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Extrathermodynamic interpretation of retention equilibria in reversed-phase liquid chromatography using octadecylsilyl-silica gels bonded to C_1 and C_{18} ligands of different densities

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Abstract

The retention behavior on silica gels bonded to C_{18} and C_1 alkyl ligands of different densities was studied in reversed-phase liquid chromatography (RPLC) from the viewpoints of two extrathermodynamic relationships, enthalpy–entropy compensation (EEC) and linear free energy relationship (LFER). First, the four tests proposed by Krug et al. were applied to the values of the retention equilibrium constants (K) normalized by the alkyl ligand density. These tests showed that a real EEC of the retention equilibrium originates from substantial physico-chemical effects. Second, we derived a new model based on the EEC to explain the LFER between the retention equilibria under different RPLC conditions. The new model indicates how the slope and intercept of the LFER are correlated to the compensation temperatures derived from the EEC analyses and to several parameters characterizing the molecular contributions to the changes in enthalpy and entropy. Finally, we calculated K under various RPLC conditions from only one original experimental K datum by assuming that the contributions of the C_{18} and C_1 ligands to K are additive and that their contributions are proportional to the density of each ligand. The estimated K values are in agreement with the corresponding experimental data, demonstrating that our model is useful to explain the variations of K due to changes in the RPLC conditions.

Keywords: Ligand density in RPLC; Extrathermodynamic relationship; Retention mechanism; Reversed phase liquid chromatography

1. Introduction

Octadecylsilyl (C_{18})-bonded silica gels are the most popular type of packing materials for RPLC [1,2]. Their chromatographic behavior depends on the modification conditions of the C_{18} and C_1 ligands bonded to the base silica gels, for instance on the density and type (monomeric or polymeric) of C_{18} ligands, and on the end-capping treatment with C_1 ligands for residual silanol groups. In some cases, the modification conditions of C_{18} and C_1 ligands are made intentionally in order to attain specific separations.

The influence of the alkyl ligand density on the chromatographic behavior has been studied from the viewpoint of the retention equilibrium [3–8]. In some papers [4–6], linear correlations have been observed between the retention factor (k') and the alkyl ligand density at low density conditions. It was reported that, although κ' initially increases with increasing density of the alkyl ligands, it tends toward a constant level at high ligand densities [4–6]. Limit values of κ' were observed for small sample compounds at high ligand densities [4]. However, the correlations between κ' and the alkyl ligand density become more nearly linear with increasing molecular size of the sample compounds or with decreasing length of the alkyl chain [4,5]. The value of κ' does not begin to plateau when the sample molecules are large enough [4]. The influence of the alkyl ligand density on the separation factor (α_{sep}) was also studied [7,8]. It was reported that the values of α_{sep} for a methylene or a phenyl unit are linearly correlated with the surface coverage of C_{18} ligands, although the increment in α_{sep} for one methylene group is relatively small [8].

The retention equilibrium in RPLC has also been studied from the thermodynamic and the extrathermodynamic points of view. The temperature dependence of κ' was analyzed using the van't

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Hoff equation to derive the changes in the enthalpy (ΔH) and entropy (ΔS) associated with the adsorption of sample molecules from the mobile phase onto the stationary phase. Analyses of retention equilibrium data are supported by a solid theoretical basis, i.e., the thermodynamics of phase equilibria. Extrathermodynamic correlations between thermodynamic parameters have also been studied to discuss retention and separation mechanisms in RPLC. For instance, mechanistic similarities of the retention behavior in RPLC were discussed on the basis of the enthalpy–entropy compensation (EEC) between ΔH and ΔS. Numerous publications have demonstrated an EEC on experimental data [9–20] and supported the possibility of an EEC on theoretical bases [21–25]. Compensation temperatures (T_c) between ca. 500 and 1000 K have been reported for retention equilibria under different RPLC conditions, different mobile phase solvents, sample compounds, and temperature ranges [10,11,15,17–20]. The existence of an EEC suggests that the retention behavior is governed by a single mechanism.

