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Journal of Chromatography B, 749 (2000) 225–231

JOURNAL OF CHROMATOGRAPHY B

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Hydrophylic surfactant–imidazole derivative association studied by RPLC using a hydroorganic solution

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Received 29 November 1999; received in revised form 10 July 2000; accepted 18 July 2000

Abstract

A high-performance liquid chromatographic (HPLC) method for an association study of imidazole derivatives in surfactant micellars using a hydrophilic detergent, i.e. Montanox DF 80 was presented. The thermodynamic results obtained showed that imidazole association in the surfactant micelles was effective over a concentration of surfactant equal to approximatively $4 \cdot 10^{-4}$ mol/l. In addition, an enthalpy–entropy compensation study revealed that the type of interaction between the solute and the RP18 stationary phase was independent of the molecular structure. The thermodynamic variations observed were considered to be the result of equilibrium displacement between the solute and free ethanol (respectively free surfactant) and its clusters (respectively to micelles) created in the mobile phase.

Keywords: Derivatization; LC; Imidazole

1. Introduction

Much research has been carried out in the area of the solute retention in micellar liquid chromatography (MLC). McCormick et al. showed that the solute retention in MLC could be reduced dramatically via the use of very wide pore stationary phases [1]. Takeuchi and Miwa examined the retention selectivity and enhanced fluorescence of dansylaminoacids on an ion-exchange induced stationary phase. Several mobile phase parameters affected the retention of the analytes, including the type and concentration of micellar agents and modifier ions and the concentration of acetonitrile [2]. Jimenez et al. reported a theoretical model for the prediction and modelling of retention factors of 27 dihydropyridines in MLC with CTAB or SDS as surfactants and 1-propanol and 1 butanol as an organic modifier [3]. The latter proposed a new physicochemical retention model that successfully described the effect on solute retention of adding 1-propanol and 1-butanol to micellar mobile phases containing CTAB or SDS [4]. Garcia and Marina [5] examined the effects of alcohol organic modifiers on the association constant and retention mechanism for hydrophobic compounds with hexadecyltrimethylammonium (CTAB). Using RPLC Escuder-Gilabert et al. [6], studied the hydrophobicity of local anesthetics. Jandera and Fischer [7] derived an experimentally verified equation describing the depen-

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0378-4347/00/$ – see front matter
PII: S0378-4347(00)00425-4

dence of solute retention on the concentration of the surfactant in both aqueous micellar and submicellar mobile phases. By using a 1-propanol-modified sodium dodecyl sulfate (SDS) mobile phase on a short-chain bonded-phase column, Kayali et al. [8] were able to separate, with a reasonable degree of retention five pAHs that were too highly retained on a long chain column. Marina et al. [9] used multiple linear regression to interpret the influence of the nature of the surfactant and concentration on the separation selectivity of 15 benzene and naphtalene derivatives with an octadecylsilica column; SDS, CTAB, and Brij-35 were employed at concentrations ranging from 0.02 to 0.1 *M*. Delgado et al. [10] measured the enthalpy and entropy of transfer of polynuclear aromatic hydrocarbons from the bulk aqueous mobile phase to the stationary phase. They compared the results obtained when alcohol modifiers were added to the mobile phase. Many imidazole derivatives are widely used or recommended for pharmaceutical use as antimycotics (clotrimazole, miconazole, etc.) and antineoplastic agents (dacarbazine . . .) [11–13]. Nevertheless, hydrophobic compounds have a weak penetration into hydrophilic human nail matrices. Their inclusion in surfactant micelles could improve this penetration considering the hydrophilic character of the exterior of the micelle. Reversed phase liquid chromatography (RPLC) has been used to study this molecular association between a hydrophobic solute and micelles. The aim of this paper was to study the imidazole retention on a C_{18} column using a hydrophilic surfactant as a mobile phase additive. A simple model based on multiple equilibria between the surfactant, the imidazole derivative and both the stationary and the hydroorganic mobile phases was built and applied to the compound behavior in this chromatographic system. Thermodynamic data were determined from Van't Hoff plots and the enthalpy and entropy compensation was studied in relation to this model

2. Experimental

The RPLC system consisted of a Waters RPLC pump 501 (Saint Quentin, Yvelines, France), fitted with a 20 μl sample loop, a Shimadzu SPD–10A (Touzart-Matignon, Vitry sur Seine, France) variable Wavelength UV spectrophotometer detector (Nogent sur Marne, France). A Lichrocart® 125 mm×4 mm I.D. RP18 column (5 μm particle size) (Merck, Darmstadt, Germany) was used with controlled temperature in an Interchim oven, TM N° 701. Mobile phase flow-rate was fixed at 1 ml/min.

