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## Molecular Dynamics Simulation of the Effects of Mobile-Phase Modification on Interactions in Reversed-Phase Liquid Chromatography

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**Abstract:** The effect of mobile phase composition (CH<sub>3</sub>OH/H<sub>2</sub>O) on the retention of solutes in RPLC was investigated by the molecular dynamics simulation method. A molecular model consisting of silica surface, solvent molecules, and a solute molecule was constructed. The conformation and the mobility of C<sub>18</sub> ligands were characterized by the relative number density profiles of ligands, C<sub>1</sub>-to-C<sub>18</sub> length distribution profiles, and the average mean squared displacement (MSD) of carbon atoms of ligands, respectively. The difference in all profiles due to a change in mobile phase composition is observed clearly, and the facts accord well with other spectroscopic and chromatographic data. The relative number density profiles of methanol and water molecules were constructed to characterize the distribution of solvent molecules around C<sub>18</sub> ligands at each composition. With the increase of methanol content in the mobile phase, solvent molecules penetrate into the bonded phase, which shows the different solvation of the bonded stationary phase. The relative number density profiles of the solute (ethylbenzene) was used to see the distribution of the solute in the separation system. The calculated solute distribution is very consistent with the actual chromatographic retention behaviors.

**Keywords:** Retention mechanisms, Mobile phase composition, Molecular dynamics, Reversed phase liquid chromatography

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## INTRODUCTION

Reversed phase liquid chromatography (RPLC) is a powerful analytical and preparative method to separate a wide range of mixtures. Many research groups have been trying to establish a method to predict the retention time and the elution order of test mixtures. Some use chromatographic retention data,<sup>[1-5]</sup> others develop a number of theoretical models on several hypotheses<sup>[6-11]</sup> where displacement and partition are most often used to represent the solute's distribution between the mobile and stationary phase. Although those works contribute much to the understanding of retention mechanism, no consensus view has emerged on what factor(s) control the retention mechanism in RPLC.

Recently, molecular simulation as a promising technology has been utilized to elucidate the separation mechanism.<sup>[12,13]</sup> Several groups have reported the applications of quantum mechanics, molecular mechanics, Monte Carlo, and molecular dynamics to the investigation of molecular behavior in the chromatographic process.<sup>[14-19]</sup> It is well known that molecular dynamics (MD) simulation is a powerful tool to study the time dependent properties of a multi-molecular system. This technique can be used to easily handle calculations for a huge molecular system, such as the stationary phase in LC, on personal computers. However, few of these reports by MD are described based on a full RPLC system comprising silica-bound hydrocarbon ligands, mixed solvent molecules, and solute molecules using all-atom models. In this paper, an MD simulation was carried out based on a full RPLC system for two purposes. First, we present a molecular level picture of stationary phase/solvent ordering and dynamics near the chromatographic interphase. Second, we can assess the validity of the partition and adsorption theories.<sup>[20]</sup> The key difference between two theories is whether the solute penetrates into the stationary phase or is retained at the interface between solvent and stationary phase. Consequently, neither the partition nor the adsorption separation mechanism fully explained the underlying mechanism for separation in the model system we have chosen. It is doubtful that one of these theories can offer a comprehensive picture of the RPLC retention mechanism.

## EXPERIMENTAL

### Molecular Model

Considering the limitation of the capability of the microcomputer and the calculation time, periodic boundary conditions with the unit cell dimension ( $25 \times 25 \times 40 \text{ \AA}$ ) were applied to generate a mimic of each molecular model, which consists of four parts: an amorphous silica surface, dimethyloctadecylsilyl ligands, solvent molecules, and an ethylbenzene molecule as the

