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## REORDERING/RESOLUTION OF SILICA IMMOBILIZED NON-HYDROGEN BONDING LIGANDS USED AS STATIONARY PHASES IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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### SUMMARY

The thermally induced reordering/resolution of  $\omega$ -haloalkyl modified surfaces are studied as a function of increasing temperature. The thermal on-set,  $T_0$ , determined from plots of  $\ln k'$  (capacity factor) vs.  $1/T$  varies with chain length and functionality of the immobilized groups. A direct correlation between  $T_0$  and boiling point of corresponding non-immobilized compound is observed. These data are explained by a three term model that accounts for energy differences which occur with reordering/resolution in terms of: (1) cohesive interaction between the bonded chains; (2) hydrophobic interaction between the bonded chains and the contact solvent, water; and (3) specific interaction resulting from hydrogen bonding between aqueous mobile phase and unreacted surface silanols.

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### INTRODUCTION

A majority of high-performance liquid chromatographic (HPLC) separations are carried out using chemically attached stationary phases which are prepared by immobilizing various organosilanes to porous silica. A number of techniques have been utilized to investigate the structure and dynamics of such surfaces<sup>1-14</sup>. The alkyl modified surfaces have received the most attention due to their popularity as reversed-phase packing. With a totally aqueous mobile phase the bonded chains assume one of two preferred orientations, an aggregated collapsed state and a resolved extended state<sup>15-17</sup>. Ligand orientation is dependent on column temperature, chain length<sup>17</sup>, silane backbone structure<sup>18,19</sup>, and solvent composition<sup>15,17,20</sup>.

In a systematic effort to extend earlier work, several monofunctional  $\omega$ -haloalkyl (chloro- and bromo-) modified surfaces have been synthesized. As in the case of bonded hydrocarbon phases these materials exhibit non-linear changes in plots of  $\ln k'$  (capacity factor) vs.  $1/T$ . The thermal on-set,  $T_0$ , of non-linearity is dependent on chain length of and attached functionality on the immobilized groups. A direct correlation between  $T_0$  and the boiling points of similar non-immobilized compounds exists. These results can be explained by a three term model which accounts for reordering/resolution of the bonded groups in terms of: (1) cohesive interaction

between the chains; (2) hydrophobic interaction between the chains and the contact solvent, water; and (3) specific interaction resulting from hydrogen bonding between water and unreacted silanols.

## EXPERIMENTAL

### *Reagents*

5-Bromo-1-pentene, 6-bromo-1-hexene, 8-bromo-1-octene, 4-penten-1-ol, 5-hexen-1-ol, allyl bromide, 1,4-dibromobutane and trichlorosilane were purchased from Aldrich. LiChrosorb Si 60 silica (mean particle size 10  $\mu\text{m}$  and surface area 550  $\text{m}^2/\text{g}$ ) was purchased from MCB.

7-Bromo-1-heptane was synthesized from allyl bromide and 1,4-dibromobutane via a Grignard reaction. An amount of 0.1 mol of allyl bromide was added to 0.1 mol of magnesium metal in 50 ml of dry ethyl ether and refluxed 4 h. Excess dibromobutane (0.2 mol) was added to this mixture which was refluxed overnight. The product was purified by distillation.

4-Penten-1-ol and 5-hexen-1-ol were converted respectively to 5-chloro-1-pentene and 6-chloro-1-hexene by reacting with thionyl chloride and pyridine. An amount of 0.1 mol of unsaturated alcohol was added slowly to a mixture of 0.15 mol thionyl chloride and 0.1 mol pyridine in 50 ml of chloroform and refluxed 4 h. The reaction mixture was diluted with 50 ml ethyl ether and washed three times with 5% hydrochloric acid and twice with distilled water. Finally the organic layer was dried over anhydrous sodium sulfate. The product was separated from the mixture by distillation. Following each preparation, the identity and purity of the materials were verified by gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance spectrometry (NMR).

