

Improvement in the Electrostatic Theory of Reversed-Phase Ion-pair Chromatography to Include the Role of Organic Modifier

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Summary: In this study, the electronic theory of ion-pair chromatography has been extended to include the role of organic modifier. This approach has increased the possibility to use organic modifier in the improvement of separation of ionic, non-ionic and ionized substances.

The results accumulated shows that both the slope and the intercept of $\ln K'$ vs. organic modifier concentration plot are predicted to increase the oppositely charged and to decrease for similarly charged solute ions.

Introduction

In reversed phase-high performance liquid chromatography (RP-HPLC), solute retention and separation are almost exclusively optimized by varying the nature and concentration of organic modifier in the mobile phase [1]. In RP ion-pair chromatography, where samples contain non-ionic, ionic and/or ionizable components, separation are optimized primarily by varying the pH and/or the pairing ion concentration of the eluent. Difficult separations of ionic solutes with similar charges and structures can only be solved by varying different types and concentration of the organic modifier [2-6], however, their effects on the separation phenomena are not understood fully.

In order to understand the electrostatic theory of ion-pair chromatography [7-10], we have attempted to improve and extend this theory to account for the

simultaneous effects of the pairing ion and the concentrations of organic modifier. Prediction from this theory are compared with experimental data for the common organic modifiers such as methanol, acetonitrile, tetrahydrofuran-buffer eluents.

Results and Discussion

Electrostatic theory

According to this theory of ion-pair chromatography, retention change of an ionic solute B is determined by the electrostatic potential difference U_0 between the stationary and mobile phase, established by the adsorbing pairing ion A. Hence, the capacity factor for B can be given as :

$$K_{CB} = K_{OB} \exp (Z_B \cdot F \cdot U_0 / RT) \quad (1)$$

where Z_B = Charge of solute B,

K'_{OB} = Capacity factor in the absence of pairing ion
and K'_{CB} = Capacity factor of solute B in presence of pairing ion.

Using the linearized Poisson-Boltzman equation for the surface potential and a surface potential modified linear isotherm equation for adsorption of the pairing ion [4], the solute capacity factor can be given as a function of the pairing ion concentration in the eluent [6] as

$$-Z_A/Z_B \ln K'_{CB}/K'_{OB} + \ln (-RT/Z_A \cdot Z_B \cdot F \ln K'_{CB}/K'_{OB}) = \ln (K_{CA}) + \ln (n_o \cdot K_{AS}) \quad (2)$$

where

Z_A = Charge of the pairing A
 C_A = pairing ion concentration in mobile phase
 n_o = monolayer capacity for adsorption
 K_{AS} = adsorption constant for pairing ion A
 K = dielectric constant

The equation can be modified to include the organic modifier concentration (∂) as recently shown by one research group (8). For constant ionic strength and $\delta \leq 0.3$,

$$\ln K'_{OB} = \ln K'_w - S \cdot \partial \quad (3)$$

where K'_w = solute retention in pure water (or buffer)
and S = Slope constant for a given solute-organic modifier, the equation is represented in Fig. 1.

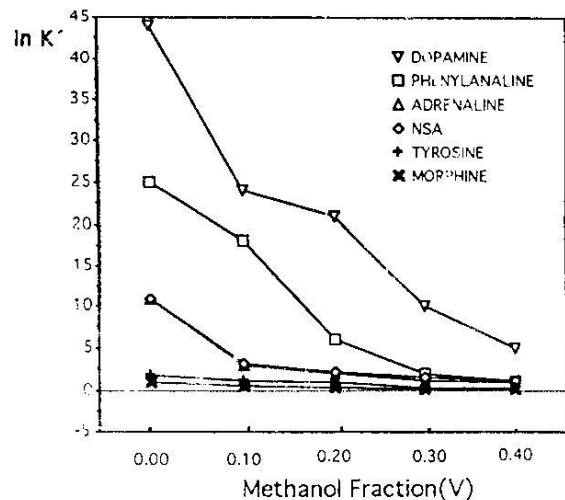


Fig. 1: Relationship between $\ln K'$ vs Concentration of methanol in absence of ion-pairing reagent.

Considering the volume fraction of organic modifier, one can formulate equation 3 as;

$$\ln (n_o \cdot K_{AS}) = C - D \cdot \partial \quad (4)$$

where C = intercept for a given pairing ion,
 D = Slope for a given pairing ion- organic modifier

and ∂ = volume fraction of organic modifier.

This equation is represented in Fig.2.