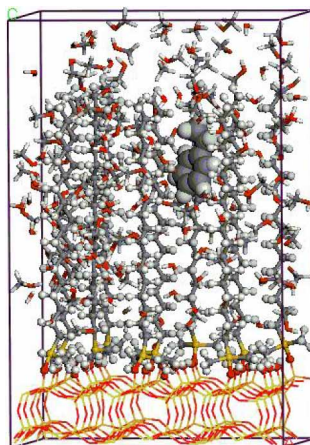
solute. Silica microparticulates form the super surface in a molecular view. Hence, a “SiO<sub>2</sub> cristobalite low” crystal was selected from the Material Studio3.0’s database to mimic the silica surface. These data should provide adequate structural parameters to simulate the suitable silica surface. Surface (1 0 1) was chosen from the calculation result of Material Studio3.0. Then a super surface (25 × 25 Å) was constructed. Atomic coordinates for the silica were fixed during the simulation. C<sub>18</sub> ligands were randomly covalently bonded to the surface at a typical ligand density of 2.6 μmol/m<sup>2</sup>.<sup>[17]</sup> The initial conformation of C<sub>18</sub> ligands was all-trans, and ligands were oriented normal to the surface. The solvent molecules were randomly positioned around ligands. The composition of mobile phase mixtures was varied in 0/100, 20/80, 45/55, 70/30, 100/0 (methanol/water, v/v). The number of molecules of each mobile phase composition was simply calculated as a function of the specific gravity, molecular weight, and Avogadro’s constant. The total number of molecules was summarized in Table 1. In all models, the position of ethylbenzene was determined by “FractionalXYZ” of the carbon atom of ethylbenzene, which connects to the ethyl group. Because of the computing time, we only considered a single molecule of ethylbenzene. The molecular model is displayed in Fig. 1. Computational results were obtained using software program Material Studio3.0, with dynamics calculations performed with Discover.

### Computational Methods

The compass force field<sup>[21]</sup> was used to approximate the potential energy of the simulated systems. A cutoff of 12 Å was used for Van der wall’s interactions and coulombic interactions were treated using the Ewald sum. To avoid bad contacts arising from the initial modeling process, the energy of each system was first minimized by the Steepest Descents followed by the Conjugate Gradients method until the maximum derivative decreased to 0.01 kcal mol<sup>-1</sup> Å<sup>-1</sup>. Then the systems were subjected to a constant

**Table 1.** The number of methanol and water molecules for each solvent composition

CH <sub>3</sub> OH/H <sub>2</sub> O (vol%)	Number of molecules	
	CH <sub>3</sub> OH	H <sub>2</sub> O
0/100	0	330
20/80	30	270
45/55	63	173
70/30	100	97
100/0	128	0



**Figure 1.** Initial state of molecular model.

volume MD simulation with periodic boundary conditions. The Berendsen method was selected as the temperature control algorithm, and the temperature of all systems was kept at 298 K during the simulation. The initial velocities were randomly assigned according to Maxwell-Boltzmann distribution for the given temperature. The equations of motion were solved by the Verlet algorithm<sup>[22]</sup> with an integration time step of 1fs. Equilibration was achieved after 20 ~ 30 ps, followed by a data collection stage of 100 ps. The atomic coordinates were stored on disk every 50 time steps for data analysis.

## RESULTS AND DISCUSSION

### Thickness of the Bonded Layer

The overall position of the alkyl ligands with respect to the surface can be characterized from the thickness of the bonded layer. The average bonded layer thickness in the systems under investigation was estimated by calculating the average distances between the terminal methyl group and the mean Si plane. The mean Si plane was constructed as an average plane containing Si atoms from the upper layer of the surface of each molecular model exemplified by the model displayed in Figure 1.