The final coupling step in the synthesis of the trichlorosilane monomers was carried out in a 50-ml stainless-steel reaction vessel which was initially cooled with dry ice-acetone. Ten drops of 0.1 *M* hydrogen hexachloroplatinate in 2-propanol were added to a 1:2.2 mixture of  $\omega$ -haloalkene-trichlorosilane. The reaction vessel was capped, removed from the dry ice-acetone, and allowed to slowly warm to ambient temperature. Subsequently the vessel was heated to 110°C and allowed to remain at this temperature for two days. The product was purified by vacuum distillation and used to chemically modify LiChrosorb silica as previously described<sup>17</sup>.

### *Column preparation*

The modified materials were packed into 25 cm  $\times$  2.4 mm I.D. stainless-steel columns using a dynamic procedure<sup>20</sup>.

### *Equipment*

An LDC (Riviera Beach, FL, U.S.A.) Model Constametric IIG LC pump with a Model SpectroMonitor III UV detector was used in all studies. Column temperature was controlled in a water bath equipped with a Tempunit (Techne., Princeton, NJ, U.S.A.) Model TU-14 zero cross-over proportional controller and a Neslab (Neslab Instruments, Portsmouth, NH, U.S.A.) Model EN-350 flowthru liquid cooler. The injection system was a Rheodyne (Cotati, CA, U.S.A.) Model 70-10 valve

and a 70-11 loop filter port, which was immersed in the water bath and maintained at the same temperature as the column. To ensure thermal equilibrium of the mobile phase, 6 ft. of 1/16 in. (0.23 mm I.D.) capillary tubing were placed between the injection valve and the column.

### Procedure

Before each evaluation cycle, all columns were conditioned with at least 100 ml of acetonitrile followed by 100 ml of water. This procedure was carried out at the lowest temperature studied. Acetonitrile was chosen as the organic conditioning solvent to minimize entrapment problems<sup>21</sup>. All columns were evaluated through a minimum of two complete cycles at a flow-rate of 1.0 ml/min, using water as the mobile phase. Retention measurements were made as a function of increasing temperature from 10°C to 80°C. Each evaluation cycle consisted of three sequential steps. (1) Initially, the retention times of phenol and resorcinol were measured every five degrees over the temperature range studied. These first measurements were always made following conditioning. (2) The column was cooled to the starting temperature and measurements were again made *vs.* increasing temperature. (3) The column was recooled to the starting temperature a second time and a limited set of measurements made over the same temperature range.

## RESULTS AND DISCUSSIONS

For linear *n*-alkyl phases thermally induced reordering/resolution of the immobilized chains have been observed when these surfaces are in contact with water<sup>17</sup>. In the current study this earlier work has been extended to  $\omega$ -haloalkyl modified silica. The immobilized chains on these latter materials also undergo similar reordering/resolution. A generalized model has been developed and used to explain previous as well as current data in terms of cohesive, hydrophobic and specific forces arising between the bonded chains, the solvent, and the underlying surface.

In carrying out the current investigation changes in the retention volumes of phenol and resorcinol were studied as a function of increasing temperature. Mean capacity factors,  $k'$ , were calculated from multiple injections of each solute. <sup>2</sup>H<sub>2</sub>O was used to determine void volume. Representative plots of  $\ln k'$  *vs.*  $1/T$  for phenol are shown in Fig. 1. These results were obtained on  $\omega$ -chloropentyl modified silica. The general shapes and positions of the curves are similar to those previously reported for *n*-alkyl systems<sup>17</sup>. During the initial evaluation step (*i.e.*, line a) a change in slope was noted at higher temperatures. The thermal on-set,  $T_0$ , for this change was determined for each of the modified surfaces and is summarized in Table I as a function of the bonded groups' chain length and attached functionality. For comparative purposes, previously reported results for mid-range linear alkyl phases also are included<sup>17</sup>. When each column was cooled to the starting temperature and the experiment rerun, a linear relationship between  $\ln k'$  and  $1/T$  was obtained over the total temperature range but it was offset to lower values along the retention axis. This is illustrated for the  $\omega$ -chloropentyl surface in Fig. 1 line b. Although not shown, when the column was cooled a second time and again data were collected they were found also to fit line b.