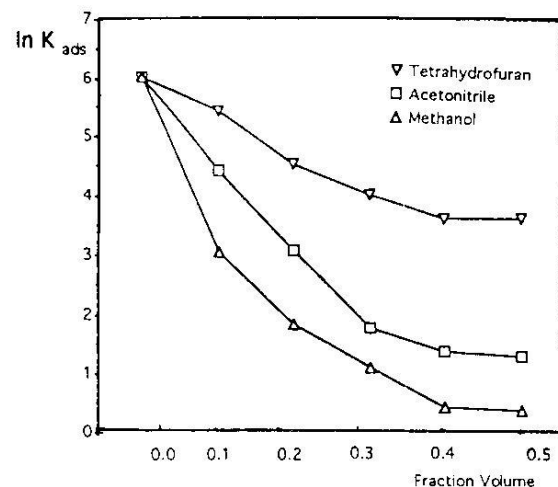


Fig. 2: Adsorption term (K_{ads}) of sodium octylsulphonate as a function of organic modifier concentrations.

Now substituting equations 3 and 4 into equation 2, it yields new retention equations which contain the mobile phase concentrations of both the pairing ion and the organic modifier.

When the solute and pairing ion have single and opposite charges ;

$$\ln K'_{OB}(\partial) = \ln K'_w + 0.5 \ln [K_1 \exp (C) C_A] - (S+0.5D) \partial \quad (5)$$

When the solute and pairing ion have single and similar charges equation 5 can be written as;

$$\ln K'_{OB}(\partial) = \ln K'_w - 0.5 \ln [K_1 \exp (C) C_A] - (S+0.5D) \partial \quad (6)$$

where K_1 is constant, C_A and C are the concentration and hydrophobicity of pairing ion.

Table 1: Experimental and predicted slope values of $\ln K'$ vs methanol concentration plots of differently charged solutes with 5 mM octylsulphonate as pairing ion (0.5D=3.6)

Solute	-S(RP)	-S(IPC) _{exp}	-S(IPC) _{cal}	Difference (%)
Phenol	4.6	4.5	-	-
NSA*	7.3	3.5	3.7	- 3.0%
Phenylalanine	6.1	10.0	9.7	-3.0%
Tyrosine	6.7	10.2	10.3	+0.9%

*NSA = 2-naphthalene sulphonic acid

Table 2: Experimental and predicted slope values of $\ln K'$ vs acetonitrile concentration plots of differently charged solutes with 5 mM octylsulphonate as pairing ion (0.5 D=7.2)

Solute	-S(RP)	S(IPC) _{exp}	S(IPC) _{cal}	Difference (%)
Phenol	6.8	6.3	-	-
NSA	14.6	8.1	7.4	- 8.6%
Phenylalanine	12.9	18.5	20.1	+8.6%
Tyrosine	16.4	22.9	23.6	+3.0%

Table 3: Experimental and predicted slope values of $\ln K'$ vs tetrahydrofuran concentration plot of differently charged solutes with 5mM octylsulphonate as pairing ion (0.5D=6.4)

Solute	-S(RP)	-S(IPC) _{exp}	-S(IPC) _{cal}	Difference (%)
Phenol	2.3	2.2	-	-
NSA	12.5	6.7	6.1	- 9.8%
Phenylalanine	9.4	15.4	15.8	+ 2.6%
Tyrosine	17.9	22.6	24.3	+ 7.5%

Testing the improved theory

A) Slope

This theory predicts that in the presence of pairing ion, the slopes of $\ln K'$ vs. ∂ plot of ionic solutes increases for the oppositely charged solutes by 0.5 D where D is the slope of the adsorption term of pairing ion according to equation 4.

Experimental and calculated slope values (S+0.5D) for differently charged solutes agree well in the three common organic modifier systems as shown in Table 1-3 and Figs. 3-5. Similar result are reported by Bertha *et al.* [8] working on different model compounds.

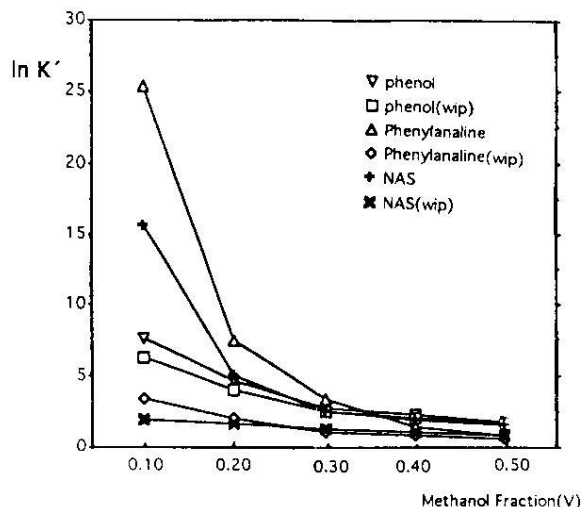


Fig. 3: Relationship between $\ln K'$ vs volume fraction of methanol in presence and absence of 5 mM octylsulphonate.

