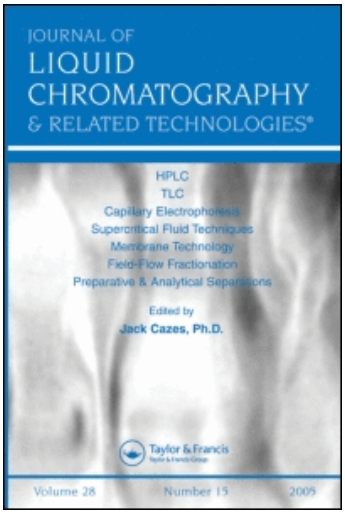

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Effects of Solvent Composition and Temperature on the Normal Phase Liquid Chromatographic Separation of Substituted Phenols

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EFFECTS OF SOLVENT COMPOSITION AND TEMPERATURE ON THE NORMAL PHASE LIQUID CHROMATOGRAPHIC SEPARATION OF SUBSTITUTED PHENOLS

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ABSTRACT

Temperature-dependent measurements of the capacity factors for the liquid chromatographic separations of position isomers of cresol and chlorophenol have been made at various concentrations of 2-propanol in n-heptane using an amino bonded phase column. Extra-thermodynamic relationships, i.e. linear free energy relationship and linear ΔH-pK_a relationships are applied to demonstrate that the basic interaction mode does not vary with the solvent composition, consistent with an earlier report by Scott and Kucera. Hydrogen bonding interaction at the molecular level is proposed.

INTRODUCTION

Recently we have demonstrated that by using extra-thermodynamic relationships, i.e. linear free energy relationship and linear ΔH-pK_b relationship, a retention mode change has occurred due to the change of mobile phase composition for the liquid chromatographic separations of several substituted anilines using an amino and a diamine bonded phase columns(1,2). This is consistent

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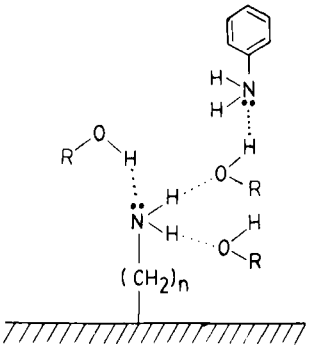
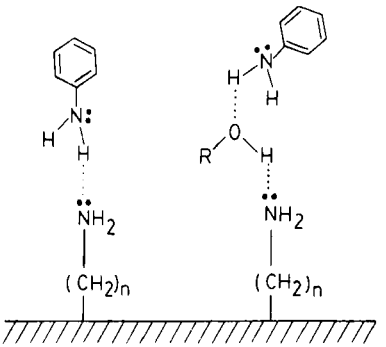
with what has been stated that for the separation of polar compounds, retention may be determined by specific solute-stationary phase and solute-mobile phase interactions, especially when high concentrations of polar eluent are added (3). Our experimental observation has further led us to propose possible interaction modes at the molecular level during the retention process. This is because a linear relationship is observed between values of pK_b of anilines and - H, i.e. the higher the $-\Delta H$ value, the lower the pK_b value using high percentage (e.g. 25%) of 2-propanol in n-heptane as mobile phase. Thus, the interaction involving hydrogen bonding is proposed in Figure 1. On the other hand, the inverse relationship observed using low concentration of 2-propanol in n-heptane as eluent implies possible hydrogen-bonding interaction modes depicted in Figure 2. These interaction modes are proposed also based on the facts that aliphatic amines are more basic than aromatic amines and N-H...O hydrogen bonding is stronger than N-H...N hydrogen bonding.

In order to examine the validity of the study and to understand more about the retention at the molecular level, we now report the effect of solvent composition and temperature on the normal phase liquid chromatographic separation of several substituted phenols using an amino bonded phase column with 2-propanol in n-heptane as eluent. Extra-thermodynamic relationships are again applied. It should be noted that several reports have appeared before this study concerning the separation of alkylphenols using several different columns (4,5,6).



1. FIGURE 1. Proposed H-bonding interaction of aniline with the amino bonded phase at high 2-propanol concentration in n-heptane eluent.



2. FIGURE 2. Proposed H-bonding interaction of aniline with the amino bonded phase at low 2-propanol concentration in n-heptane eluent.

However, the effects of temperature on the separation have not been included.

EXPERIMENTAL

A. Apparatus and Reagents

A Micromeritics (Norcross, GA., USA) model 7500 liquid chromatography system was used. This system was equipped with a model 750 solvent delivery system, a model 752 ternary solvent mixer, a model 731 column compartment with a universal sample injector and a variable temperature controller from ambient to 150°C, and a model 786 variable wavelength (200-600nm) detector with a deuterium lamp. A Waters (Milford, MA., USA) HPLC system equipped with two model 6000 A solvent delivery systems, a model 660 solvent programmer and a model 440 absorbance detector (254nm) was also used.

