Quantitative study of retention processes in reversed-phase liquid chromatography by means of reaction kinetics

Brian S. Ludolph, Chawn-Ying Jeng, Alexander H.T. Chu[*] and
Stanley H. Langer[*]

Department of Chemical Engineering, University of Wisconsin–Madison, Madison, WI 53706 (USA)

ABSTRACT

Degrees of deviation from idealized partition and adsorption retention mechanisms are quantified for reversed-phase liquid chromatographic (RPLC) columns. Rate constants for an *in situ* chemical reaction were measured in octylsilyl and octadecylsilyl bonded stationary phases with a methanol mobile phase. The retention behavior of several types of relevant solute molecules was investigated as a function of chain length and column temperature. With a bonding density of 2.7–3.0 μmol/m^2 on the same support (Beckman Ultrasphere at 200 m^2/g), the chain length (C_8 *vs.* C_{18}) does not appear to impact the retention process, suggesting similar stationary phase characteristics and compositions. However, increased temperature tends to shift retention behavior toward adsorption. The degree of hydrocarbon ligand participation in retention and the phase ratio were also determined to provide a more complete description of the stationary phase composition in RPLC.

INTRODUCTION

Although many retention mechanisms have been proposed for reversed-phase chromatography [1–7], most fall within the two broad categories of partition and adsorption. Experimentally, retention mechanisms have generally been studied by observing retention behaviors of various compounds, often members of homologous series. Subsequently, important system parameters such as mobile phase composition and polarity, bonded-phase chain length and density, and temperature have been varied systematically to infer molecular information about the stationary phase environment [8–16]. Because conventional chromatographic retention studies often fail to provide the information

needed to distinguish between theories, much recent experimental work has focused on alternative approaches for obtaining information about the stationary phase. These encompass the use of spectroscopic techniques such as NMR, IR and fluorescence spectroscopy [17–23] as well as *in situ* chemical reaction methods [24–26].

During an investigation of the base-catalyzed methanolysis of tetrachloroterephthaloyl chloride (TCTPCl$_2$) on a reversed-phase liquid chromatographic (RPLC) column with an octadecylsilane (C_{18}) bonded phase, it was observed by Bolme and Langer [24] that this reaction proceeded faster than might be explained by reaction occurring in the mobile phase volume alone. Since chemical reaction apparently was occurring in the stationary phase, it was suggested that such reaction might provide a probe for characterizing the stationary phase environment. In a subsequent study by Chu and Langer [25], information about the bonded C_{18} phase was inferred by evaluating stationary phase rate

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* Corresponding author.
[*] Present address: Abbott Laboratories, N. Chicago, IL 60064, USA.
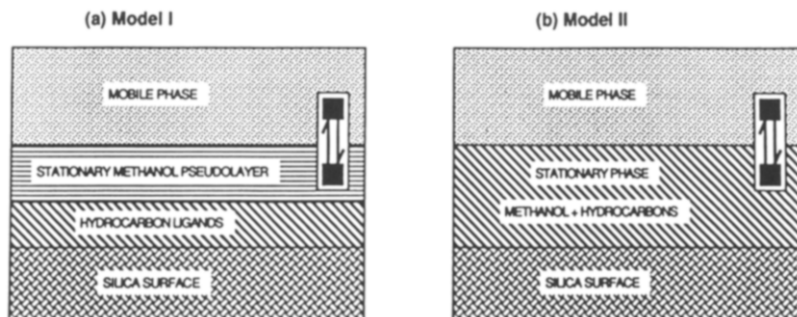
Fig. 1. Molecular retention in RPLC. (a) "Model I". Solute molecules interact with a methanol "pseudo-layer" without direct participation of bonded hydrocarbon ligands. (b) "Model II". Solute molecules interact through a dispersion type of mechanism with a composite stationary phase of methanol sheathing bonded hydrocarbon ligands.

constants, which were then compared with what might be expected from the extreme, generalized retention mechanisms of Knox and Pryde [2] (adsorption type, Fig. 1a) and Yonker et al. [3,4] (partition type, Fig. 1b). The observed rate constants fell between the two models, but much closer to the partition type model. It also appeared that temperature increase tended to shift the retention mechanism toward the adsorption model. Later, the phase ratio of the bonded-phase system was deduced through measuring reaction kinetic measurements for both mobile and stationary phases [27].

In this investigation, the bonded stationary phase of interest is octylsilane (C_8), and reaction kinetics in liquid chromatographic columns have been measured and analyzed in a fashion analogous to that with the earlier bonded phase. These experiments were performed to attempt to answer the following questions: (1) does the shorter chain length in C_8 relative to C_{18} significantly influence the retention mechanism in these systems? and (2) can the temperature effects observed in the C_{18} investigation also be observed with C_8 packings? In addition, the phase ratio and the degree of hydrocarbon ligand and associated methanol participating in retention were evaluated to determine phase compositions for these bonded-phase systems.

