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Solvent effects on the retention mechanisms of an amylose-based sorbent

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ABSTRACT

The mobile phase, when used in combination with a polysaccharide-based sorbent, often contains hydrocarbons with polar modifiers. Studies have investigated the effect of solvent on the recognition mechanism of polysaccharide-based sorbents, but it remains unclear how these modifier molecules affect solute retention behavior. This study used an amylose 3,5-dichlorophenylcarbamate–based sorbent to systematically investigate the retention behavior of various solutes as a function of the concentration of four polar modifiers: ethanol, isopropanol, methyl *tert*-butyl ether, and acetone. The thermodynamic properties of adsorption for the four modifiers were thoroughly investigated using retention factor data, van't Hoff plots, and adsorption isotherms of the modifier molecules. The adsorption data of acetone and isopropanol followed the Langmuir isotherm, whereas the bi-Langmuir isotherm more accurately fit the ethanol data. For methyl *tert*-butyl ether, the Brunauer–Emmett–Teller adsorption isotherm indicated multilayer adsorption with a low saturation capacity of the first adsorption layer. A multivalent retention model interpreted slopes of the plots of the logarithm of the retention factor versus the logarithm of the modifier concentration. Because the molecular polarity of acetone is stronger than that of methyl *tert*-butyl ether, the limiting absolute slope values of tetrahydrofuran at a very high acetone modifier concentration were smaller than that when methyl *tert*-butyl ether was used as the polar modifier. The higher absolute value of the slope for *tert*-butanol suggested the potential of C=O groups of acetone molecules to form bifurcated H bonds on a *tert*-butanol molecule. When alcohol was employed as the polar modifier, the results suggested that the effect of isopropanol self-association on the retention factor was stronger than the effects of solute–IPA complexation and isopropanol adsorption. For alcohol modifiers, U-shaped retention curves were obtained for all aromatic solutes. When acetone or methyl *tert*-butyl ether was used, the absence of a U-shaped curve indicated the weak polarity of those modifiers.

1. Introduction

Chiral separation in liquid chromatography has advanced considerably and become one of the prevailing methods of obtaining pure enantiomers. Polysaccharide-based (PS-based) chiral stationary phases (CSPs) are commonly employed in enantioselective liquid chromatography. These CSPs can resolve numerous structurally different compounds and generally have high loading capacities. The effectiveness and versatility of these sorbents are due to their high-order structure, a high proportion of which has well-defined chirality [1]. Although much research has studied the recognition mechanisms of these sorbents, further investigation is required to advance the fundamental understanding of such mechanisms.

PS-based CSPs have been recommended for chiral separations in the normal-phase mode [2,3]. This is because hydrogen-bonding interactions are the primary contributors to recognition mechanisms, and these interactions are weakened in aqueous media [4–7]. The mobile phase, used in combination with PS-based CSPs, often contains nonpolar hydrocarbons with low-molecular-weight polar modifiers, such as isopropanol (IPA), ethanol (ETH), tetrahydrofuran (THF), and acetone (AC). A polar modifier is commonly used to modulate the strength of solute–sorbent interactions, which also provides insight into the types of noncovalent bonds involved. Retention factors (k) generally decrease with increasing modifier concentration and depend strongly on the type of polar modifier. Numerous studies have investigated the retention behav-

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ior of solutes with PS-based CSPs and hydrocarbon–polar modifier mobile phases [2,3,6–17].

Ma et al. investigated the enantiomeric separation of two aromatic α-substituted alanine esters on the PS-based CSP Chiralpak AD-H and discovered that enantiomeric elution order (EEO) reversal for one of the compounds depended on the concentration of ETH or *n*-propanol in the mobile phase [18]. By using vibrational circular dichroism spectrometry and density functional theory calculations, they concluded that the alcohol content in the mobile phase affects sorbent conformation by breaking some intramolecular hydrogen bonds. Both the content and type of polar modifier affect the EEO [8]. Okamoto and Nakazawa observed that EEO reversal of pyriproxyfen on cellulose-ester-based CSPs was dependent on the bulkiness of the alcohol modifier used. They found that insertion of the mobile-phase modifier into the chiral cavities of the CSP induced changes in the dominant chiral recognition mechanism, leading eventually to inversion of the EEO. Less bulky alcohols could be inserted into the cavity of the CSP more easily. Despite decades of research on this phenomenon, study results remain inconclusive on the major recognition mechanisms behind it. To understand these mechanisms, this study investigated the effect of polar modifiers on retention behavior.

Several retention models have been proposed to describe the effect of solvent composition on retention behavior for normal phase [3,9–11,15,19–21]. These models are employed to explain the generally linear plots of the logarithm of the solute retention factor versus the molar concentration of a competitive modifier in a nonpolar solvent. However, using a sufficiently wide modifier concentration range, Tsui et al. obtained slightly concave downward retention curves [10,11]. Nonlinearity cannot be explained by the conventional displacement models. A more sophisticated model was developed by Tsui et al. that accounted for self-association of modifiers, multivalent solute adsorption, multivalent solute-modifier complexation, modifier adsorption, and solute intra H-bonding, which might occur in some solutes. Although the model fit the experimental data reasonably well for solutes with strong hydrogen bonding, the fit was poor for those with substantial aromatic interactions. Hsieh et al. investigated the retention behavior of five simple aromatic molecules as a function of IPA concentration using a Chiralpak IA sorbent [15]. U-shaped retention curves were obtained for all aromatic solutes. The aromatic interactions of the solutes with the CSPs were suggested to involve both electrostatic interactions and dispersion forces. At low IPA concentrations, aromatic solute adsorption was dominated by electrostatic interaction, and the retention time decreased when the IPA concentration was increased. Higher than a certain IPA concentration, solvophobic interactions dominated and the retention factor increased as the IPA concentration was increased further. The authors concluded that solvophobic interactions should be considered when investigating the retention behavior of solutes associated with aromatic groups.