Pressure-Lok series C-160 (Precision Sampling Corp., LA., USA) 10 μl and 25 μl syringes were used. Chromatograms were recorded with a Linear model 555 single-channel recorder.

Amino bonded phase, Rosil-NH_2, was purchased from Alltech Associates (Deerfield, IL., USA). All other chemicals were reagent grade and obtained from various sources.

B. Column Packing

The columns were packed by using an up-flow method with a Micromeritics Model 705 stirred-slurry Column packer. Carbon tetrachloride was used as the solvent for column packing.

Before packing, the bonded phases were dispersed in the solvent and treated by an ultrasonic vibrator for 1 minute so

that a well-mixed suspension was formed (7). The procedures for column packing were described in a previous publication (8).

C. Chromatographic Procedures

Before the separation experiments, the columns were washed by 1% 2-propanol in n-heptane. A flow rate of 1 ml/min. was used for the washing process. After washing 12 hours, the column was allowed to stand for another 12 hours. The process was repeated 3-4 times. After pre-equilibrium was achieved, a flow rate of 2 ml/min. was used. The temperature-dependent measurements of capacity factor (k) were performed starting with a lower polar modifier concentration and then progressing to higher polar modifier concentrations. Additional washing with a flow rate of 2 ml/min. for 8 hours was performed when the concentration of polar modifier was changed.

The separation of a mixture of o-, m-, and p-isomers of cresol and chlorophenol was pre-tested. Depending on the separation condition, solute mixtures were prepared so that overlapping peaks did not occur and good resolutions (R) were obtained (i.e. R > 1.0)

In each separation, solute mixtures were prepared using the eluent. The concentration of each solute was ca. 1-2 mg/ml. An amount of 5μl of the solute mixture was injected into the system. A back pressure less than 1000 psi was usually observed throughout the experiment.

All data points were collected by averaging more than three reproducible separations and treated with normal statistical methods. p-Xylene or benzene was used to determine t_o for each column.

It is necessary to point out that the temperature-dependent measurements were performed by a very careful control of temperature. Generally, the mobile phase was preheated before it entered into the column. With the pre-heat-equilibrated solvent in the column and additional heating of the column body, it was found that the temperature was fairly uniform. Two observations supported this conclusion. First, the temperatures in the heater measured by a thermometer and by a thin wire probe (Kane-May Measuring Instrument, Inc. Albuquerque, NM) around the tubing and around the column were within ±0.1°C. Secondly, the chromatograms obtained under the set temperature were reproducible to within ±1% in terms of capacity factors calculated for each peak.

RESULTS AND DISCUSSION

I. Elution Sequence.

The retention of the position isomers of cresol and chlorophenol in the amino column follows the increasing order: p < m < o for chlorophenol and o < m ~ p for cresol (Table I). In general, chlorophenols always elute later than cresols. This is attributed to the presence of the chlorine atom on chlorophenol which serve as a second site for both hydrogen bonding and dipole-dipole interactions. The resulting low pK_a values for chlorophenols due to the presence of chlorine atoms certainly are also very significant.

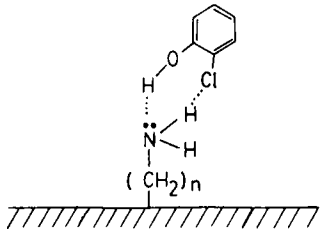
Two other observations are also apparent. First, o-cresol elutes faster than the meta- and para-isomers because the steric

Table I. Log k and -ΔH (Kcal) Values for the Separation of Chlorophenol and Cresol Isomers Using An Amino Bonded Phase Column At Varous 2-Propanol Composition.*

		percentage of 2-propanol in n-heptane		
		7.5%	15%	20%
chlorophenol,	ortho-	1.19(5.59)	0.90(5.58)	0.76(5.23)
	meta-	0.81(2.68)	0.44(3.14)	0.28(2.87)
	para-	0.73(2.07)	0.37(2.58)	0.20(2.37)
cresol,	ortho-	0.25	-	-0.21
	meta-	0.38	0.04	-0.09
	para-	0.36	0.05	-0.08