The average bonded layer thickness values for the systems under investigation are presented in Table 2. It can be seen that the layer thickness values increase along with the increase of the methanol content in the mobile phase. This result indicates that the equilibrium positions of the ligands at high methanol content systems are normal to the surface or

**Table 2.** The bonded layer thickness for each solvent composition

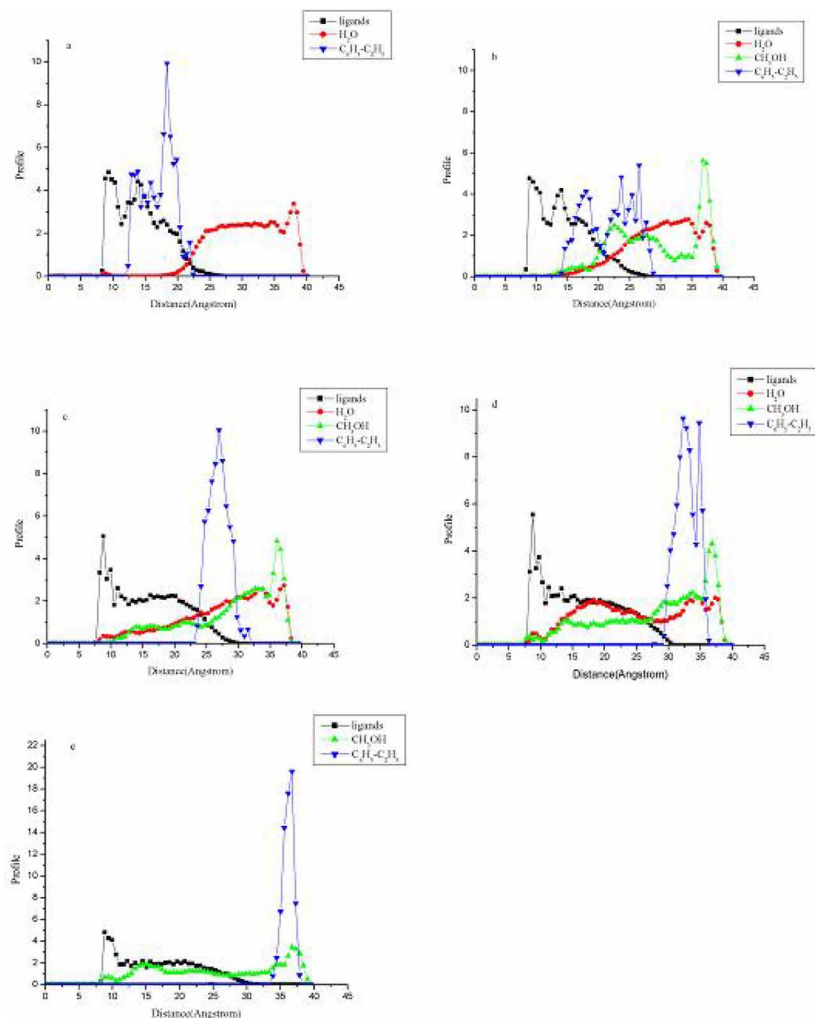
CH <sub>3</sub> OH/H <sub>2</sub> O (vol%)	Bonded layer thickness (Å)
0/100	14.18
28/80	15.38
45/55	19.82
73/30	21.28
100/0	21.44

slightly tilted. It may be related to the penetration of solvent molecules into the bonded phase, which leads to a 'swollen' alkyl layer. This effect was observed by Martire and Boehm,<sup>[23]</sup> who considered the alkyl bonded stationary phases as a 'breathing' surface in which the alkyl ligands become 'swollen' by solvent penetration and are extended away from the support surface in the presence of nonpolar solvents, but collapse upon one another and toward the support surface in the presence of more polar solvents, so as to continually maintain a relatively nonpolar surface character.

### Relative Number Density Profiles of the Ligands, the Solvent Molecules and the Solute

Following the MD simulation, the density profiles of the ligands, solvent molecules, and solute (the average along Z of all alkyl ligands, solvent and solute atoms densities) were derived to characterize the conformation of C<sub>18</sub> ligands and the dynamic behavior of solvent and solute at each solvent composition.