2.1. Solvents and samples

RPLC grade ethanol (Carlo Erba, Val de Reuil, France) was used without further purification. Water was obtained from an Elgastat option I water purification system (Odil, Talant, France), fitted with a reverse osmosis cartridge. The mobile phase used for these studies was a ethanol–aqueous phosphate buffer (40:60, v/v) mixture, adjusted at pH=3.00 with phosphoric acid with various surfactant concentrations (c) varying from 0 mol/l to $9.25 \cdot 10^{-4}$ mol/l. This surfactant, an ethoxylated mono-oleate sorbitan (Montanox DF 80) was purchased from SEPPIC (Paris, France) (Fig. 1). The phosphate buffer was composed of sodium hydrogenphosphate (0.01 *M*) and sodium dihydrogenphosphate (0.02 *M*). Clotrimazole (1), bifonazole (2), econazole (3), sulconazole (4), and miconazole (5) (Sigma, Paris, France) were dissolved in acetone to obtain a concentration of 1 mg/ml (Fig. 1). Each solute or a mixture of these was injected and the retention times were measured using a Merck D 2 500 chromato-integrator. Deuterium oxide was used as a dead time marker (Merck, Nogent sur Marne, France). Between the different mobile phases, the variation coefficients of the dead time values obtained were <0.8% indicating that the dead time was independent of the surfactant concentration. Surface tension measurements (γ) were performed using a K 6 tensiometer (Krüss, Hamburg, Germany) with the ring method. The interfacial tensiometer was calibrated with double distilled water (surface tension at 25°C=71.4 mN/m). Experiments were run on the mobile phase over the surfactant concentration range (0 mol/l–$9.25 \cdot 10^{-4}$ mol/l) at 25°C.

2.2. Temperature studies

Compound retention factors were determined at temperature values of 25°C, 30°C, 35°C, 40°C, 45°C.