THEORY

In a chromatographic reactor for kinetic studies, a reactant is injected as a pulse onto a column where it proceeds to form product(s)

while passing through the column [28,29]. If an inert reference material, I, is added to the reactant mixture it has been shown that for first-order reactions [28,29]

$$\ln\left(\frac{A_R}{A_I}\right) = \ln\left(\frac{A_R}{A_I}\right)_{t=0} - k_{app}t_R \qquad (1)$$

where A_R and A_I are peak areas of reactant and inert standard, t_R is the reactant retention time, and k_{app} is the apparent rate constant for the column.

The apparent rate constant is related to rate constants in the mobile and stationary phases, k_M and k_S, by

$$k_{app} = k_M\left(\frac{t_M}{t_R}\right) + k_S\left(\frac{t_S}{t_R}\right) \qquad (2)$$

A plot of ln (A_R/A_I) vs. t_R gives a straight line slope $-k_{app}$ from eqn.1. Since the residence time ratio between the mobile and stationary phases t_M/t_S is constant for a given chromatographic separation, k_M and k_S cannot be decoupled simply by flow-rate variation. Where k_M is known (e.g. from batch kinetic experiments in pure mobile phase), k_S can be determined using eqn. 2.

Once the stationary phase rate constant has been determined experimentally for a given set of conditions, it can be compared with values from proposed models. For this purpose, two generalized retention mechanism models compared and discussed by Chu and Langer [25] for characterization of the C_{18} phase are used here.

EXPERIMENTAL

Liquid chromatographic system

The basic liquid chromatographic array was similar to the one described earlier and operated in a similar manner [25] except that PTFE tubing [8 in. × 1/8 in. I.D. (1 in. = 2.54 cm), with 10 μm solvent filter] connected the pump inlet and the mobile phase reservoir. Approximately 4 ft. (1 ft. = 30.48 cm) of this tubing were submersed in a constant-temperature water bath as a pre-warming stage for the mobile phase. The HPLC pump outlet was connected to the injector with 10 ft. × 1/16 in. O.D. × 0.020 in. I.D. stainless-steel tubing. Approximately 6 ft. of this tubing were submerged in the constant-temperature water bath to bring the mobile phase to a temperature closer to that of the columns. A 0.5-μm pre-column filter was placed before the two analytical chromatography columns in series.

The liquid chromatographic columns of this study, 2 × (25 cm × 4.6 mm × 5 μm) Altex Ultrasphere Octyl (C_8) were connected as before [25]. Two columns in series gave good separation of reactants and products. Each column contained 3.2 g of 5-μm particles with surface area 200 m^2/g and average pore diameter of 80 Å. The C_8 columns used here are 6.4% (w/w) carbon and 2.7 μmol/m^2 bonded phase ligand [30]. Both types of packings were derivatized with monochlorodimethyl alkylsilanes to give monofunctional coverage. The columns were housed in glass water jackets (25 cm × 20 mm O.D. × 18 mm I.D.) insulated with approximately 3/4 in. layer of glass wool type insulation held in place by aluminum foil.

Reagents and kinetic measurements

Reagents and their preparation have been described earlier [25].

Kinetic studies of $TCTPCl_2$ methanolysis reaction with pyridine and 4-picoline catalysts were performed in the two C_8 column array at both 25 and 35°C. The columns were first equilibrated with the mobile phase for at least 1.5 h at approximately 0.9 ml/min flow-rate, corresponding to about 25 column volumes. The reactant mixture (ca. 0.24 mM $TCTPCl_2$, 26 mM 1-phenyloctane inert standard and 0.2 M tetrahydrofuran in methanol) was prepared immediately before injection to prevent experimental variations in the extent of uncatalyzed reaction. About ten experiments (flow-rates ranging from 0.1 to 0.9 ml/min) were performed at each temperature with several different catalyst concentrations. In addition to the columns, the other difference between C_8 and C_{18} experiments [25] was the use of a smaller sample loop size, 5 μl for C_8 and 20 μl for C_{18}.

Determination of associated methanol volume in the stationary phase

The amount of associated stationary phase methanol for the C_8 columns was determined in a manner similar to that for C_{18} columns [25] (see also refs. 3, 4, 32, 33). The total system volume between the pump drawoff valve and detector inlet was determined by equilibrating the system with methanol at 0.9 ml/min at the specified temperature for at least 1 h. The UV detector inlet fitting was then disconnected and the total system methanol volume flushed into a 100-ml volumetric flask, containing 10.0 ml of absolute ethanol internal standard. Flushing was performed with dioxane at 0.5 ml/min for GC analysis. No residual methanol was found in a second 100-ml aliquot. The volume of methanol associated with the stationary phase, V_{MeOH}, is given by

$$V_{MeOH} = V_{T,MeOH} - (V_M + V_{EC}) \qquad (3)$$

where $V_{T,MeOH}$ is the total amount of methanol between the pump and detector inlet, V_M is the column mobile phase volume plus all dead volumes between the injector and detector inlet found from the unretained species method (Uracil) and V_{EC} is the extracolumn dead volume between the pump and injector. The injector inlet was disconnected and the extracolumn volume was measured after equilibrating it with methanol which was then flushed with dioxane into a 25.0-ml volumetric flask containing 2.5 ml ethanol internal standard.

