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Apparent and true enantioselectivity of single- and binary-selector chiral stationary phases in gas chromatography

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Abstract

Almost all gas-chromatographic chiral stationary phases (CSPs) are complex systems containing one or more chiral selector(s) dissolved in, or bonded to, an achiral solvent such as squalane or poly(dimethylsiloxane). The presence of different components in the total CSP, interacting independently with the analyte enantiomers, impairs the elucidation of enantiorecognition mechanisms and complicates the optimization of enantioseparations. In the present work a quantitative analysis of the influence of different factors on the observed enantioselectivity is performed. The parameters varied in this study were the composition of the CSP, the concentration and the enantiomeric excess of the chiral selector(s) and the presence of achiral selectors (including racemic compositions). Special attention is given to the determination of distribution and association constants, as well as apparent and true enantioseparation factors.

Keywords: Enantioselective gas chromatography; Apparent and true enantioselectivity; Apparent and true enantioseparation factor; Binary-selector chiral stationary phase; Mixed chiral stationary phase; Association and distribution constant; Nonenantioselective interactions

1. Introduction

In contemporary enantioselective gas chromatography (enantio-GC), a chiral selector A, e.g. modified cyclodextrin, is either dissolved in [1,2], or chemically bonded to [3,4] an achiral solvent S (often a poly(dimethylsiloxane)) and is used in the undiluted form only in exceptional cases [5]. The distribution equilibria of the selectand enantiomers D and L between the gas phase (designated with subscript g) and the neat achiral solvent (designated with subscript s) are determined by the nonenantioselective distribution constants, K_D^o and K_L^o, respectively. Due to the achiral nature of S, K_D^o and K_L^o are identical:

$$D_g \overset{K_D^o}{\rightleftarrows} D_s, \qquad L_g \overset{K_L^o}{\rightleftarrows} L_s$$

The association equilibria between the selectand enantiomers D and L and the enantiopure selector A are determined by the association constants, K_{AD}^{assoc} and K_{AL}^{assoc}, respectively. When the enantiopure selector A is enantioselective towards the selectand enantiomers D and L, the constants K_{AD}^{assoc} and K_{AL}^{assoc} are rendered nonequivalent:

$$A + D_s \overset{K_{AD}^{assoc}}{\rightleftarrows} AD, \qquad A + L_s \overset{K_{AL}^{assoc}}{\rightleftarrows} AL$$

The ratio of the association constants determines the *true enantioseparation factor* of the selectand enantiomers D and L enantioseparated on the chiral stationary phase (CSP) containing selector A, i.e. $\alpha_{true} = K_{AD}^{assoc}/K_{AL}^{assoc}$, which is related to the enantioselectivity of the *chiral selector* A. The Gibbs energy difference between the diastereomeric associates AD and AL is determined by the expression $-\Delta_{AD,AL}(\Delta G) = RT \ln(\alpha_{true})$, where R is the gas constant and T is the absolute temperature.

If the chiral selector A is dissolved in, or bonded to, an achiral solvent S, the distribution of the selectand enantiomers D and L between the gas phase and the CSP, A in S, is now described by the apparent distribution constants, $K_D^{A\,in\,S}$ and $K_L^{A\,in\,S}$, respectively. When the enantiopure selector A is enantioselective towards the selectand enantiomers D and L, the apparent distribution constants $K_D^{A\,in\,S}$ and $K_L^{A\,in\,S}$ are rendered

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nonequivalent:

$$\mathrm{D_g} \overset{K_{\mathrm{D}}^{\mathrm{A\,in\,S}}}{\rightleftarrows} \mathrm{D_{A\,in\,S}}, \qquad \mathrm{L_g} \overset{K_{\mathrm{L}}^{\mathrm{A\,in\,S}}}{\rightleftarrows} \mathrm{L_{A\,in\,S}}$$

The ratio of the apparent distribution constants $K_{\mathrm{D}}^{\mathrm{A\ in\ S}}$ and $K_{\mathrm{L}}^{\mathrm{A\ in\ S}}$ determines the *apparent enantioseparation factor* of the selectand enantiomers D and L enantioseparated on the CSP, A in S, i.e. $\alpha_{\mathrm{app}} = K_{\mathrm{D}}^{\mathrm{A\,in\,S}}/K_{\mathrm{L}}^{\mathrm{A\,in\,S}}$. The enantioselectivity of the *total chiral stationary phase*, A in S, is quantitatively determined as the Gibbs energy difference between the transfer of the selectand enantiomers D and L from the mobile phase into the total CSP, A in S, by the expression $-\Delta_{\mathrm{D,L}}(\Delta G) = RT\ln(\alpha_{\mathrm{app}})$, where R is the gas constant and T is the absolute temperature.