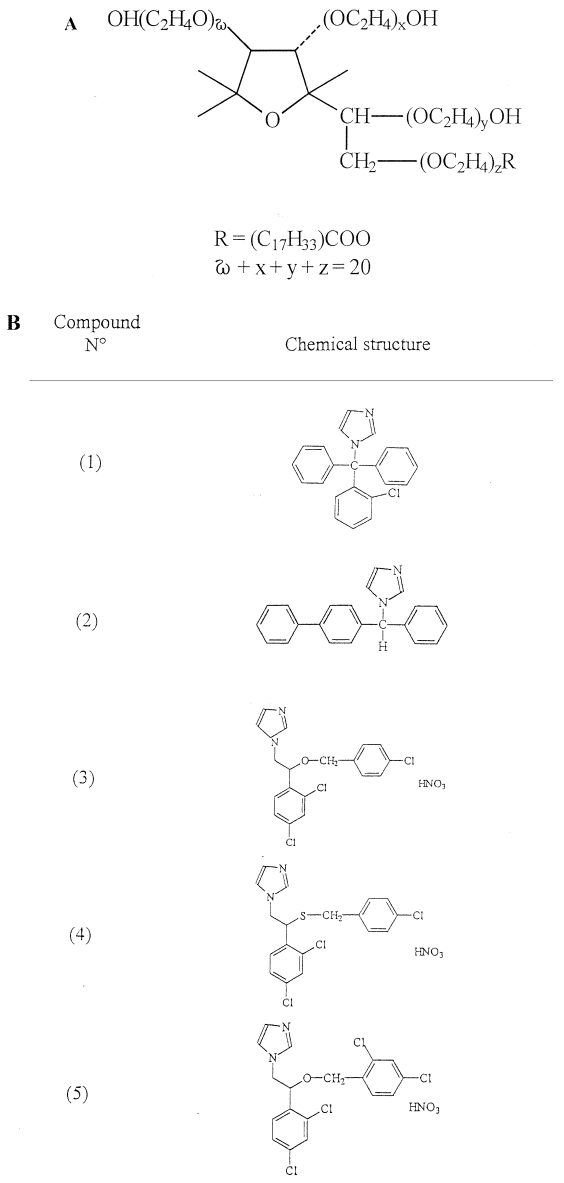

Fig. 1. Montanox DF 80 (A), five imidazole derivatives (B) structures.

The chromatographic system was allowed to equilibrate at each temperature for at least 1 h prior to each experiment. To study this equilibrium process, the compound retention time of the bifonazole was measured every hour for 7 h and again after 22, 23 and 24 h. The maximum relative difference in the retention times of this compound between these different measurements was always 0.6%, making the chromatographic system sufficiently equilibrated for use after 1 h. All the solutes were injected in triplicate at each temperature and surfactant concentration.

3. Results and discussion

The surface tension measurements of the mobile phase were repeated five times showing a perfect reproductibility of the results. γ decreased with C until the critical micellar concentration (CMC) was reached and then remained constant. CMC was determined to be equal to $\cong 4 \cdot 10^{-4}$ mol/l at $T=298$ K. This result corroborated the data obtained in a previous paper [14] where the formation of micelles using montanox DF80 with high organic solvent conditions was demonstrated. It was shown that the plot of the ''free'' surfactant (not in a micelle) concentration in the mobile phase vs. the total surfactant concentration presented an inflexion point for $\cong$ CMC. This surfactant had a high hydrophilic–lipophilic balance [15] and thus a hydrophilic character. This fact was corroborated by its low retention time ($\cong$1.5 min). Therefore, in the studied surfactant concentration range the modification of the stationary phase by adsorption of the detergent can be neglected [14,16–19]. To examine the concentration dependencies of imidazole derivative retention corresponding to the binding capacity of the RP18 stationary phase, retention measurements were related to varying amounts of injected solutes at all surfactant concentrations in the mobile phase at 298 K (20 μl with solute concentration varying from 0.5 to 3 mg/ml). There was no significant change in the k' retention factor values for any of the solutes over this range. Thus, 20 μl of each solute were injected at a concentration of 1 mg/ml where the retention was sample concentration independent (i.e, linear elution conditions). An example of a chromatogram of the 5 imidazole derivatives is given in Fig. 2. All the imidazole derivatives exhibited a similar variation for the retention factor with the surfactant concentration. The retention decreased with an increase of the surfactant concentration. Fig. 3 repre-