Shown in Fig. 2 are plots of  $T_0$  *vs.* carbon number of the immobilized chains.

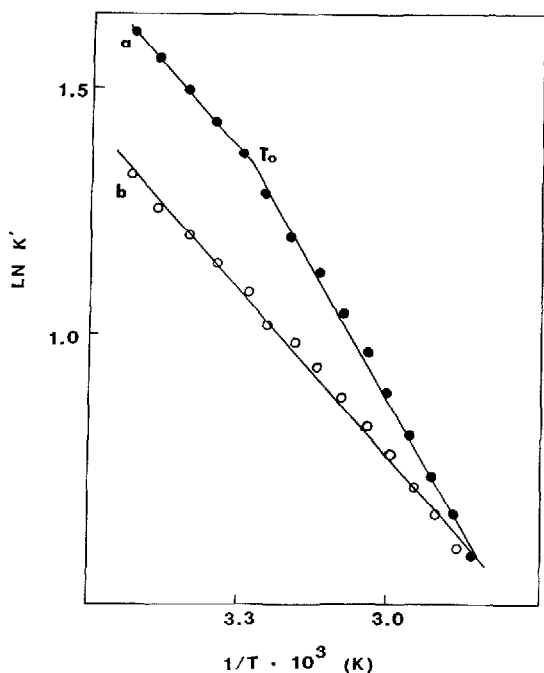


Fig. 1. Graph of  $\ln k'$  vs.  $1/T$  for a chloropentyl modified surface. Test solute: phenol. Evaluation cycles: (a) initial run, (b) rerun.

For a given bonded ligand type (*i.e.*, *n*-alkyl,  $\omega$ -chloroalkyl, or  $\omega$ -bromoalkyl groups)  $T_0$  increased about  $10^\circ\text{C}$  for each methylene added to extend the chain. Likewise, between the group types, the  $\omega$ -chloroalkyl and  $\omega$ -bromoalkyl surfaces were shifted respectively about  $20^\circ\text{C}$  and  $30^\circ\text{C}$  to higher temperatures compared to the normal alkane phases. As noted above, the *n*-alkyl data have been explained in terms of

TABLE I

EFFECT OF CHAIN LENGTH AND FUNCTIONALITY ON CHAIN RESOLUTION

Compound	On-set temperature ( $^\circ\text{C}$ ) <sup>*</sup>	Boiling point ( $^\circ\text{C}$ ) <sup>**</sup>
<i>n</i> -C <sub>8</sub>	41	126
<i>n</i> -C <sub>9</sub>	52	151
<i>n</i> -C <sub>10</sub>	60	174
<i>n</i> -C <sub>5</sub> -Cl	35	108
<i>n</i> -C <sub>6</sub> -Cl	44	135
<i>n</i> -C <sub>5</sub> -Br	43	135
<i>n</i> -C <sub>6</sub> -Br	55	155
<i>n</i> -C <sub>7</sub> -Br	65	179
<i>n</i> -C <sub>8</sub> -Br	none	201

<sup>\*</sup> Determined from linear fits of  $\ln k'$  vs.  $1/T$  data labeled point  $T_0$  in Fig. 1 for chloropentyl surface.

<sup>\*\*</sup> Boiling points of corresponding *n*-alkanes,  $\omega$ -chloroalkanes or  $\omega$ -bromoalkanes<sup>22</sup>.