A Hewlett-Packard (HP) 5890A gas chromatograph with a thermal conductivity detector was used for the "associated stationary phase methanol" determination. The column used was

297–177 μm diameter Poropak Q packed in 180 cm × 2 mm O.D. × 0.6 mm I.D. Pyrex coiled to fit the apparatus. The column wall was silylated to quench residual silanol groups which tend to cause peak tailing. Samples of 0.1 μl containing methanol and dioxane diluent were injected at 28.5 ml/min helium carrier flow and 160°C oven temperature. Starting at 2.5 min after injection, the oven temperature was programmed at a rate of 70°C/min until it reached 230°C to facilitate dioxane elution. An HP 3396A recording integrator was used for collecting and analyzing data.

RESULTS AND DISCUSSION

A typical series of liquid chromatographic reactor chromatograms for the first step in the base-catalyzed methanolysis of TCTPCl$_2$ for the C$_8$ system are illustrated for 4-picoline in Fig. 2.
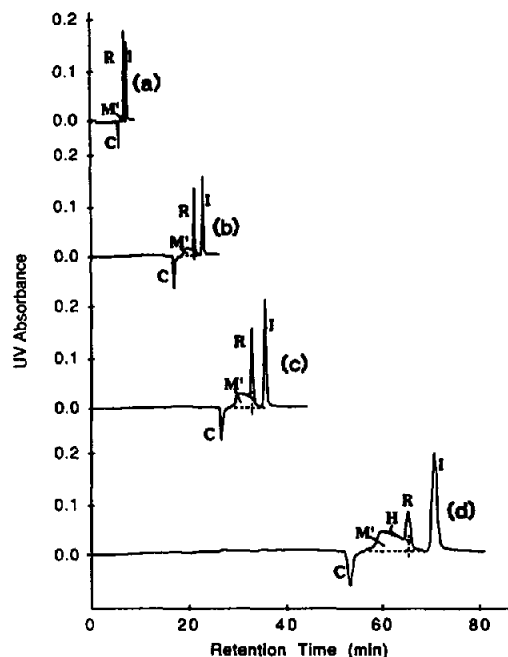

Fig. 2. Series of liquid chromatograms for first step in TCTPCl$_2$ (R) esterification catalyzed by 6.07 mM 4-picoline in methanol at 25°C (see eqn. 4b). The chromatographic system consists of two 25 cm × 4.6 mm Altex Ultrasphere Octyl (C$_8$) columns in series; mobile phase flow-rates (ml/min) were: (a) 0.995, (b) 0.33, (c) 0.228 and (d) 0.114. M' = Quaternary salt; I = inert standard; C = catalyst vacancy peak from injection; H = methanolic half ester impurity.

These resemble those for the C$_{18}$ system of Chu and Langer [25]. Mechanistic details for the complete methanolysis of TCTPCl$_2$ were studied previously [31]. In the present study, we are considering only the first steps in the organic base-catalyzed sequence:
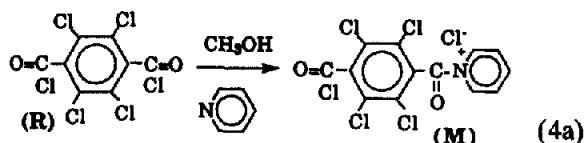
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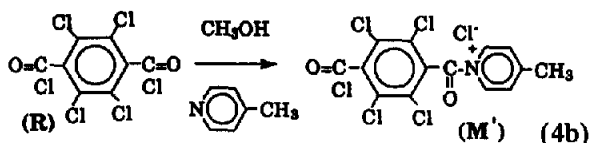
Here TCTPCl$_2$(R) reacts with base (pyridine or 4-picoline) to form the quaternary ammonium salt (M or M') intermediate which is stabilized by solvating methanol [31]. The reaction is second order, first order in reactant and first order in catalyst. As Illustrated in Fig. 2 for 4-picoline, quaternary ammonium salt product M' forms as a continuous "wave" which grows with increasing retention time or decreasing flow-rate. The indicated methanolic half ester product H, Me,Cl-TCTP is original sample impurity conveniently manifested and separated from R as a "bump" on the product wave. The identity of this peak was confirmed in both C$_8$ and C$_{18}$ experiments through comparison of retention times with those of isolated half ester prepared earlier in our laboratories. The small, albeit significant, product interference correction is indicated in Fig. 2.

Determination of amount of methanol associated with the stationary phase

For model predictions of the stationary phase rate constants, the amount of methanol associated with the stationary phase had to be determined. Procedural details for this adapted from Westerlund and Theodorsen [32], McCormick and Karger [33], and Yonker *et al.* [3,4] are discussed in the Experimental section. Table I

TABLE I

VOLUMES ASSOCIATED WITH ULTRASPHERE OCTYL AND OCTADECYL STATIONARY PHASES

Columns, 25 cm × 4.6 mm I.D.