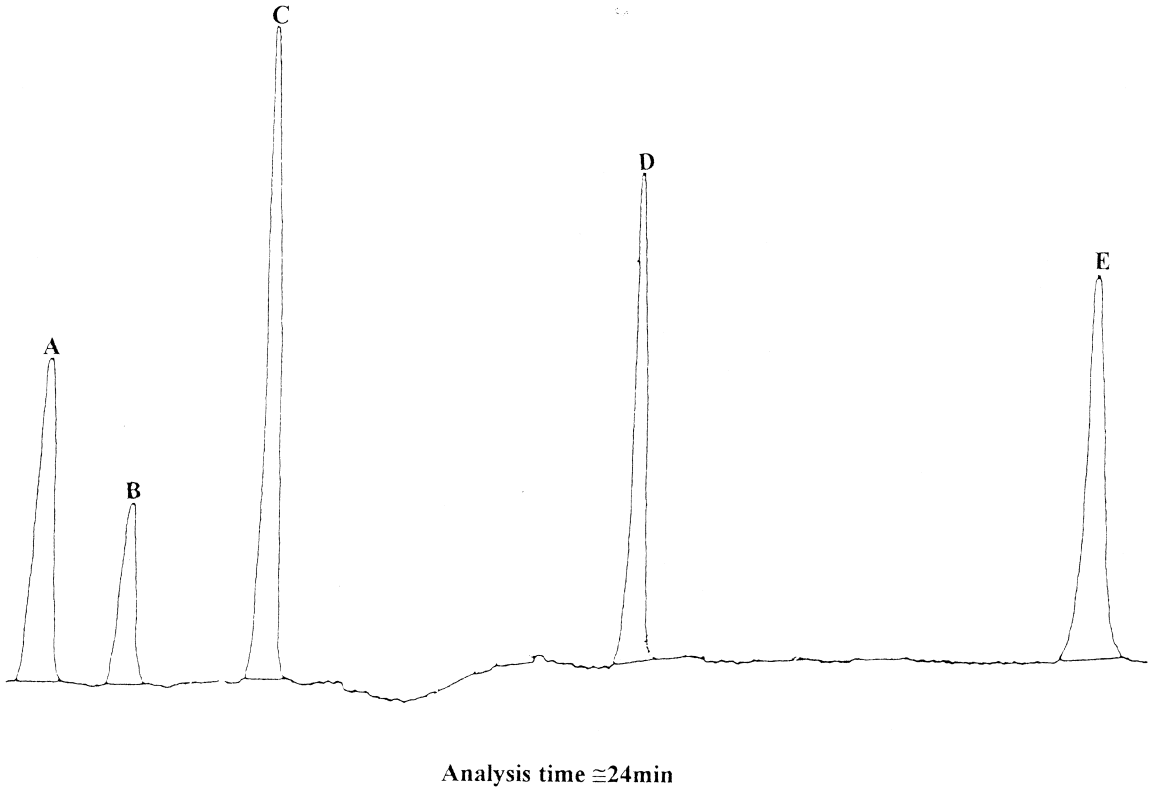

Fig. 2. Representative chromatogram of the five imidazole derivatives at $T=298$ K for a surfactant concentration equal to $3.06\cdot10^{-4}$ mol/l. A, clotrimazole; B, bifonazole; C, econazole; D, sulconazole; E, miconazole.

sents the variation curve for all the solutes at $T=303$ K. As can be seen from this figure, clotrimazole was the lowest compound retained and miconazole the highest. This can be explained by the fact that the mobile phase was dominant in governing retention changes in weak polar solutes in RPLC [20]. The structure of the ethanol–water mixture depended on the hydrogen bonds between the bulk water and ethanol [21–24]. It was demonstrated that an ethanol–water mixture consisted of free water (W), free ethanol (E), the highest hydrophobic species and ethanol–water clusters [21–25]. For a water fraction equal to 0.60 it was demonstrated that the solute was preferentially solvated by the free ethanol and the clusters of stoichiometry 1-1 [21–24]. Bifonazole and clotrimazole were well solvated and had the greatest affinity for the mobile phase. Therefore, they were retained less on the column than the three other derivatives econazole sulconazole and miconazole.

The dependency of the k' values on the temperature is given by the Van't Hoff equation:

$$\ln k' = -(\Delta H^\circ/RT) + (\Delta S^\circ/R) + \ln \varnothing \quad (1)$$

where $\varnothing$ is the phase ratio (volume of the stationary phase divided by the volume of the mobile phase), ΔH° (respectively ΔS°) is the enthalpy (respectively entropy) of transfer of the solute from the mobile to the stationary phase, T is the temperature and R is the gas constant. From the slope and the intercept, ΔH° and $(\Delta S^\circ/R)+\ln \varnothing = \Delta S^{\circ *}$ can be calculated. This provided a convenient way of calculating the thermodynamic parameters for a chromatographic system if the phase ratio is known or can be calculated [25]. Usually, ΔS° is not provided due to the ambiguity in the calculation of the phase ratio for the commercial columns. The correlation coefficients r for the Van't Hoff curves were at least equal to