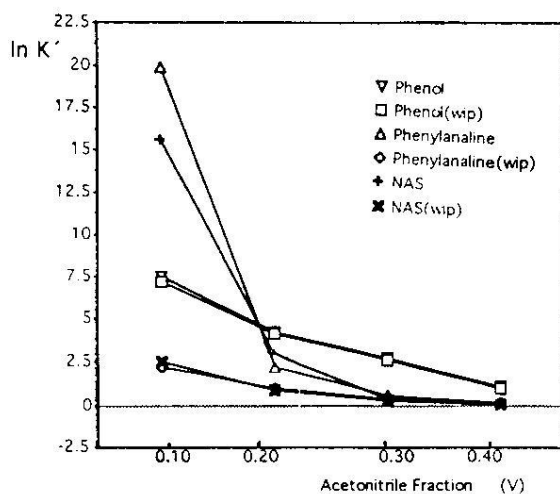


Fig. 4: Relationship between $\ln K'$ vs acetonitrile concentration of differently charged solutes in presence and absence of 5 mM octylsulphonate.

B) Intercept

According to equations 5 and 6, the intercept values of $\ln K'$ vs. ∂ plots of charged solutes were influenced by both the concentration and hydrophobicity of pairing ion. It also shows that the retention is a linear function of $\ln C_A$ with a slope of 0.5 at a constant organic modifier concentration and secondly when the pairing ion is replaced with more hydrophobic one, it results in a parallel shift of $\ln K'$ vs $\ln C_A$ plot of charged solutes.

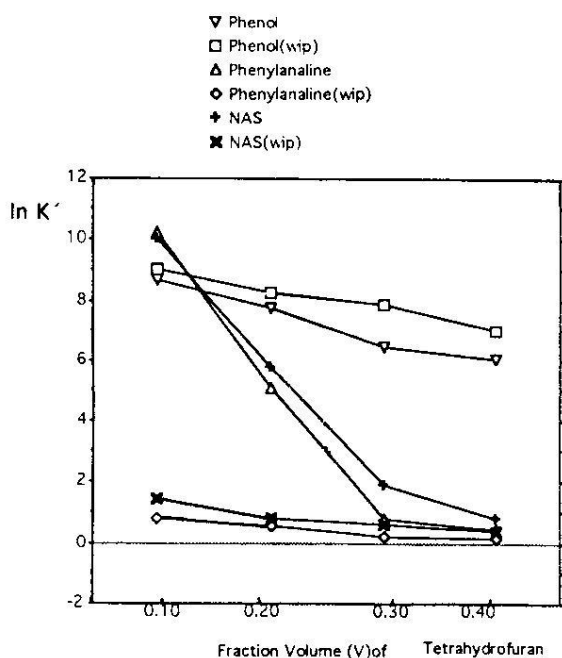


Fig. 5: Relationship between $\ln K'$ vs concentration of Tetrahydrofuran plots of differently charged solutes in presence and absence of 5 mM octylsulphonate.

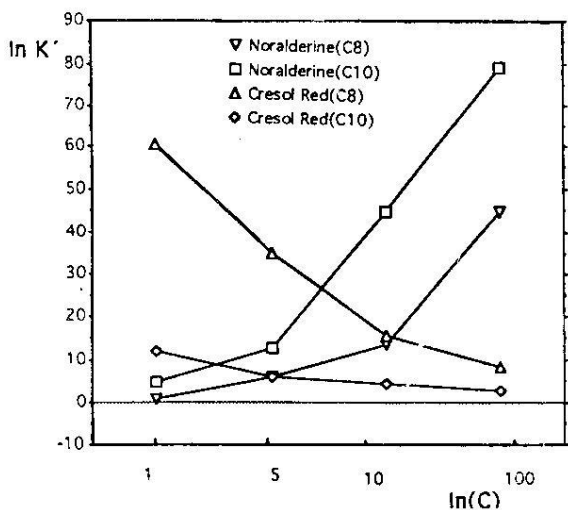


Fig. 6: Relationship between $\ln K'$ vs concentration of ion-pairing reagent in mobile phase containing 40% methanol in 0.1 M phosphate buffer (pH=21).

Experimental solute retention data shown in Fig. 6 verify these predictions. Both for the positively and negatively charged solute ions, using either

sodium octylsulphonate (C8) or decylsulphonate (C10) as ion-pairing reagent.

In conclusion, the understanding of electrostatic theory of ion-pair chromatography has been extended to include the effects of organic modifiers. This improved theory is shown to optimize RP-HPLC method in presence of ion-pairing reagent on the basis of organic modifier concentrations.

Experimental

An isocratic HPLC system considered of HPLC pump (LKB 2115) from LKB, Bromma, Sweden; an analytical column 5 μm Microsorb C18 (150 x 4/6 μm i.d.) from Pharmacia, Uppsala, Sweden; and universal injector with 50 μl loop from Rheodyne, California, USA and a UV detector set at a fixed wavelength of 254 nm (model 2048) from Beckman, New York, USA.

All chemicals used were of high purified quality obtained from various commercial sources. All separations of commercial standards were performed at the flow rate of 1 ml/min at room temperature. Until unless stated, all standard were of concentration of 1 $\mu\text{mol/l}$.

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