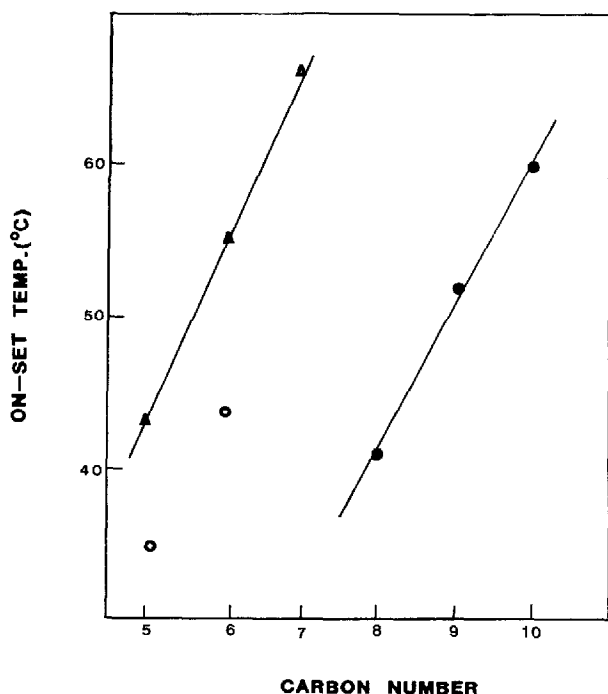


Fig. 2. On-set temperature for reordering/resolution as a function of chain length of the bonded groups for different ligand types. ● = *n*-Alkanes; ○ = *ω*-chloroalkanes; △ = *ω*-bromoalkanes.

changes in the immobilized chains orientation between a collapsed aggregated configuration and a resolved extended configuration<sup>15-17</sup>. The *ω*-haloalkyl data are also consistent with this model.

Perhaps, the most interesting and significant feature emerging from the current study is a direct correlation between the thermal on-set of chain reordering/resolution ( $T_0$ ) and boiling point of the corresponding non-immobilized compound (*i.e.*, *n*-alkanes, *ω*-chloroalkanes, and *ω*-bromoalkanes). This relationship is shown in Fig. 3 where  $T_0$  is plotted against boiling point of the corresponding non-immobilized compound. Changes in  $T_0$  with chain length, shifts in  $T_0$  with functional substitution, and the linear relationship between  $T_0$  and boiling point can be explained qualitatively by a three term model which accounts for energy differences which occur with chain reordering/resolution in terms of chain-chain (cohesive forces), chain-solvent (hydrophobic forces), and solvent-surface (specific forces) interactions. A fourth term is needed for immobilized ligands containing strongly interacting specific groups (*e.g.*, nitrile, amine, etc.). This is the subject of a forthcoming report in which the experimental work is now in progress for hydrogen bonding ligands.

A generalized expression for the total system energy of non-hydrogen bonding ligands,  $E_t$ , is:

$$E_t = E_c + E_h + E_s \quad (1)$$

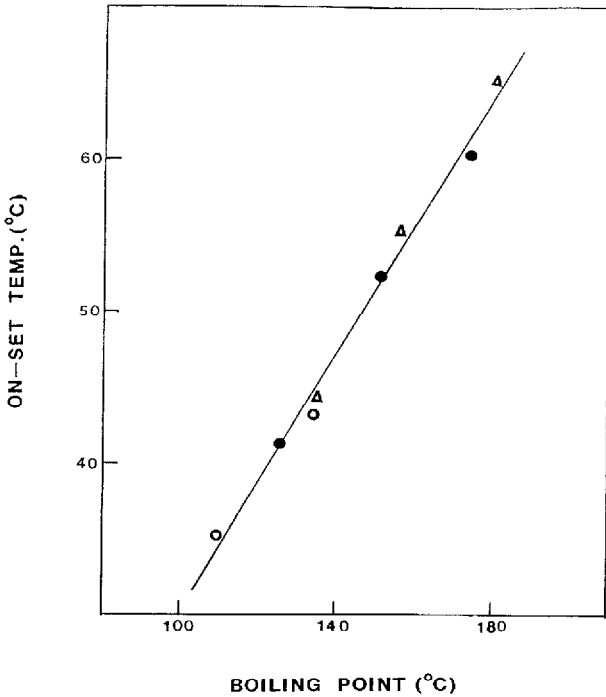


Fig. 3. On-set temperature for reordering/resolution for the bonded groups vs. boiling point of the corresponding *n*-alkanes (●),  $\omega$ -chloroalkanes (○) or  $\omega$ -bromoalkanes (△).