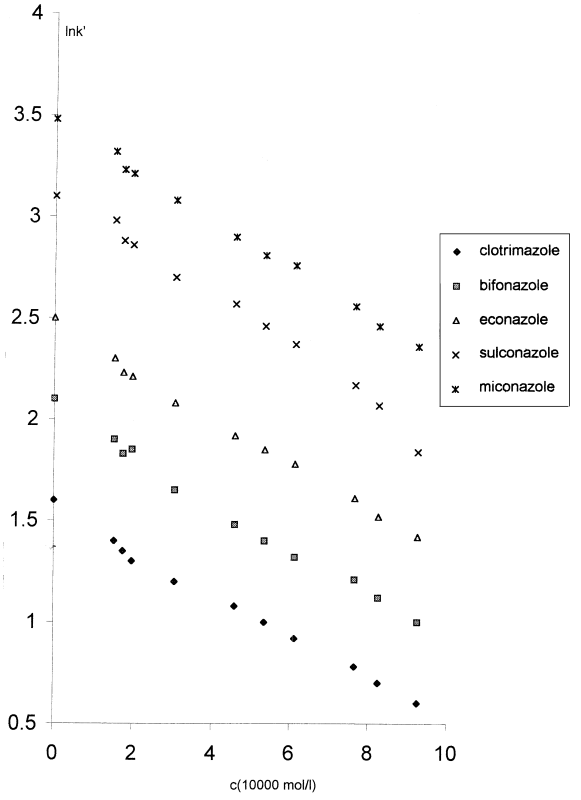

Fig. 3. Influence of the hydrophylic surfactant concentration c (mol/l) on ln k' for the five imidazole derivatives at $T=303$ K.

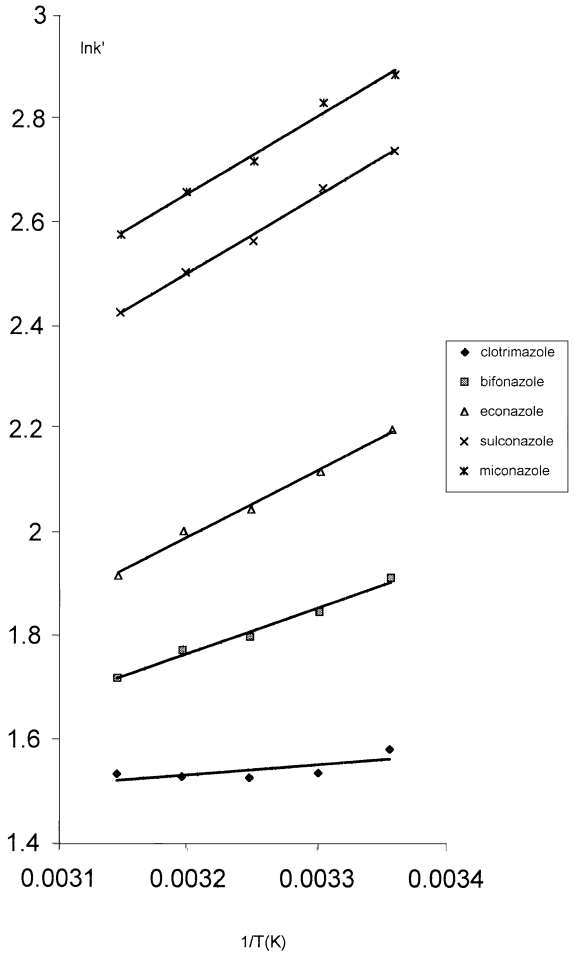

Fig. 4. Van't Hoff plots at a surfactant concentration equal to $3.06\cdot10^{-4}$ mol/l for the five imidazole derivatives.

0.98. Fig. 4 shows the van't Hoff plots for all solutes at a surfactant concentration equal to $3.06\cdot10^{-4}$ mol/l. In order to gain further insight on the validity of the proposed model, the enthalpy–entropy compensation was examined. This approach has been previously used in chromatographic procedures to analyze and compare the retention mechanism for a group of compounds [26–30]. The enthalpy–entropy compensation is a term used to describe a compensation temperature which is system independent for a class of similar experimental systems. Enthalpy–entropy compensation compensation can be expressed by the formula:

$$\Delta G^{\circ}_{\beta} = \Delta H^{\circ} - \beta\,\Delta S^{\circ} \qquad (2)$$