*The associated error for the ΔH (values in parenthesis) determination is estimated to be ±0.5 kcal. The precison of log k determination is ±1%

repulsion effect of the methyl group at the ortho position. Second, the reason why o-chlorophenol elutes slower than its position isomers may be twofold: (1) The o-isomer is more acidic. (2) There is a possibility of bi-functional interaction for the o-chlorophenol with the amino groups on the bonded phase, (Figure 3). The possibility of bi-functional interaction is further proved by the temperature-dependent measurements of the capacity factors (k). According to the van't Hoff equation:



3. FIGURE 3. Possible bi-functional interaction between o-chlorophenol and the amino bonded phase.

$$\ln k = - \frac{\Delta H}{R} \cdot \frac{1}{T} + \frac{\Delta S}{R} + \rho$$

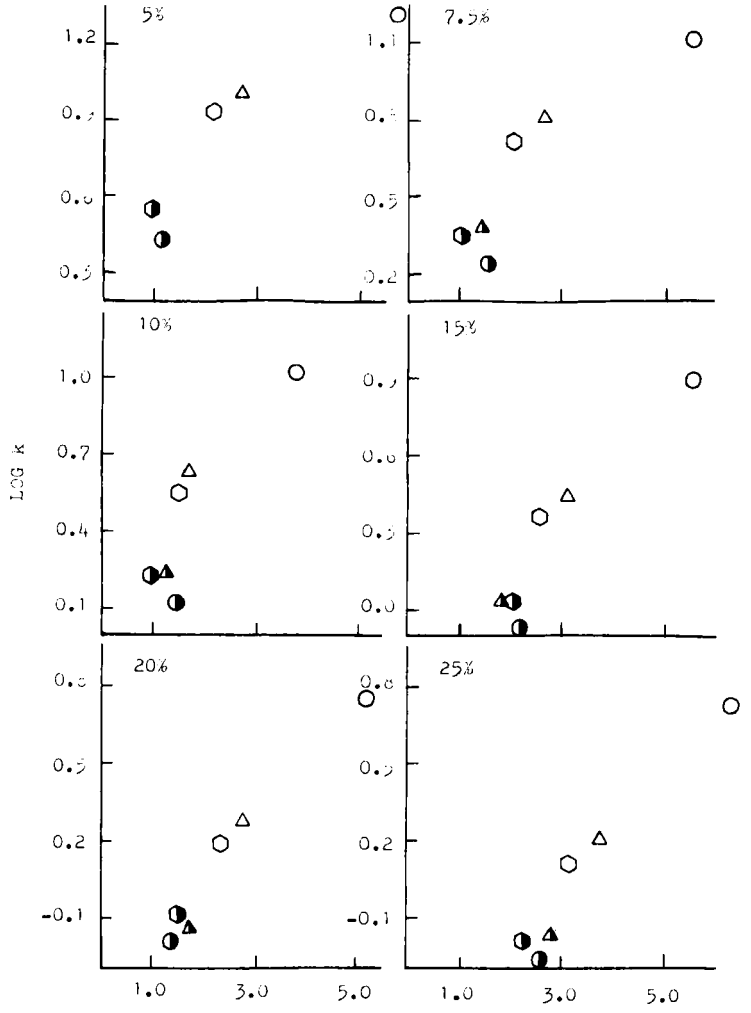
the ΔH values obtained by plotting $\ln k$ vs. $\frac{1}{T}$ for o-chloropohenol at several eluent conditions are always much more negative for o-chlorophenol than those of its position isomers. In fact, they are about twice in terms of absolute values. This is consistent with what was reported previously concerning the interaction of catechol with a diamine bonded phase column (9). Similar observation is also found when nitrophenol isomers were separated using a diamine bonded phase column with a much more acidic eluent (10).