where  $E_c$ ,  $E_h$  and  $E_s$  are the cohesive, hydrophobic and specific interaction energies respectively. Since the chains can assume one of two preferred orientations (*i.e.* aggregated configuration which is designated state 1, and extended configuration which is designated state 2), the total energy of each state can be written as follows:

$$\text{state 1, } E_{t_1} = E_{c_1} + E_{h_1} + E_{s_1} \quad (2)$$

$$\text{state 2, } E_{t_2} = E_{c_2} + E_{h_2} + E_{s_2} \quad (3)$$

When reordering/resolution occurs, the change in energy,  $\Delta E$ , is given by the difference between these two states:

$$\Delta E = E_{t_2} - E_{t_1} = (E_{c_2} + E_{h_2} + E_{s_2}) - (E_{c_1} + E_{h_1} + E_{s_1}) \quad (4)$$

Each of the above individual interaction terms can be expressed as the product of the energy per unit area,  $E'$ , and a function of the total interaction area,  $f(A)$ . Thus  $E = E'f(A)$ . After making this substitution into eqn. 4:

$$\Delta E = [E'_c f(A_{c_2}) + E'_h f(A_{h_2}) + E'_s f(A_{s_2})] - [E'_c f(A_{c_1}) + E'_h f(A_{h_1}) + E'_s f(A_{s_1})] \quad (5)$$

Upon rearrangement,

$$\Delta E = E_c[f(A_{c_2}) - f(A_{c_1})] + E_h[f(A_{h_2}) - f(A_{h_1})] + E_s[f(A_{s_2}) - f(A_{s_1})] \quad (6)$$

The first two terms favor the initially formed associated or aggregated state. Since the immobilized moieties are anchored on the silica surface by covalent bonds, they are separated by some average distance. In the collapsed state, the degree of overlap or association between the anchored chains is determined by spacing and chain length. This thus results in an average cohesive interaction energy for a given chain length and spacing (*i.e.*, average reaction density). After resolution the anchored groups are separated by solvent molecules, reducing the attractive interaction between chains,  $f(A_{c_2}) < f(A_{c_1})$ . Inversely, the hydrophobic interaction, which is repulsive in nature, increases during resolution. This is the result of an increase in the solvent-hydrocarbon contact area for extended *vs.* clustered chains. Thus if only cohesive and hydrophobic forces are considered, the collapsed state is energetically favored. However, experimentally this is not the case as noted previously<sup>17</sup> and by the current data. This is illustrated in Fig. 1.

Once the bonded chains undergo thermally induced reordering/resolution, they remain in the extended state even when the temperature is decreased below  $T_0$ . These results imply that the aggregated state is a metastable state. Thus an increase in the specific interactions between the solvent, water, and unreacted silanol groups (*e.g.* third term in eqn. 6) must be the dominant factor in accounting for differences in stability between the initially formed collapsed aggregated state and the resoluted extended state. Under totally aqueous conditions, water hydrogen bonds with unreacted silanol groups on the surface. In the collapsed state, a portion of these silanol groups are shielded from the solvent by the clustered alkyl chains<sup>21</sup>. However, in the extended state, the covered silanols are exposed and are accessible to solvent, which results in an increase in the total number of silanols sterically available for hydrogen bonding. This has been demonstrated by solvent entrapment studies<sup>21</sup>. An increase in hydrogen bonding area after resolution,  $f(A_{s_2}) > f(A_{s_1})$ , more than compensates for unfavored cohesive and hydrophobic interactions,  $E_c$  and  $E_h$ . When the system is recooled below  $T_0$ , the chains remain in their more stable extended configuration. The above model is diagrammatically illustrated in Fig. 4. Shown in Fig. 4a is an idealized picture of clustered chains shielding an underlying silanol. Similarly, Fig. 4b shows the resoluted chains and an exposed silanol.