As shown in Fig. 2, with the increased methanol content in mobile phase, more overlap of profiles of solvent molecules with that of the ligands is observed, indicating the increased mixing of solvent and bonded stationary phase. This confirms the conclusions about the bonded stationary phase solvation of previous publications by several groups,<sup>[24,25]</sup> that the amount of organic solvent extracted into the bonded stationary phase is dependent on its concentration in the mobile phase. The profiles of ligands become widely extended, which means an increased bonded phase thickness. This is consistent with the results acquired through other methods,<sup>[26–30]</sup> that a partial extension away from the silica surface in a brush like configuration of the ligands occurs in high methanol mixtures. The profiles of the solute overlap less with that of ligands and more with that of bulk solution, which means that the solute-ligands interaction becomes weaker than the solute-solvent interaction as a result of competition of ligands with solvent to 'solvate' the solute. Thus, the retention of the solute is weaker, which is



**Figure 2.** Relative number density profiles of the ligands, methanol, water, ethylbenzene at each solvent composition. a, 0/100 (methanol/water); b, 20/80; c, 45/55; d, 70/30; e, 100/0.

consistent with RPLC experiments, where increasing the methanol concentration in the mobile phase decreases the retention.

For the molecular model at 100% water (Fig. 2a), the densities of ligands decrease sharply from 17.5 Å with three relatively high peaks at 7.5 Å, 14.0 Å, and 17.5 Å, respectively. This means that ligands are situated mainly below about 17.5 Å, concentrating in three disordered layers near 7.5 Å, 14.0 Å, and 17.5 Å, respectively. In other words, the ligands collapse upon one

another and cover the available silica surface, so it is largely impenetrable to water; the majority of water molecules are positioned above the ligands. It is all due to the repulsive force between the hydrophobic, non-polar alkyl ligands and the relatively polar water molecules. Meanwhile, the small, non-polar solute is trapped on the silica surface by a “tent” of ligand chains, thus the hydrophobic solute is solvated completely by ligands and is retained strongly. This indicates that partition domains the retention mechanism.

As the content of methanol in mobile phase increases (Fig. 2b, c, d, e), methanol molecules preferentially begin to overcome the weak inter ligand dispersion interaction and solvate the aliphatic (alkyl) ligands, because the lipophilicity of methanol is stronger than that of water. Water molecules penetrate into the bonded stationary phase caused by hydrogen bonds among water and methanol molecules, including  $\text{H}_2\text{O}-\text{H}_2\text{O}$ ,  $\text{CH}_3\text{OH}-\text{CH}_3\text{OH}$ ,  $\text{H}_2\text{O}-\text{CH}_3\text{OH}$  hydrogen bonds. The preferential penetration of methanol molecules into bonded phase may result in the composition difference between bulk solution and solvent in bonded stationary phase. The relative density profiles of ligands distribute nearly uniformly between 10 ~ 21 Å, and become widely extended with the increasing methanol content in the mobile phase. That indicates a partial extension away from the silica surface in a brush like configuration of the ligands occurs in high methanol mixtures. It may be caused by preferential penetration of methanol, which “unzips” the ligands interaction from top to bottom of the alkyl ligand chains, the interaction between methanol and ligands becomes stronger than inter-and/or intra-molecular interactions among ligands. Consequently, the ligands become more “erect”, which induces trans conformation. This “brush” like structure increases the ability of the ligands to undergo van der Waals’ interaction with methanol, as there is a larger carbon surface area available for the interaction. More methanol can be brought into the bonded phase by a synergistic effect: van der Waals’ interaction and hydrogen bond, and more water can be brought along with it. Additionally, the open structure of ligands makes the residual silanols on the silica surface more accessible to water, silanol-water hydrogen bond can explain the higher and wider peak of water in highly methanol mobile phase (Fig. 2d). Meanwhile, the solute can no longer be surrounded by the ligands and only a fraction of the solute surface can be in contact with the ligands. Consequently, the solute is partially solvated by solvent and ligands, respectively. This reflects a greater contribution to retention by the adsorption mechanism as the content of methanol in mobile phase increases.