We have also studied the influence of the C_{18} ligand density on some RPLC characteristics [18,26]. With increasing density of C_{18} ligand, the retention equilibrium constant (K), the absolute value of the isosteric heat of adsorption (the enthalpy change due to retention) (Q_{st}), and the activation energy of surface diffusion increase while, in contrast, the surface diffusion coefficient decreases. There is a critical carbon content of the stationary phase above which these four parameters no longer significantly change with increasing C_{18} ligand density. This level probably depends on the size of the sample molecules. It was also suggested that one sample molecule probably interacts with a single C_{18} chain at low C_{18} ligand densities whereas all the C_{18} ligands do not necessarily contribute to the retention behavior of the sample molecules at high ligand densities. The possibility of the interaction of one sample molecule with several C_{18} ligands was denied even when the density of C_{18} ligand is high enough [26]. An explanation for the retention behavior on C_{18}-silica gels is the assumption that the sample molecules penetrate into the layer of C_{18} ligands [27,28]. However, the actual retention behavior in RPLC could be more complicated and we have not yet sufficiently interpreted the influence of the modification conditions of the stationary phase, i.e., the length and density of alkyl ligands, on retention equilibria, notably from the thermodynamic and extrathermodynamic viewpoints.

This paper is concerned with the retention behavior of several C_{18}-bonded silica gels the surface of which is modified with C_{18} and C_1 ligands at different densities. First, it was attempted to explain the experimental retention data of sample molecules on the C_{18} ligands on the basis of the solvophobic theory. We assumed independently parallel contributions of the C_{18} and C_1 ligands on the retention equilibrium. Then, we introduced a new coefficient, κ, the equilibrium constant K normalized by the alkyl ligand density (σ), because the densities of both the C_{18} and the C_1 ligands is changed in this study. We tried to demonstrate the presence of a true EEC relationship for the retention equilibrium in RPLC by analyzing the temperature dependence of κ, according to the four methods proposed by Krug et al. [21–23]. Finally, we derived a new model to account for the influence of several experimental parameters on the retention behavior in RPLC. It was demonstrated that the new model provides a comprehensive interpretation of the variations of K with some RPLC conditions.

2. Experimental

2.1. Columns

Table 1 lists some physico-chemical properties of the stationary phases, i.e., five C_{18}-silica gels (ODS, #1–#5) and one C_1-silica gel (TMS, #6). The RPLC columns packed with these separation media and most of the information were obtained from YMC (Kyoto, Japan). All the stationary phases are synthesized from the same base silica gel. The five C_{18}-silica gels are probably monomeric type packing materials. The density of C_{18} ligand was calculated from the carbon content of the packing materials and the BET surface area of the base silica gel (290 $m^2\,g^{-1}$). The carbon content of the C_{18}-silica gels from #1 to #4 increased from 0.9 to 3.4 wt.% upon end-capping treatment with trimethylsilyl ligands. The end-capping caused no substantial increase in the carbon content of the C_{18}-silica gel #5.

The density of C_{18} ligand was estimated in the range between 0.26 and 3.2 $\mu mol\,m^{-2}$. Although this range is not extremely

Table 1
Physico-chemical properties of RP stationary phases

Packing material/column no.	1	2	3	4	5	6
Main alkyl chain	C_{18}	C_{18}	C_{18}	C_{18}	C_{18}	C_1
Particle density, ρ_p ($g\,cm^{-3}$)	0.67	0.69	0.71	0.79	0.86	0.74
Porosity (ε_p)	0.65	0.61	0.57	0.50	0.46	0.62
Carbon content (%)						
Before end-capping	1.6	3.6	6.4	12.8	17.1	4.1
After end-capping	5.0	6.6	8.6	13.7	17.1	–
C_1 ligand, C_{C_1}	3.4	3.0	2.2	0.9	0	4.1
C_{18} ligand density, $\sigma_{C_{18}}$ ($\mu mol\,m^{-2}$)[a]	0.26	0.59	1.1	2.3	3.2	–
Distance between C_{18} ligands (nm)[a]	2.9	1.9	1.4	1.0	0.81	–
Ratio of silanol group treated with C_{18} ligands (%)[b]	3.2	7.3	13	29	40	–

[a] Calculated from the carbon content before end-capping and the BET surface area of the base silica gel (290 $m^2\,g^{-1}$).

[b] Calculated from the C_{18} ligand density and the density of silanol groups on the surface of the base silica gel (assumed to be 8 $\mu mol\,m^{-2}$).