where ΔG°_{β} is the Gibbs free energy of a physicochemical interaction at a compensation temperature β. ΔH° and ΔS° are respectively the corresponding standard enthalpy and entropy. According to Eq. (2), when enthalpy–entropy compensation is observed with a group of compounds in a particular chemical interaction, all the compounds have the same free energy ΔG°_{β} at temperature β. If, therefore, enthalpy–entropy compensation is observed for the imidazole derivatives, then they will all have the same net retention at the compensation temperature β, although their temperature dependency may differ. Combining Eqs. (1) and (2), the following equation is obtained:

$$\ln k'_{T} = \ln k'_{\beta} - \Delta H^{\circ}/R\,(1/T - 1/\beta) \qquad (3)$$

where,

$$\ln k'_{\beta} = -\Delta G^{\circ}{}_{\beta}/R\beta_{+} \ln \varnothing \quad (4)$$

Eq. (3) Shows that if a plot of $\ln k'_T$ against $-\Delta H^{\circ}$ is linear, then the imidazole derivatives are retained by an essentially identical interaction mechanism. A plot of $\ln k'$ (for $T=323$ K) calculated for each imidazole derivatives against $-\Delta H^{\circ}$ without a surfactant in the mobile phase and for two different values of the surfactant concentration was drawn ($c_1<$CMC and $c_2>$CMC). The r values for the fits were 0.90 for $c=0$ and 0.95 and 0.93 for $c_1=1.98\cdot 10^{-4}$ mol/l and $c_2=9.25\cdot 10^{-4}$ mol/l respectively. Cole and Dorsey [27] demonstrated that this degree of correlation can be considered adequate to verify enthalpy–entropy compensation. Nevertheless, if sulconazole and miconazole are not taken into account, these linear fits are better (>0.97). Therefore, the retention mechanism can be thought to be independent of the molecular structure with or without surfactant in the mobile phase below and above the critical micellar concentration. This is in agreement with previous work in which this type of interaction was found to be the same for various imidazole derivatives using a similar chromatographic system and enthalpy–entropy compensation [31]. Using Eq. (3) the β values were calculated from the slopes and went from approximatively 398 K to 430 K over the surfactant concentration range. Figs. 5 and 6 show the variation in ΔH° and $\Delta S^{\circ *}$ with c for the imidazole derivatives. The surfactant modified the solvation sphere of imidazole derivatives by free ethanol and its clusters. When the surfactant concentration varied from 0 to $9.25\cdot 10^{-4}$ mol/l the following can be given:

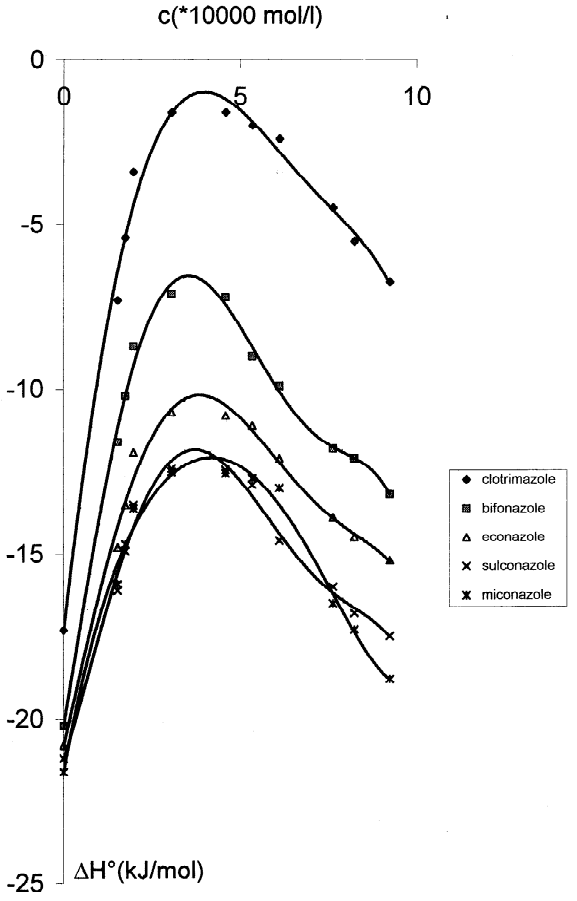

Fig. 5. Influence of the hydrophilic surfactant concentration c (mol/l) on ΔH° (kJ/mol) for the five imidazole derivatives.