The expression $-\Delta_{\mathrm{D,L}}(\Delta G) = RT\ln(\alpha_{\mathrm{app}})$ has often been erroneously used for the assessment of the Gibbs energy difference between the diastereomeric associates AD and AL, i.e. for the determination of the enantioselectivity of the chiral selector A. Since the selector A is diluted with S and the latter contributes to the retention of the selectand enantiomers to the same extent, the apparent enantioseparation factor α_{app} does not correspond to the enantioselectivity of the chiral selector A. This notion has been expressed many times in the literature [6–12]. Thus, whereas the apparent enantioseparation factor α_{app} is important from the practical point of view because it reflects the observed separation capabilities of the entire enantioselective GC system, the true enantioseparation factor α_{true} is of theoretical importance and can be used for the elucidation of enantiorecognition mechanisms. Thus, α_{app} is related to the enantioselectivity of *the total CSP*, composed of a chiral selector dissolved in, or bonded to, an achiral solvent, while α_{true} is related to the enantioselectivity of *the chiral selector* A present in the solvent. As the true enantioseparation factor is always higher than the apparent enantioseparation factor, the enantioselectivity of chiral selector A is often underestimated in the literature.

In 1975, Laub and Purnell [13] suggested the use of mixed stationary phases in GC for the improvement of separation of complex mixtures of achiral compounds. Since then many reports on the preparation and use of CSPs containing more than one chiral selector appeared in the literature [9,14–21]. The notion behind the binary-selector CSP approach is the endeavor to combine complementary enantioselectivities of two (or more) different selectors in one stationary phase, thereby expanding the scope of the enantioseparations [9,19–21]. In binary-selector CSPs, due to the presence of two chiral selectors, A and B, diluted in an achiral solvent S, the apparent enantioseparation factor α_{app} depends not only on the concentration of the chiral selectors but also on the molar ratio between them. Moreover, the enhancement (‘matched case’) and the compensation (‘mismatched case’) of enantioselectivity have to be considered.

In the present treatise, a comprehensive theoretical treatment of the enantioseparation on single- and binary-selector CSPs is presented. Methods for the determination of the apparent and true enantioseparation factors are described for both single- and binary-selector CSPs. Analyses of different factors influencing the apparent enantioseparation factor α_{app} obtained on single- and binary-selector CSPs are presented.

1.1. Terms and abbreviations used in the paper

In the present treatise the gas-chromatographic column constitutes an open tube coated with a thin film of a CSP. The total CSP consists of a chiral selector A or B, or a mixture of them, A and B, diluted in, or bonded to, an achiral solvent S. Such CSPs will be referred to as CSP, A in S, B in S, and, AB in S, respectively. The CSPs containing a single selector will be termed single-selector CSPs, the CSPs containing two selectors will be called binary-selector CSPs and the CSPs composed of more than two selectors will be called multi-selector CSPs. The chiral selectors A and B are considered to be strictly enantiomerically pure. However, at the end of this paper the case of enantiomerically impure selectors will be discussed. The chiral selectand, D and L, (considered to be racemic) consists of the first eluted enantiomer L and the second eluted enantiomer D, irrespective of their absolute configuration, at a given temperature. The occurrence of peak coalescence at the isoenantioselective temperature T_{iso} and peak reversals below and above T_{iso} [22] will not be considered.

The subscripts “g” and “s” refer to the gas phase and the achiral solvent S, respectively. $\mathrm{D_g}$ ($\mathrm{L_g}$) and $\mathrm{D_s}$ ($\mathrm{L_s}$) correspond to the enantiomer D (L) present in the gas phase and in the achiral solvent S, respectively. AD and BD (AL and BL) relate to the corresponding associates (complexes) formed between selectors A or B and selectand enantiomer D (L), respectively. All following terms correspond equally to the L enantiomer provided the subscript “L” is used instead of “D”.

The terms c_{A}, c_{AD} and c_{Ds} represent the concentrations of A, AD and $\mathrm{D_S}$ in the achiral solvent S. It should be noted that if measurements are performed at different temperatures, molality (mol/kg) instead of molarity (mol/L) should be used because of the possible thermal expansion of the stationary phase. The use of concentrations instead of activities is a simplification which is only allowed at a high dilution of A and D in S [23]. $K_{\mathrm{D}}^{\mathrm{o}}$ is the distribution constant of D corresponding to its partitioning between the gas phase and the achiral solvent S devoid of the chiral selector A. $K_{\mathrm{D}}^{\mathrm{A\,in\,S}}$ is the apparent distribution constant of D corresponding to its partitioning between the gas phase and the CSP, A in S. $K_{\mathrm{AD}}^{\mathrm{assoc}}$ is the association constant corresponding to the reversible formation of the associate AD. $\beta^{\mathrm{A\ in\ S}}$ is the phase ratio of the column containing the CSP, A in S. $k_{\mathrm{D}}^{\mathrm{A\,in\,S}}$ and $k_{\mathrm{St}}^{\mathrm{A\,in\,S}}$ are retention factors of the selectand D and an achiral standard compound “St” that does not interact with the selector A, respectively, obtained on the CSP, A in S. $r_{\mathrm{D}}^{\mathrm{A\,in\,S}}$ refers to the retention of D relative to the inert standard compound St on the CSP, A in S. $\Delta_{\mathrm{AD,AL}}(\Delta G)$ is the Gibbs energy difference between the transient associates AD and AL. R is the gas constant and T is the absolute temperature. For binary selector systems, $x_{\mathrm{A}}^{\mathrm{AB}}$ and $x_{\mathrm{B}}^{\mathrm{AB}}$ are the mole fractions of A and B in the mixture of A and B, respectively. n_{A} and n_{B} are the mole amounts of A and B, respectively.