II. Retention Mode.

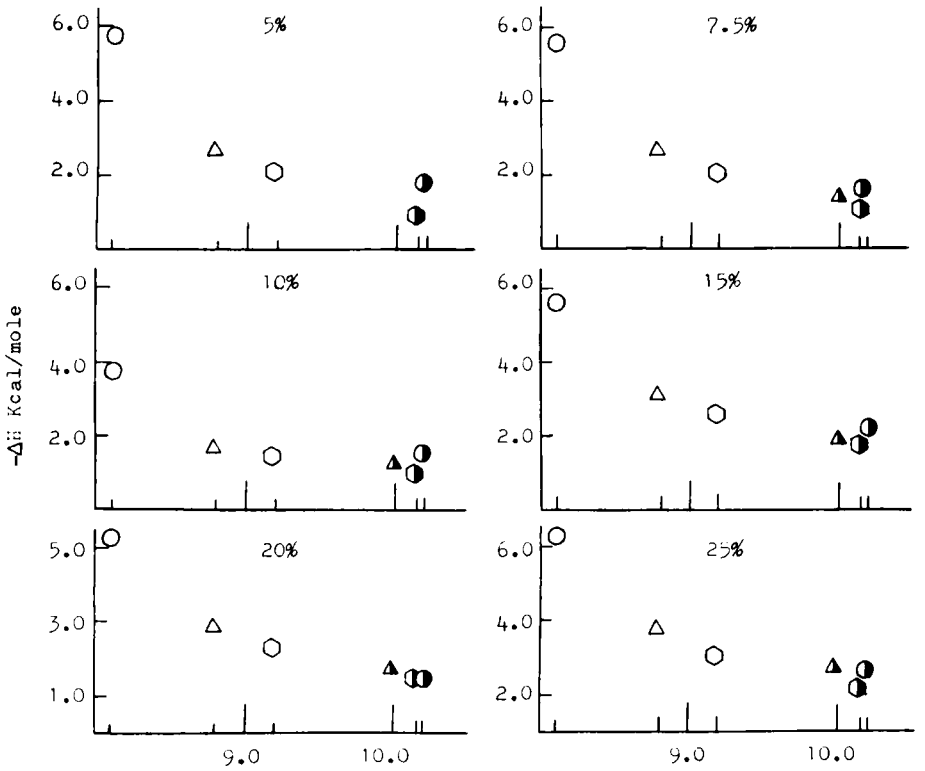
In many cases that were previously reported, linear free energy relationships (enthalpy-entropy compensation) were observed, i.e. plots of log k vs. ΔH values are linear. The slopes of enthalpy-entropy compensation plots have been used to interpret the retention mechanisms in reverse phase chroma-

tography (11). In the previous report concerning the separation of substituted anilines, a progressive change of the slopes has prompted one to propose an interaction mode change as the eluent concentration of 2-propanol (in n-heptane) is increased (2). Figure 4 shows similar plots and interestingly, the slopes remain roughly unchanged. This may be an indication of the fact that as the 2-propanol concentration in eluent is varied, the basic retention mode is not changed. The conclusion is further supported by the plot of ΔH values vs. pK_a values of solutes (Figure 5). Although the measured ΔH values have relatively large associated errors, the plots indicate that the more acidic the compound, the more negative the ΔH value obtained, regardless of the solvent composition.

A proposed interaction at the molecular level between the solutes and the amino bonded phase is proposed in Figure 6. Because phenols are inherently much more acidic than 2-propanol in the eluent, they will always occupy the lone pair electron site of the amino group upon interaction irrespective of the concentration of 2-propanol in the eluent. Thus, a "displacement" or a "direct interaction" involving hydrogen bonding always occur: When the 2-propanol concentration in n-heptane is low, there still are unoccupied lone pair electron sites of amino groups, "direct interaction" may occur. On the other hand, at high concentration of 2-propanol, most lone pair electron sites are taken, phenol will "displace" the less acidic alcohol on the bonded phase. Both type of interactions are between phoneol and

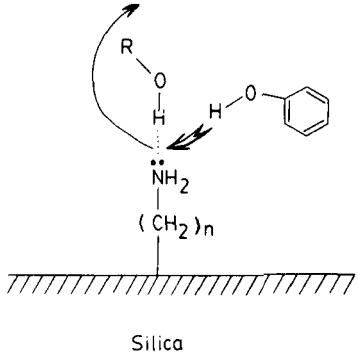


4. FIGURE 4. Plots of log k vs. -ΔH in an amino column using 2-propanol/n-heptane mixture as eluent for the separation of several substituted phenols. % 2-propanol is on the upper-left of each plot. Solutes: o-chlorophenol (O), m-chlorophenol (Δ), p-chlorophenol (⬡), o-cresol (◑), m-cresol (▲), p-cresol (◐).



-ΔH vs. pK_a FOR CHLOROPHENOL ISOMERS

5. FIGURE 5. Plots of -ΔH vs. pK_b value of solutes in an amino column using 2-propanol/n-heptane mixture as eluent for the separation of several substituted phenols. Solute symbols are the same as Figure 4.



6. FIGURE 6. More acidic phenol displaces the adsorbed 2-propanol on an amino stationary phase.

amine which explains why the interaction mode remains unchanged throughout the 2-propanol concentration range studied. It should also be pointed out that the "displacement" mode is consistent with an earlier report of Scott and Kucera (12).

Finally, it is noted that the pK_a values are taken from those measured in aqueous solution. However, it has been noted that the relative pK_a values for various Bronsted acids in a given (aprotic) solvent parallel their classical pk_a values and their relative position in one solvent are usually the same in the other media (13). It should be exercised with caution to extend the same type of study to other systems.

ACKNOWLEDGEMENT

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