At a methanol/water ratio of 100/0 (Fig. 2e), methanol equably positioned around the ligands from the top to bottom due to the van der Waals’ interaction between the ligands and methanol molecules, which indicates that the ligands seem to be soluble in methanol. The profile of solute is far away from the profile of ligands, which means the solute-mobile phase interaction becomes much stronger, the retention process can be described by an adsorption like mechanism.



### Distance Distribution Between C<sub>1</sub> and C<sub>18</sub> Atoms

The conformation of the hydrocarbon chains was further characterized by constructing the distribution profiles of the C<sub>1</sub>-to-C<sub>18</sub> atoms distance. The maximum C<sub>1</sub>-to-C<sub>18</sub> distance for the C<sub>18</sub> ligand chains in an all trans conformation is about 22 Å. As is evident in Fig. 3, for highly aqueous systems (0/100, 20/80, 45/55), shorter C<sub>1</sub>-to-C<sub>18</sub> lengths in the range of 16 ~ 18 Å corresponding to the gauche conformation occur with the higher probability, while the more extended chains (20 Å or more) has a relatively low probability. In contrast, an increase in the probability of the chains existing in a more extended arrangement for the higher methanol content cases (70/30, 100/0) is observed. This also reveals that collapsed geometries dominate in water rich mobile phase, but that the extended chains conformations are more likely to occur in organic modifier rich mobile phase.

### Ligand Chain Mobility

The mobility of even carbon atoms of ligands was investigated by the self diffusion coefficient of an atom undergoing random Brownian motion in

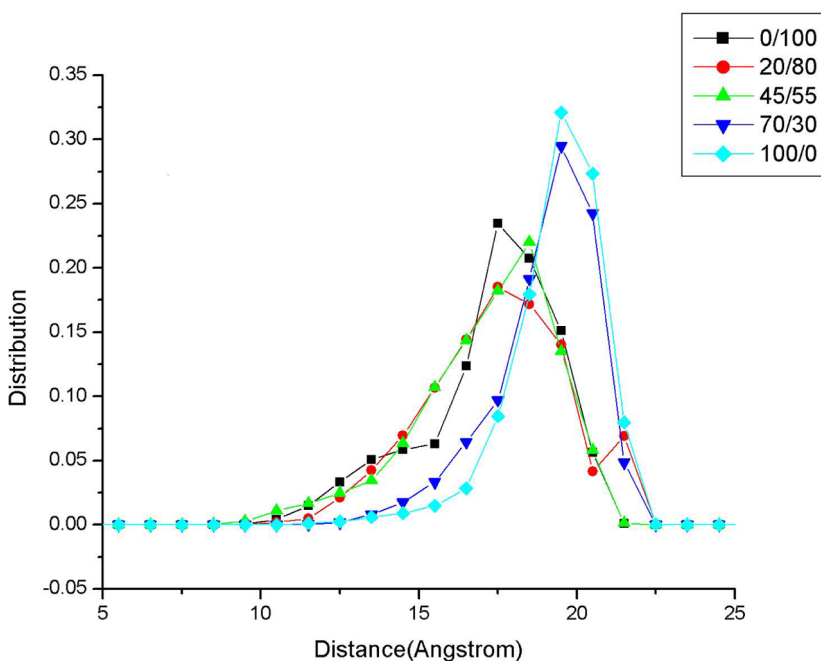
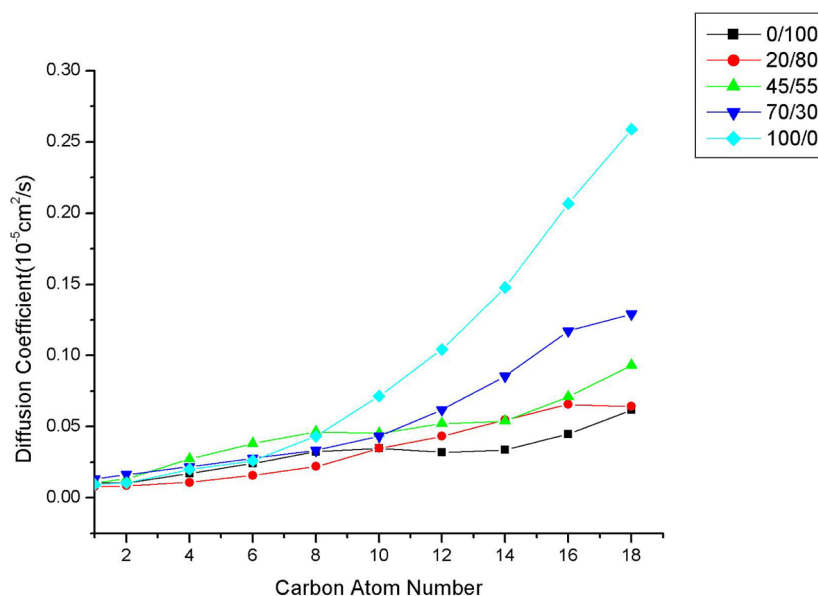