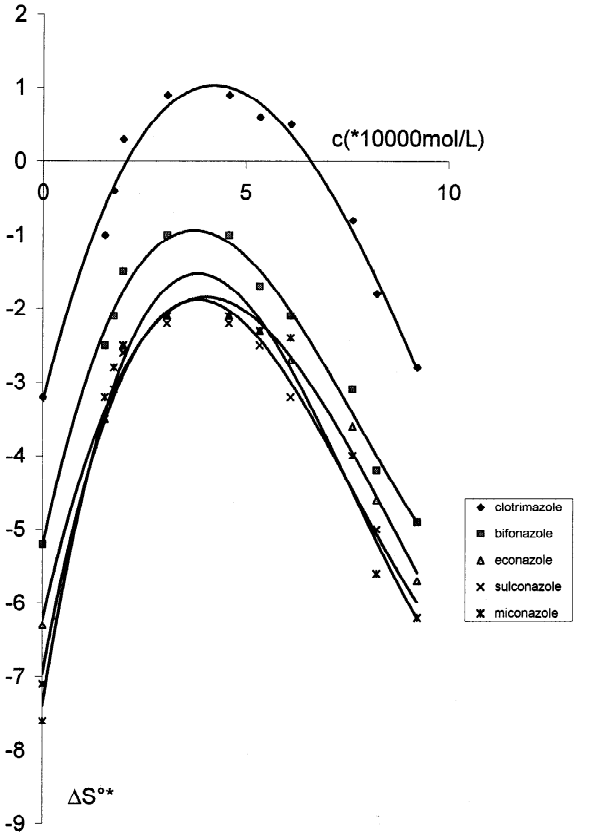

Fig. 6. Influence of the hydrophilic surfactant concentration c (mol/l) on $\Delta S^{\circ *}$ for the five imidazole derivatives.

Below CMC; the addition of the surfactant in the mobile phase displaced the equilibration of solvatation of the solute with ethanol and its clusters in the direction of the free solute. Therefore, the direct interaction between the free hydrophilic surfactant (not in a micelle) and the imidazole solute was facilitated. This stronger hydrogen bonding and/or van der Waals interactions replaced the solute and free ethanol (or ethanol/water cluster) interactions and ΔH° increased (Fig. 5). The observed trend of $\Delta S^{\circ *}$ to increase with C (Fig. 6) can be explained by an increase in the entropy in the mobile phase due to an increase in the polar interaction of the imidazole derivative with the polar residues of the free hydrophilic surfactant.

Above the CMC; the solvation equilibria were completely displaced in the direction of the free solute which can interact with the surfactant micelles created in the mobile phase by an association process. Thus, the retention process corresponded to a simple hydro-organic chromatographic system in reversed-phase as shown for a surfactant concentrations equal to $5.35 \cdot 10^{-4}$ mol/l and $9.25 \cdot 10^{-4}$ mol/l. When the weak polar imidazole derivative was transferred from the micelles in the mobile phase to the RP18 stationary phase, stronger van der Waals interactions replaced the micelle–imidazole solute interactions and ΔH° decreased (Fig. 5). The greater immobilization effect following the binding process explained the decrease in $\Delta S^{\circ *}$ (Fig. 6).

4. Conclusion

This work used RPLC to investigate the inclusion mechanism of a series of imidazole derivatives in surfactant micellars using a hydrophilic detergent, Montanox DF80. With this simple treatment, it was possible to describe the different equilibria which were implied when the imidazole derivative was transferred from the mobile to the stationary phase. Thermodynamic result obtained showed that the solute inclusion in the surfactant micelles was effective above a surfactant concentration equal to approximatively $4 \cdot 10^{-4}$ mol/l. Finally enthalpy–entropy compensation revealed that the imidazole derivative retention mechanism is independent of the molecular structure.

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