Although numerous studies have investigated the solvent effect on the recognition mechanism of PS-based sorbents, few have systematically investigated the retention behavior of solutes with different functional groups as a function of concentration of various polar modifiers. Such a systematic investigation would improve our understanding of the molecular recognition mechanism of PS-based CSPs. Therefore, in this study, the retention behavior of various monovalent solutes was evaluated using ETH, IPA, methyl *tert*-butyl ether (MTBE), and AC separately as polar modifiers. The solutes investigated were AC, THF, MTBE, methanol (MET), *tert*-butanol (TBA), benzene (BZN), naphthalene (NPL), and mesitylene (MSL). Although PS-based CSPs were not used to separate small molecules, these monovalent solutes were employed as probes to study interactions of the modifiers with the stationary phase. AC, THF, and MTBE contain oxygen groups which can form H bonds with the NH functional groups of the sorbent, whereas the hydroxyl groups of alcohols may form H bonds with sorbent C=O groups. Aromatic solutes were used for probing possible π-π interactions with the sorbent. One of the most widely used amylose carbamate CSP, Chiralpak IE (IE), was chosen for the investigation [22]. The sorbent backbone has a conformation of an amylose helical structure. Three hydroxyl groups of each monomer are substituted with 3,5-dichlorophenylcarbamate groups. The polymer was covalently bonded onto the surface of silica beads as a thin film, which considerably enhanced the solvent resistance and mechanical stability of the sorbent [3,8,22–24]. Therefore, the solvent swelling phenomenon of the sorbent was assumed to be negligible in this study. This study used chromatographic retention models and thermodynamics to investigate the retention behavior of PS-based CSPs with different solvent strength modifiers for understanding the effect of solvent on retention behavior and providing insight into the complex interaction mechanisms of chiral molecules.

2. Theory

To model the dependence of normal-phase retention behavior on polar modifier concentration, Tsui et al. developed retention models by considering modifier self-association, solute–modifier complexation, and the competitive adsorptions of the solute and modifier [10,11]. For a solute with multiple H-bonding functional groups, the retention model can be expressed as

$$\ln k = -\ln\left(1 + K_{\mathrm{SL-M}} C_{\mathrm{M}}^{y}\right) - x\ln\left(1 + K_{\mathrm{M}} C_{\mathrm{M}}\right) + \ln\left(K_{\mathrm{SL}} q_{\mathrm{M},max}{}^{x} \phi\right) \quad (1)$$

$$C_{\mathrm{M}}^{0} = C_{\mathrm{M}} + nK_{\mathrm{Mn}} C_{\mathrm{M}}^{n} \quad (2)$$

where K_{SL}, K_{M}, and $K_{\mathrm{SL-M}}$ (all in units of M^{-1}) are the equilibrium constants for the solute–sorbent, modifier–sorbent, and solute–modifier interactions, respectively; K_{Mn} (in M^{1-n}) is the equilibrium constant for modifier self-association; $q_{\mathrm{M},max}$ (in mol/L of solid volume, or M') is the saturation capacity of the adsorbed modifier; C_{M} (in M) is the free modifier monomer concentration in the mobile phase; $C_{\mathrm{M}}{}^{0}$ (in M) is the total modifier concentration in the mobile phase; ϕ is the ratio of solid volume to liquid volume in the column; x is the number of modifiers displaced from the sorbent surface upon solute adsorption; y is the number of modifiers displaced from the solute–modifier complex upon solute adsorption; and n is the average modifier self-association number in the solution.

In this model, both the solute and modifier are assumed to interact homogeneously with the sorbent. Furthermore, one-to-many solute–modifier complexation and solute–sorbent adsorption are assumed. A one-step aggregation process with aggregation number n is assumed to describe modifier self-association behavior. The dependence of k on $C_{\mathrm{M}}{}^{0}$ isdetermined by the four equilibrium constants and the parameters x, y, n, φ, and $q_{\mathrm{M},max}$

When the total modifier concentration is large enough or zero, Eqs. (1) and (2) are reduced to Eqs. (3) or (4):

$$\ln k \approx -\frac{x+y}{n}\ln\left(C_{\mathrm{M}}^{0}\right) + \ln\left[\frac{K_{\mathrm{SL}}\cdot\left(nK_{\mathrm{Mn}}\right)^{x+y/n} q_{\mathrm{M},max}{}^{x}\cdot\phi}{K_{\mathrm{SL-M}} K_{\mathrm{M}}{}^{x}}\right] \quad (3)$$

$$k = K_{\mathrm{SL}} q_{\mathrm{M},max}{}^{x} \phi \quad (4)$$

where C_{SL} (in M) is the free solute concentration. When the total modifier concentration is large enough, the absolute value (B) of the slope is close to $(x + y)/n$. When the modifier concentration is negligible, the retention factor is equal to $K_{\mathrm{SL}} q_{\mathrm{M},max}{}^{x}\phi$, and Eq. (4) is obtained.