Figure 3. C<sub>1</sub>-C<sub>18</sub> distance distribution of C<sub>18</sub> ligands at each solvent composition.

three dimensions evaluated from the limiting slope of the mean square displacement as a function of time, that is:

$$D = \frac{1}{6N} \lim_{t \rightarrow \infty} \frac{d}{dt} \sum_{i=1}^N \langle [r_i(t) - r_i(0)]^2 \rangle \quad (1)$$

where  $r_i$  denotes the coordinates of atom  $i$ , and the angular brackets denote averaging over all choices of time origin within a dynamics trajectory. It can be seen from Fig. 4, that at all mobile phase compositions the mobility increases from the  $C_1$  atom to the  $C_{18}$  atom. Atoms closer to the silica surface is largely “frozen” into a solid/glossy state, while the chain tails which are distant from the surface exhibit liquid like behavior. Variation of the state of the bonded stationary phase with distance from the surface may have a large impact on retention behavior. These results are in agreement with Pfeleiderer and Zeigler.<sup>[31,32]</sup> In addition, the mobility of the  $C_{10}$ - $C_{18}$  atoms of the ligand chains increases along with the increase of the methanol content in the solvent mixtures. In high water composition systems, the chains self assemble (collapse) and form strong contacts with the silica surface, which reduces a lower chain mobility. In high methanol composition cases, preferential segregation of methanol into the bonded stationary phase induces a more extended chain configuration, which results in a higher mobility as observed. However, the mobility of the  $C_1$ - $C_9$  atoms of the



**Figure 4.** Diffusion coefficients of even carbon atoms of  $C_{18}$  ligands at each solvent composition.

ligands seems not to be effected significantly by the mobile phase composition. Those atoms are so close to the silica surface that they are restricted greatly. In conclusion, change of the mobility of the chains with mobile phase composition may have, to some extent, influence on retention behavior. Larger mobility may result in greater accessibility of the residual silanols on the silica surface to solvent.

## CONCLUSION

An MD simulation has been carried out to investigate the influence of the mobile phase composition on the conformation, mobility, and solvation of the ligands, and the retention of ethylbenzene as the test probe in RPLC. The result of the simulation is consistent with published spectroscopic data and chromatographic measurements. With the increase of methanol content in mobile phase, the ligands extends more in a higher mobility; solvent molecules penetrate more into the bonded stationary phase due to the methanol-water hydrogen bond and the van der Waals interaction between ligands and methanol molecules, and the retention of the solute decreased. To summarize, the retention mechanism tends to be a transition from partition like to adsorption like as the percentage of methanol in mobile phase increases.

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