Phase	Temperature (°C)	Methanol associated with stationary phase (see Experimental), V_{MeOH} (ml/column)	Hydrocarbon volumes in stationary phase based on %C, V_{HYD} (ml/column)	Calculated methanol molecules per bonded hydrocarbon chain
C_8	25	0.23 ± 0.06	0.225 [30]	3.3
	35	0.26 ± 0.07	0.225	3.7
C_{18}[a]	25	0.56 ± 0.07	0.50	7.2
	35	0.64 ± 0.06	0.50	8.1

$V_{MeOH}(C_{18})/V_{MeOH}(C_8) = 2.43 \pm 0.71$ (at 25°C), 2.46 ± 0.70 (at 35°C)

[a] Adapted from ref. 25.

presents a summary of volumes of methanol associated with the stationary phases of interest in this work. Included are volumes of methanol associated with the stationary phase at two temperatures for the two systems determined by GC, as well as the hydrocarbon volumes of the stationary phases estimated from percentage carbon loading and density data. It can be noted that the ratios of $V_{MeOH}(C_{18})/V_{MeOH}(C_8)$ are approximately the same for 25 and 35°C and are about equal to 18/8 or 2.25, the ratio of bonded ligand chain lengths. This would indicate that the methanol volume extracted into the stationary phase is approximately proportional to the bonded phase ligand volume over the C_8 to C_{18} range. This result is not obvious. Several investigators [3,34,35] found that the eluent volume extracted into the stationary phase was *not* proportional to the carbon chain length for mixed aqueous–organic eluents. For pure organic eluent systems of the type used here, it would appear that hydrocarbon ligands are sufficiently solvated by methanol to establish a stable methanolic sheath around these chains. The methanol extracted into the stationary phase, then, indeed might vary in direct proportion to total bonded ligand volume (*i.e.* increasing with chain length for a constant degree of surface coverage).

Actually, the number of associated methanol molecules per bonded hydrocarbon chain (C_8 or C_{18}) can be calculated:

$$N_{MeOH} = \frac{V_{MeOH} d_{MeOH}/M_r (= 32 \,g/mol)}{3.2 \,g \times 200 \,m^2/g \times surface\,coverage\,(mol/m^2)}$$

(5)

where d_{MeOH} is the density of methanol at either 25 or 35°C. As shown in Table I, there are approximately 7.2–8.1 methanol molecules associated with each C_{18} ligand *versus* 3.3–3.7 molecules with the C_8 ligand. Considering the molecular volumes of both methanol (67.6 Å³) and the straight chains of C_8 (*ca.* 220 Å³) and C_{18} (*ca.* 430 Å³), the bonded hydrocarbon moieties may be visualized as essentially covered by the intercalated methanol to provide a "liquid" layer or sheath. For the model system here, it is likely that this layer acts as the effective partition medium for solute retention as Dorsey and co-workers have indicated [6,14].

In the stationary phase formation scenario postulated by Yonker et al. [3,4], methanol binding to residual surface silanol groups is believed to be a driving force for stationary phase development in RPLC. Under completely non-aqueous conditions methanol access to the silanol surface is expected to be roughly the same for both C_{18} and C_8 systems. This has been described as "unzipping" of the C_{18} chains to form a brush-like structure, with hydrocarbon ligands more or less extended and perpendicular to the surface [3–5]. A consequence is an in-
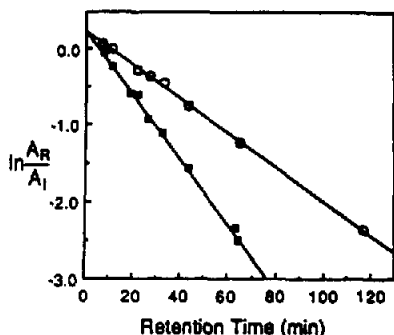
Fig. 3. First-order plot: area ratio A_R/A_I vs. reactant retention time on a semilogarithmic scale for TCTPCl$_2$ reaction in a C$_8$ system with 6.07 mM 4-picoline in mobile phase at 25°C (O) and 35°C (■).

crease in C$_{18}$–methanol Van der Waals dispersion interactions (compared to mixed aqueous systems) with a concomitant increase in total methanol brought into the stationary phase. Observing an associated methanol volume approximately proportional to ligand chain length suggests that the ligand–methanol dispersion interactions with C$_{18}$ ligands resemble those of the more restricted C$_8$ chains.

Calculation of rate constant values in the stationary phase

The apparent rate constant k_{app} was determined from first-order plots of logarithm of A_R/A_I vs. reactant retention time as illustrated for 4-picoline in Fig. 3. Peak areas were measured by planimetry because of the need for correction from product overlap in the reactant area

[25,36], as well as the lack of a software package for this specialized integration. Effects from internal diffusion and sorption kinetics have been shown to be negligible for this system [37].