The above model can be used to explain increases in on-set temperature with alkyl chain length and functionality (Fig. 2) as well as correlation between  $T_0$  and boiling point (Fig. 3). In order to simplify the current data set, we assume that the reaction density is within a narrow range for both the hydrocarbons and  $\omega$ -halohydrocarbons of different chain length. Although this assumption certainly is not valid over a wide range of chain lengths and differences in ligand shape and attachment chemistry, it is reasonable in the current work for immobilized groups of similar size if reaction conditions are carefully controlled. Experimentally this assumption has been found to be reasonable for mid-range linear groups by microcarbon analysis. Therefore, in the current study the total number of unreacted silanols are nearly constant and to a first approximation  $\Delta E_s$  varies little with small changes in chain length. Conversely,  $\Delta E_c$  and  $\Delta E_h$  are chain length dependent. Both are smaller for

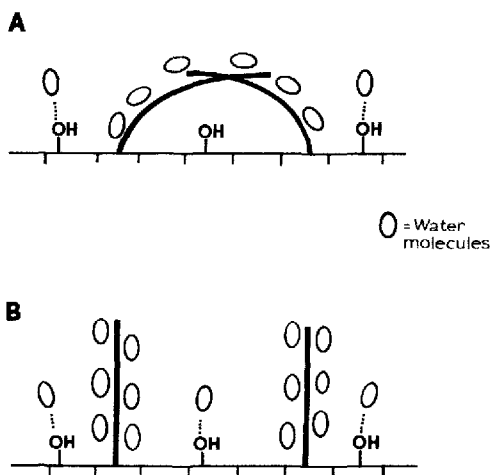


Fig. 4. (A) an aggregated collapsed state. (B) a resolved extended state. — = Immobilized ligands.

shorter chains due to a reduction in the total interaction area and each incrementally increases with addition of a methylene to the chain<sup>23,24</sup>. Thus,  $\Delta E_i$  which directly reflects a shift to higher temperature, increases with increasing chain length. Similarly, for a given chain length,  $\Delta E_s$  of *n*-alkyl chains and  $\Delta E_s$  of  $\omega$ -haloalkyl chains are about the same, but  $\Delta E_c$  decreases in the order  $-\text{Br} > -\text{Cl} > -\text{CH}_3 > -\text{H}$ , due to a reduction in van der Waals forces<sup>23</sup>. Although hydrophobic forces decrease in the order of  $-\text{CH}_3 > -\text{Br} > -\text{Cl} > -\text{H}$ , the relative change with functionality is small compared to relative change in cohesive forces with functionality. Thus the shift in  $T_0$  to higher temperatures respectively for the  $\omega$ -chloroalkyl and  $\omega$ -bromoalkyl surfaces compared to *n*-alkyl surfaces is due principally to cohesive interactions (eqn. 6).

Since the anchored groups are separated by several chemical bonds, the degree of overlap is less than that for free molecules of equivalent chain length. Thus  $T_0$  should be less than but linearly related to the boiling point of the corresponding free molecule. Additionally, the cohesive interaction arising from the monolayer structure of the immobilized groups is more nearly two dimensional compared to the solution state for free molecules. Hence, the approximately 10°C/methylene incremental increase in  $T_0$  for the immobilized chains (*i.e.*, compared to about a 25°C/methylene increase in boiling point of similar non-immobilized compounds, see Table I) is reasonable based on structural differences between the immobilized groups and free molecules.

## CONCLUSION

The thermally induced reordering/resolution of  $\omega$ -haloalkyl modified surfaces under totally aqueous conditions exhibit similar behavior to previously studied *n*-alkyl surfaces. A three term model has been proposed to explain observed experimental results for non-hydrogen bonding ligands in the current study. However, in a forth-



coming report, a fourth term is needed for immobilized ligands containing hydrogen bonding groups (e.g., nitrile, amine, etc.).

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