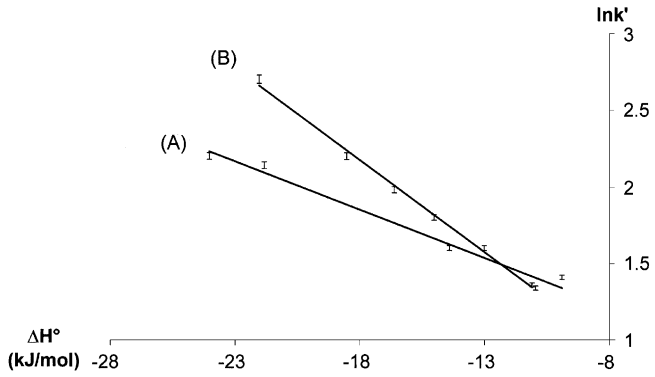

Fig. 6. The plot of $\ln k'$ vs. ΔH° (kJ/mol) when $x < x_c$ (A) and $x > x_c$ (B) for the methoxyphenol on the PGC surface at 20 °C.

- On the PGC surface:

$$x < x_c \text{ (i.e. region I)}: \quad \ln k' = -0.0618\,\Delta H^\circ + 1.0901 \quad (r = 0.998)$$

$$x > x_c \text{ (i.e. region II)}: \quad \ln k' = -0.1182\,\Delta H^\circ + 0.0366 \quad (r = 0.996)$$

- On the C18 stationary phase in the entire concentration range:

$$\ln k' = -0.1691\,\Delta H^\circ + 5.2084 \quad (r = 0.997)$$

According to these regression analyses, the following conclusions can be drawn:

- For the PGC surface, the slopes of the linear plots were different for the two regions ($\beta_{PGC,\text{region I}} = 357\,K \neq \beta_{PGC,\text{region II}} = 340\,K$) confirming well a change on the phenol derivative–PGC binding mechanism in these two regions [21].
- On the C18 stationary phase, the plot was linear for all the salting-out agent concentrations in the bulk solvent showing that the phenol derivative retention process on the C18 stationary phase was independent of the saccharose concentration in the bulk solvent.

4.2. Water concentration effect on the retention mechanism

To gain further insight into the saccharose influence on the PGC retention, the saccharose effect for another methanol fraction in the bulk solvent on the mobile phase was analysed (a 0.8 (v/v) methanol fraction) with the two chromatographic methods (i.e. thermodynamic and Langmuir approach). Using a non-linear regression and for each saccharose concentration, the non-linear coefficients of Eq. (2) were determined for all phenol derivatives ($r^2_{\mathrm{PGC\,0.8}}$). Table 1 gives the values obtained for methoxyphenol for all the saccharose concentrations in the mobile phase and for the PGC stationary phases. For the PGC stationary phase, the r^2 values of Eq. (2) were always >0.992 showing that the use of the simplified relation of Langmuir theory (Eq. (2)) was sufficient to described accurately the association mechanism of phenol derivative with the C18 stationary phase (Table 1). It corresponded to the case in which the phenol derivatives bound only on the free PGC stationary phase. It was confirmed by the linear $\ln k'$ versus $\ln x$ plot (Fig. 3). For the PGC stationary phase, over the entire saccharose concentration range, when the saccharose increased, the $\ln k'$ decreased (Fig. 3). The plot obtained for the C18 stationary phase was similar to the one with a 0.7 (v/v) methanol fraction in the mobile phase. Then, contrary to the results obtained with a classical reversed phase, the saccharose addition is unfavourable on the retention process. This is typical of a competition between saccharose and molecules to bind on the PGC surface without saturation phenomena. It corresponds to the case when the influence of the change in water activity is negligible in relation to the direct saccharose effect on the PGC surface. Then, the use of Wyman equation [24] is sufficient to fit the saccharose dependence on the solute retention factor k'. For a given temperature, k'_T can be linked to the change in saccharose concentration, x, using the following equation [24–26]:

$$\left(\frac{\mathrm{d}(\ln k')}{\mathrm{d}(\ln x)}\right)_T = \Delta n \tag{11}$$

where the release parameter Δn is related to the difference in the number of saccharose molecules bound in the PGC–solute interface between the two state of equilibrium. Rearranging Eq. (11) gives [24–26]:

$$\ln k' = \gamma + \Delta n \ln x \tag{12}$$

where γ is a constant. The Δn values, for all phenol derivatives were determined from the slope of the linear plot ($r^2 > 0.956$) $\ln k'$ versus $\ln x$ (Fig. 3) and are reported in Table 3. The negative values of Δn reflected the direct competition of saccharose species for the PGC surface (effect (i)). It must be pointed out that the Δn values varied similarly to the elution order of the solute molecules, except for the nitrophenol which contains a voluminous substituent. Therefore, the Δn value can be considered as an affinity marker of phenol derivatives for the PGC surface. It is of interest to note that Δn can be fitted to a van't Hoff equation [24,25]:

$$\ln(\Delta n) = -\frac{E_a}{kT} + \ln \bar{w} \tag{13}$$

where $\bar{w}$ is a pre-exponential factor, k the Boltzmann constant, and E_a an activation energy term. The activation energy was determined only for the three most retained molecules on the PGC surface (Table 4). The van't Hoff plots of $\ln(\Delta n)$ versus $1/T$ were drawn for the three most retained compounds. Similar linear plots were observed for the three molecules with $r^2 > 0.977$. The E_a values for the other compounds were not shown, because they were not sufficiently accurate enough as a result of small retention time. $E_a < 0$ indicated that when T increased, the n value decreased due to a decrease in the solute transfer from the bulk solvent to the PGC surface. The magnitude of E_a corresponded to a dipolar–dipolar interaction of high energy (Table 4). The thermodynamic parameters variations were calculated using van't Hoff plots. In order to gain further information on this retention mechanism, ΔH° and ΔS° versus $\ln x$ were plotted for all the compounds and the thermodynamic data variations with x were similar for all the phenol derivatives. As example, Fig. 7 reports the curves of ΔH° versus $\ln x$, respectively, for nitrophenol and the two stationary phases. Similar variation were observed for the plot ΔS° versus $\ln x$. For the C18 stationary phase, the thermodynamic data increased due to the salting-out character of saccharose. For the PGC surface, when x increased ($x < x_c = 0.03$ M), the ΔH° and ΔS° values increased on the entire range of saccharose due to: (i) the competition effect; and (ii) the salting-out character of saccharose. The difference of the results can be explained by an increase in the solute solvation by methanol–water cluster

Table 3
Δn values (at $T = 20\,^\circ$C) corresponding to the number of excluded saccharose molecules for a 0.8 (v/v) methanol fraction in the mobile phase

Solute molecule	Δn
Benzoquinone	−0.18
Catechol	−0.15
Ethoxyphenol	−0.14
Hydroquinone	−0.19
Hydroxyquinone	−0.18
Methoxyphenol	−0.14
Nitrophenol	−0.17
Phenol	−0.17
Resorcinol	−0.16

Standard deviation <0.02.

Table 4
E_a (kJ/mol) values for the most retained compounds

Solute molecule	E_a (kJ/mol)
Ethoxyphenol	−6.7
Methoxyphenol	−10.2
Nitrophenol	−11.7

Standard deviation <0.1.

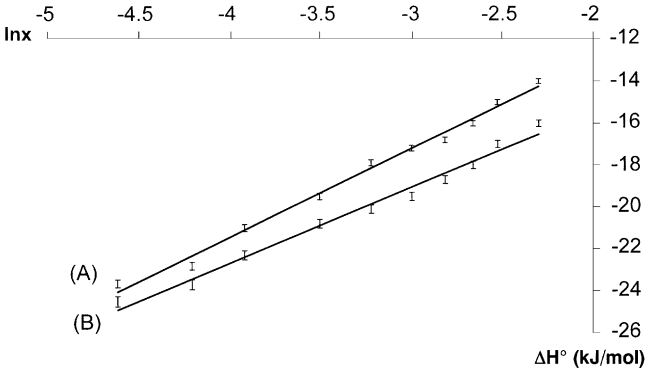

Fig. 7. ΔH° (kJ/mol) vs. $\ln x$ for nitrophenol and for a 0.8 (v/v) methanol fraction in the mobile phase on the PGC surface (A) and the C18 stationary phase (B).

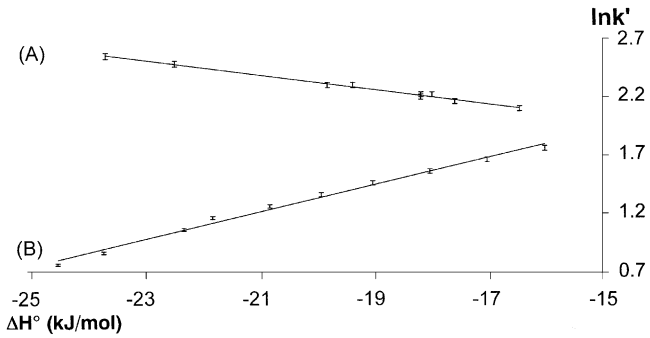

Fig. 8. The plot of $\ln k'$ vs. ΔH° (kJ/mol) at $T = 30\,^{\circ}$C for nitrophenol and for a 0.8 (v/v) methanol fraction in the mobile phase for the PGC surface (A) and the C18 stationary phase (B).

when the mobile phase polarity decreases [27,28]. In order to verify that with a 0.8 (v/v) methanol fraction in the mobile phase, an enthalpy–entropy was investigated. Fig. 8 shows the $\ln k'$ values plotted in relation to ΔH° for the nine saccharose concentrations and for ethoxyphenol and for each stationary phase at 30 °C. The correlation coefficient for the linear fit was 0.996. This degree of correlation can be considered to be adequate to verify enthalpy–entropy compensation indicating that the interaction mechanism is independent of the saccharose concentration in the mobile phase for both the PGC surface and the C18 stationary phase. This tends to confirm that contrary to a 0.7 (v/v) methanol fraction in the mobile phase, the possible association of phenol derivative with the adsorbed saccharose seems to be negligible.

5. Conclusion

This paper explores the change in the PGC–solute association as the saccharose concentration in the bulk solvent is modified. From the Langmuir approach, thermodynamic data and the Wyman concept, it is demonstrated the importance of the use of a multiple equilibrium model that takes into account the saccharose adsorption on PGC surface and the contributions of the surface tension of both the PGC and the bulk solvent. These different contributions explained the possible saccharose antagonist effect on the phenol derivative molecule association with the PGC surface obtained for specific saccharose concentration in the bulk solvent. Therefore, studying wide and large saccharose concentration and temperature ranges, appears very important to provide valuable information about the relative contributions of different interactions involved in the ligand–PGC association.

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