The experimental rate constants for the stationary phase, k_S, as calculated from eqn. 2 are shown in Table II (25°C) and Table III (35°C). The stationary phase rate constants based on model I and model II calculated as explained earlier (eqns. 16 and 17 in ref. 25) are provided in these tables for comparison. Because values for the proposed model I and II rate constants vary with experimental conditions, a test statistic Y was used to quantitatively treat the effects of experimental variables on solute retention mechanism:

$$Y = \frac{[k_S - (I + II)/2]}{[(II - I)/2]} = 1 \quad \text{if} \quad k_S = k_S(II)$$
$$-1 \quad \text{if} \quad k_S = k_S(I) \qquad (6)$$

where I and II correspond to the stationary phase rate constants predicted by models I [$k_S(I)$] and II [$k_S(II)$], respectively. The test statistic Y is useful because it is dimensionless and independent of chemical kinetics; linear interpolation from model predictions is used to obtain the experimental Y value.

Comparison of experimental and model predicted stationary phase rate constants in Table II (25°C, C$_8$ system) supports a generalized retention mechanism involving the bonded C$_8$ ligands with associated methanol pseudo-layer. This is because although the experimental

TABLE II

STATIONARY PHASE RATE CONSTANTS AT 25°C IN THE C$_8$ SYSTEM, EXPERIMENTAL AND MODEL PREDICTIONS

Base catalyst	Base concentration (mM)	Batch k_M (10^{-4} s^{-1})	k_S (10^{-4} s^{-1})			Test statistic Y
			Experimental	Model I	Model II	
4-Picoline	6.07	4.02 ± 0.06	2.90 ± 0.07	6.52 ± 1.37	1.65 ± 0.58	0.49
	8.18	5.42 ± 0.09	3.55 ± 0.47	8.79 ± 1.85	2.23 ± 0.78	0.60
Pyridine	4.98	1.44 ± 0.05	0.64 ± 0.11	1.86 ± 0.39	0.47 ± 0.17	0.76
	5.10	1.47 ± 0.06	0.62 ± 0.07	1.91 ± 0.40	0.48 ± 0.17	0.81
	7.35	2.12 ± 0.08	0.89 ± 0.15	2.75 ± 0.58	0.70 ± 0.24	0.81
	7.55	2.18 ± 0.08	0.79 ± 0.06	2.82 ± 0.59	0.72 ± 0.25	0.93

TABLE III

STATIONARY PHASE RATE CONSTANTS AT 35°C IN THE C_8 SYSTEM, EXPERIMENTAL AND MODEL PREDICTIONS

Base catalyst	Base concentration (mM)	Batch k_M (10^{-4} s^{-1})	k_s (10^{-4} s^{-1})			Test statistic Y
			Experimental	Model I	Model II	
4-Picoline	2.93	3.46 ± 0.06	3.27 ± 0.16	5.00 ± 1.05	1.42 ± 0.50	−0.04
	4.12	4.86 ± 0.08	3.69 ± 0.24	7.03 ± 1.48	2.00 ± 0.70	0.33
	6.07	7.16 ± 0.11	6.36 ± 0.18	10.35 ± 2.17	2.94 ± 1.03	0.08
Pyridine	4.98	2.54 ± 0.10	2.34 ± 0.09	2.93 ± 0.62	0.83 ± 0.29	−0.44
	7.55	3.85 ± 0.15	3.15 ± 0.13	4.44 ± 0.93	1.26 ± 0.44	−0.19

stationary phase rate constants fall between the two model extremes, they are always closer to model II at 25°C. The data of Table III (35°C, C_8 system) further support a stationary phase operating with features between models I and II, but now shifting toward model I. These findings are consistent with the results of others [6,7,22,23]. Effects on retention models from variation of temperature, alkyl chain length, catalyst type and concentration are discussed below.

Effect of temperature

The retention mechanism for C_8 apparently shifts away from model II (partition type) toward model I (adsorption type) with a temperature increase from 25 to 35°C, consistent with a similar shift toward model I with increasing temperature observed for the earlier C_{18} stationary phase [25]. Fig. 4 shows a Y vs. temperature plot for all data in both studies. The C_8 and C_{18} data are plotted with different symbols and
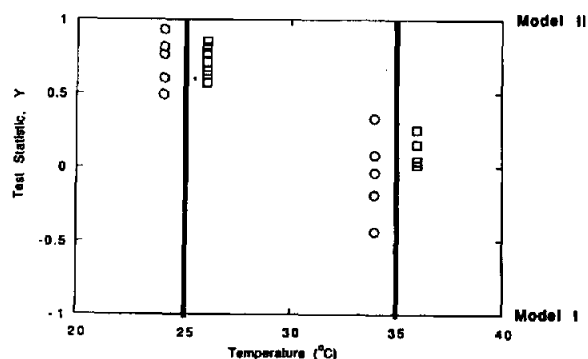

Fig. 4. Plot of test statistic Y values (see eqn. 6) vs. temperature. $O = C_8$; $\square = C_{18}$.

artificially spread apart on the temperature axis to allow comparison (all data were taken at either 25.0 or 35.0°C). Although the data scatters are admittedly large, the retention mechanism shift with temperature change is marked. The lower stationary phase volume for C_8 may well explain greater scatter than with C_{18}. In Table I, the amount of methanol associated with the C_8 stationary phase was found to increase slightly with temperature, similar to the trend found with C_{18}. An increase in thermal energy imparted to the bonded phase ligands at 35°C was postulated to induce chain stretching with accompanying increase in ligand–methanol dispersive interactions [25]. The experimentally determined rate constants might well be indicating that solute molecules preferentially interact with methanol molecules to shift retention toward model I behavior.

Although the temperature effect is difficult to interpret, there may well be one or more interacting factors worthy of consideration. The most plausible are discussed below:

(a) Heat-induced structural changes in the bonded phase layer were suggested by Hammer and Verschoor [16] and Yang and Gilpin [38] for aqueous conditions. For the octyl surface, a sigmoidal change was observed to occur at approximately 40°C (discontinuity temperature). For the octadecyl surface, this "transitional temperature" would probably increase to ⩾70°C [15,17]. Since operational temperatures here are lower than 40°C, the structural change may not be significant enough to account for all retention behavior changes. Of course, the extent of

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change also might be related to bonded chain density (surface coverage) and mobile phase composition differences. However, with the methanol environment here, the amounts incorporated into the bonded octyl and octadecyl layers are relatively constant from 25 to 35°C (see Table I), suggesting a structurally similar surface. Therefore, the magnitude of the retention change from 25 to 35°C indicated in Fig. 4 cannot be explained simply by conformational shift (or folding and unfolding of bonded ligands) resulting from temperature change.

(b) Adsorption interaction with residual silanol groups has been frequently invoked in RPLC literature to explain retention behavior observations [11,39–42]. For the octyl-modified silica surface, thermally induced reordering and/or resolvation has been shown by Gilpin and Wu [43] to be a function of microscopic silanol distribution. Retention contributions from silanophilic interactions have been postulated to be a function of the surface coverage (density) of bonded ligands [23,44]. For surface coverages between 2.7 and 3.0 μmol/m^2 studied here, about one third of maximum lateral packing density (8.1 μmol/m^2) in alkane crystals [6,7], steric constraints among neighboring bonded ligands would tend to force ordering or chain molecule alignment normal to the silica surface, thus facilitating solute access to the silanol functionalities. Therefore, it is possible that silanol groups act as "active sites" to bind both methanol and product base (M or M', pyridinium chloride type of quaternary intermediate salt) molecules to provide a mixed or dual mechanism [45] manifested by the shifting between model I (adsorption) and model II (partition) with changing thermal conditions. With temperature increasing from 25 to 35°C and the increased thermal energy favoring straightening of the bonded ligands, the silanol interactions became more important through improved access of product molecules to silica surface functionalities.

Effect of alkyl chain length. Bonded alkyl chain length does not appear to be a major influence on retention mechanism over the range of experimental conditions here. This may be due to the relatively small solute probe size, so

that the bonded ligand chains with their methanolic sheaths here would be sufficiently deep for solute immersion in the stationary phase environment. This observation is consistent with Tchapla et al. [46] who observed no marked change in selectivity when solute alkyl length was shorter than those of bonded hydrocarbon ligands. Although there is a difference in motional freedom with the alkyl chain distance from the silica surface for C_8 relative to C_{18} [47], the overall retention behavior based on kinetic analysis showed little impact of mobility on solute retention. Similar retention behavior for small solutes in a comparison between C_8 and C_{18} phases was observed by Melander et al. [11] in their study of retention energetics on alkyl bonded stationary phases using unmodified methanol eluent, but not with C_2 alkyl groups. In contrast to these short, highly restricted chains, C_8 chains are probably long enough to permit the same types and degrees of dispersion interactions with solute/solvent molecules observed in the C_{18} stationary phase.

Similarity of retention behavior for alkyl chain lengths greater than eight carbons has been discussed by others [6,7,48]. Comparable stability behavior for chain length greater than C_8 has also been reported [49]. Depending on size and polarity of solute and solvent composition, critical chain lengths can vary from seven to fourteen carbons [13,50,51] in terms of specific underlying retention mechanisms. However, it seems reasonable to conclude that reversed-phase chromatographic retention is generally a partition process [6,7], whereas adsorption is an extreme situation resulting from low surface hydrocarbon coverage [14] or short carbon chain length relative to solute molecular size [11].

Finally, neither catalyst type nor its concentration appear to have significant effects on the retention mechanism as indicated by the relatively constant Y values over the range of variables covered in this work (Tables II and III). Regression analysis of Y values in the 2^4 factorial design provided additional evidence that these effects are statistically insignificant (both t ratios less than 2). Furthermore, the observed negligible concentration effect provides some assurance that the UV detector response was linear

throughout the high catalyst concentration range.

Interpretation of stationary phase rate constants in terms of the fraction of operative stationary phase ligands and associated methanol. The hydrocarbon ligand volumes used in the model II predictions of stationary phase rate constants were made on the basis of 100% ligands participation in retention, and therefore represent upper limits. If some portion of the ligands did not contribute to the retention mechanism, as a result of steric hindrance or non-uniform sheathing with methanol for example, then the operative ligand fraction would be expected to be lower. However, there is probably an overcompensation for the amount of methanol employed in model predictions. Methanol concentration might well be expected to increase near the mobile phase boundary, and decrease close to the restricted access area of the stationary phase support surface [6,25]. Some methanol extracted into the stationary phase might be unavailable for interactions with solute molecules as a result of steric hindrance or other factors. It, therefore, becomes appropriate to consider any effects from overcompensating for hydrocarbon ligand amounts with associated methanol on model predictions for stationary phase rate constants.

In Fig. 5 a hypothetical plot is presented of model I and II stationary phase rate constants *vs.* associated methanol volume for several different volumes of active hydrocarbon ligands in the C_8 system, using 6.07 m*M* 4-picoline as a typical example. Model I is represented by the uppermost curve, where the volume of active hydrocarbon ligands, V_{HYD}, is essentially zero. Model II represents a situation where 100% of the hydrocarbon ligands (all 0.45 ml from the two Beckman columns) are assumed active and results in the lowest curve. Between these extremes are results from model II predictions where 0.35, 0.25 and 0.15 ml of the total 0.45 ml hydrocarbon ligands in the two columns participate in the retention mechanism. The experimentally determined stationary phase rate constant as determined with eqn. 2 is plotted as a single point at the measured amount of stationary phase methanol (0.46 ml) for comparison. In
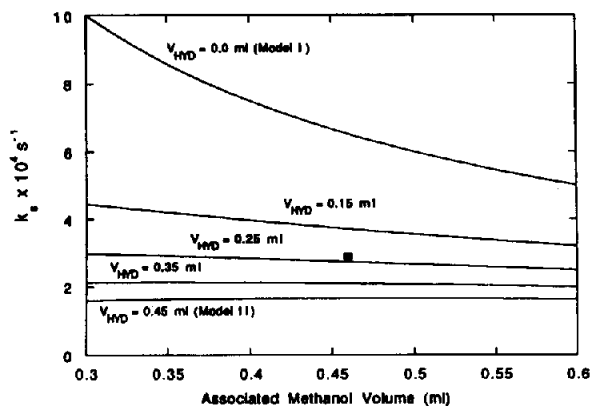

Fig. 5. Expected effects for operative stationary phase hydrocarbon ligands with associated methanol volume on model predictions for the stationary phase rate constant k_s. 6.07 m*M* 4-picoline in the mobile phase, 25°C for the C_8 system; k_s (experimental) = $2.90 \cdot 10^{-4}$ s^{-1}, denoted by ■; V_{MeOH} = 0.46 ml; V_{HYD} (from %C loading) = 0.45 ml.

Fig. 5, the curve with 0.25 ml of the 0.45 ml hydrocarbon ligands active is seen to pass near the experimentally determined stationary phase rate constant. Therefore, if 100% of the methanol is operative in the retention mechanism, the data for this experiment could be explained by a generalized stationary phase in which about 0.25/0.45 or 0.55 fraction of the hydrocarbon ligands participate in solute retention.

With Fig. 5, it is also seen that the experimentally determined stationary phase rate constant cannot be rationalized on the basis of an overcompensated measure of associated methanol and model II with 100% of the hydrocarbon ligands active. While a smaller associated methanol volume might at first be thought to result in an increase in model stationary phase rate constant, the hydrocarbon ligand dilution effect counterbalances this yielding a model II rate constant which is relatively insensitive to the considered volume of associated methanol. Even using only 0.15 ml of the 0.45 ml stationary phase ligands, the model II predicted rate constant is still a weak function of associated methanol. It seems reasonable, therefore, to disregard non-participating methanol effects in the stationary phase, and to use the measured GC value without correction. The fraction of active hydrocarbon ligand and a fairly good estimate of the

column phase ratio can be calculated, therefore, as described below.

Phase ratio determination in C_8 and C_{18}. An alternative approach to rationalizing the results above is to calculate the phase ratio and "degree of bonded phase ligand participation". The data then would be interpreted using a degree of participation, δ, of bonded phase ligands defined by

$$\delta = (V_S - V_{MeOH})/V_{HYD} \tag{7}$$

where V_{MeOH} is the methanol volume associated with the stationary phase determined by GC and V_{HYD} is the ligand volume calculated from percentage carbon loading and ligand density. The effective bonded phase ligand volume participation in retention then becomes δV_{HYD}. Where $\delta = 1$, ligands participate fully in the retention mechanism and the system is equivalent to the partition type model II. With $\delta = 0$, ligands do not participate in the retention and adsorption type model I is fully operative. Values of δ reflect the fraction of ligands participating in partition.

The phase ratio, ϕ, is then

$$\phi = V_S/V_M = (V_{MeOH} + \delta V_{HYD})/V_M \tag{8}$$

where V_S is the stationary phase volume participating in retention, and V_M is the mobile phase volume determined by either the unretained species or homologous series method. The

value of δV_{HYD} can be calculated as shown earlier (eqn. 17 in ref. 27) using V_{HYD}.

Table IV is a summary of δV_{HYD}, V_S and ϕ values from experiments here and earlier [25]. The phase ratio increase resulting from lengthening the bonded ligand chain from 8 to 18 is illustrated in the table. The phase ratio is approximately proportional to bonded phase chain length ($\phi_{C18}/\phi_{C8} = 2.2$ to 3.0) over the 8–18 range studied here. An approximate directly proportional increase can be explained by similar and significant methanol–ligand dispersive interactions in the C_{18} and C_8 stationary phase systems [3,4]. This could be a consequence of the well solvated chains developed in the non-aqueous stationary phase environment of this investigation. Thus, the proportionality between phase ratio and bonded phase chain length results from bonded hydrocarbon volume increase to the surface combined with methanol volume associated with the operative ligand surface area.

It is of further interest to compare the phase ratios here with the capacity factor ratios for the eluites from the C_{18} and C_8 systems as listed in Table V. Void volumes were obtained using Uracil as an unretained species. These retention volumes are essentially independent of temperature and mobile phase composition over the range of the study. The capacity factor ratio between the two columns for these eluites is of special interest. With the retention mechanism of

TABLE IV

PHASE RATIO CALCULATIONS; SUMMARY

Column	Temperature (°C)	Base	Total ligand volume V_{HYD} (ml)	Effective ligand volume δV_{HYD} (ml)	Effective stationary phase volume V_s (ml)	Phase ratio, ϕ
C_8	25	Pyridine	0.45	0.35	0.81	0.16
	25	4-Picoline	0.45	0.24	0.70	0.14
	35	Pyridine	0.45	0.08	0.59	0.12
	35	4-Picoline	0.45	0.15	0.66	0.13
C_{18}	25	Pyridine	1.00	0.49	1.61	0.35
	25	4-Picoline	1.00	0.63	1.75	0.38
	35	Pyridine	1.00	0.43	1.71	0.37
	35	4-Picoline	1.00	0.45	1.73	0.37

TABLE V

SOLUTE RETENTION VOLUMES FOR INDICATED PHASES

Compound	Retention volume (ml)		Capacity factor, k'		Ratio C_{18}/C_8
	C_8	C_{18}	C_8	C_{18}	
TCTPCl$_2$	7.1	9.1	0.38	0.98	2.58
1-Phenyloctane	7.6	10.7	0.47	1.33	2.83
Pyridine	5.8	5.2	0.11	0.13	1.18
4-Picoline	5.9	5.4	0.14	0.17	1.21
Product salts[a]					
Pyridinium salt (M)	6.3	6.6	0.22	0.43	1.95
4-Picolinium salt (M')	6.5	7.1	0.26	0.54	2.08
Void volume (Uracil)	5.2	4.6	–	–	–

[a] See eqns. 6a and 6b.

any given solute molecule identical for both C_8 and C_{18} stationary phase systems, then we might expect that

$$k'_{C18}/k'_{C8} = \phi_{C18}/\phi_{C8} \qquad (9)$$

for that molecule. As can be seen in Table V the capacity factor ratio for the two columns for reactant TCTPCl$_2$ (2.58) and 1-phenyloctane (2.83) inert standard are close to the ratio of the column phase ratios supporting the hypothesis of a similar retention mechanism for these two compounds on these columns.

The capacity factor ratios for catalyst bases pyridine (1.18) and 4-picoline (1.21), on the other hand, are considerably lower than the ratio of column phase ratios. This may well indicate that the retentions for these bases are influenced by residual silanol groups to shift retention mechanisms toward adsorption on the support. Bonded ligand chain length then may also play a role in terms of access. Strong interactions between nitrogeneous bases and residual silanol/siloxane groups on the support frequently are cited to explain irregular retention behavior of these compounds [40] (including significant peak tailing for the two bases used in this study). The capacity factor ratios for the quaternary ammonium salt products (1.95–2.08) fall between

those for catalyst bases and reactant/inert standard. Quaternary ammonium compounds also might strongly interact with silanol groups to experience a dual retention mechanism [45]. Eluite interactions with silanol groups are expected to be greater under non-aqueous conditions where such interactions are not moderated by strongly adsorbed water [11,39]. On the other hand, for both C_8 and C_{18} columns, the resultant "relatively polar" quaternary ammonium type products (M or M') are still retained less than the non-polar compounds (TCTPCl$_2$ and 1-phenyloctane), suggesting non-retention in the bulk bonded phase for the former and that non-specific dispersive interaction between eluite molecules and the bonded hydrocarbon ligand composite phase still dominates retention for the latter. However, the hydrocarbon volume increase from C_8 to C_{18} still resulted in longer retention for the quaternary ammonium salts than for the bases (pyridine and 4-picoline), the capacity factors of which increase little with the change from C_8 to C_{18}.

CONCLUSIONS

It is shown that a kinetic analysis allows a quantitative approach to retention mechanisms for octylsilyl and octadecylsilyl bonded chro-

matographic systems. A dynamic picture involving both stationary phase formation and particular solute interactions emerges. The participation of intercalated methanol with grafted hydrocarbon ligands and sometimes residual silanols gives a complex chromatographic scenario. Results here should assist in formulating a broad-based approach to put retention behavior on a quantitative basis. Although RPLC retention is a function of solvent composition, silica surface bonding chemistry, temperature, etc., it seems reasonable to conclude that it can be considered generally to be a partition process with adsorption becoming operative where possibilities for specific strong interactions become available.

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