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Comparison of two methods for the gas chromatographic determination of thermodynamic parameters of enantioselectivity

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Abstract

The thermodynamic parameters responsible for the formation of diastereomeric associates between enantiomers of methyl lactate and two Lipodex E columns [with 5.37% or 10.08% of octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin in SE 54] were calculated using two different methods. Method A was based on the direct determination of $\Delta(\Delta G^0_{R,S})$ from experimental values of the chiral separation factor α obtained at different temperatures for the separation of the enantiomers of methyl lactate on two Lipodex E columns. Method B relies on the determination of the relative retention of the enantiomers of methyl lactate in respect to an inert reference standard on a reference column containing only the solvent and that of the enantiomers of methyl lactate to the same reference standard on the reactor column containing the cyclodextrin derivative in the solvent S. SE 54 was used as solvent for Lipodex E and *n*-heptane, *n*-octane and *n*-nonane were used as reference standards. Obtained results for $\Delta_{R,S}(\Delta G^0)$ show that the data calculated by Method A are too small and are dependent on the concentration of the chiral selector in the diluted stationary phase. Since the dependence of ln α on $1/T$ for methyl lactate enantiomers on Lipodex E columns was nonlinear, it was not possible to calculate $\Delta_{R,S}(\Delta H^0)$ and $\Delta_{R,S}(\Delta S^0)$ values by this method. Results obtained by Method B are essentially independent on the choice of reference compound and the concentration of the chiral selector in the mixed stationary phase.

Keywords: Enantiomer separation; Enantioselectivity; Thermodynamic parameters; Methyl lactate

1. Introduction

For the separation of enantiomers various chromatographic and electrophoretic methods are employed [1,2]. The understanding of mechanistic aspects of chiral recognition in chromatography is important because it allows one to design improved chromatographic systems and it addresses fundamental concepts in chiral recognition also for disciplines outside the separation science. Chiral recognition mechanisms in chromatography have been extensively studied using various methods such as computational chemistry [3,4], quantitative structure retention relationships [5], chemometric tools [6] or NMR methods [7].

The direct enantiomer separation is based on the formation of reversible diastereomeric associates or complexes which are created by intermolecular interactions of enantiomers with a chiral selector [8,9]. This formation process can be characterized by Gibbs–Helmholtz thermodynamic parameters (ΔG^0, ΔH^0, and ΔS^0) which are different for the *R* and *S* enantiomer. These can be calculated for both enantiomers according to the general equation:

$$\Delta G^0_i = -RT \ln K_i \quad (1)$$

where K_i is the association constant of a diastereo-

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meric associate of considered enantiomer (selectand) with a chiral selector [9,10].

As there are some problems with the measurement of the association constants of individual enantiomers, differences [$-\Delta_{R,S}(\Delta G^0)$, $-\Delta_{R,S}(\Delta H^0)$ and $-\Delta_{R,S}(\Delta G^0)$] are usually determined [11].

The aim of this work was to calculate thermodynamic parameters responsible for the formation of diastereomeric associates between enantiomers of methyl lactate on two Lipodex E columns coated with 5.37% or 10.08% of octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin in the polysiloxane SE 54 by gas chromatography. For the calculation of thermodynamic parameters two different methods published in the literature were compared. Method A is based on the direct determination of $-\Delta_{R,S}(\Delta G^0)$ from experimental values of the chiral separation factor α obtained at different temperatures by the separation of the enantiomers of methyl lactate on two Lipodex E columns while Method B relies on the determination of the relative retention of the enantiomers of methyl lactate in respect to an inert reference standard on a reference column containing only the solvent and that of the enantiomers of methyl lactate to the same reference standard on the reactor column containing the cyclodextrin derivative in the solvent S. SE 54 was used as solvent for Lipodex E and *n*-heptane, *n*-octane and *n*-nonane were used as reference standards.

2. Theoretical

2.1. Method A

In this method, $-\Delta_{R,S}(\Delta G^0)$ is calculated from the chiral separation (selectivity) factor α according to the general equation [10]:

$$-\Delta_{R,S}(\Delta G^0) = RT \ln \alpha \quad (2)$$

where α relates the retention factors of the enantiomers *R* and *S* ($\alpha = k_R/k_S$) and *S* refers arbitrarily to the first-eluted, and *R* to the second-eluted enantiomer, respectively. Thus, α is used as a criterion to express the enantioselectivity of the chiral stationary phase for the enantiomers. Eq. (2) is valid for undiluted chiral selectors [9].

2.2. Method B

This method, introduced in gas chromatography for diluted selectors by Schurig et al. [11–14], is based on the determination of a retention increment (i.e., a chemical capacity factor) R' and $-\Delta_{R,S}(\Delta G^0)$ is calculated from the equation:

$$-\Delta_{R,S}(\Delta G^0) = RT \ln (R'_R/R'_S) = RT \ln (K_R/K_S) \quad (4)$$

where *K* is the association constant between selectand and selector in the solvent S. The retention increment R' is experimentally accessible from relative adjusted retention data of the enantiomers and proper reference standards on a reactor column containing the selector (CSP) in the solvent S (r) and a reference column containing only S (r_0), respectively:

$$R' = K \cdot m = \frac{r - r_0}{r_0} \quad (5)$$

where m = molality (mol/kg) of the selector in the solvent S (dissolved or chemically bonded). A reference column is coated with the same solvent (achiral stationary liquid) as that used for dilution of the selector (CSP). The concept of the retention increment R' allows to separate the achiral contributions of the solvents and the chiral contributions of the selector to the overall retention. The chiral separation factor α is concentration dependent [11].

$$\alpha = \frac{R'_R - 1}{R'_S - 1} = \frac{K_R m - 1}{K_s m - 1} \quad (5a)$$

3. Experimental

3.1. Instrumentation

A gas chromatograph Fractovap 4100 (Carlo Erba, Milan, Italy) equipped with a flame ionization detection (FID) system and a split/splitless injection port has been used for the gas-chromatographic enantiomer separations. The temperature was checked on the oven display and with an additional conventional thermometer. Hydrogen of 99.99% purity with approximately 50 cm s^{-1} carrier gas flow was used. The FID signal was monitored by a HP

3390A integrator (Hewlett–Packard, Avondale, USA).

3.2. Capillary column

The following fused-silica capillary columns were used:
Reference column: 50m×0.25 mm I.D. capillary coated with an 0.5 μm film of a SE 54.
Column I: 25 m×0.25 mm I.D. capillary coated with a mixed stationary phase [5.37% (w/w) of octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin dissolved in SE 54].
Column II: 25m×0.25 mm I.D. capillary coated with a mixed stationary phase [10.08% (w/w) of octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin dissolved in SE 54].
Prior to coating, the inner surface of fused-silica capillary columns was modified by an acidic leaching, deactivated by 1,3-diphenyl-1,1,3,3-tetramethyldisilazan and coated with the stationary phases by the static method. Thermodynamic measurements were performed at isothermal conditions in a temperature interval of 50–95°C with 5°C intervals.

3.3. Compounds

The racemic mixture and the *R*-enantiomer of methyl lactate, *n*-heptane, *n*-octane and *n*-nonane were delivered by Fluka.

Octakis(3-*O*-butanoyl-2,6-di-*O*-pentyl)-γ-cyclodextrin, introduced by König et al. [8], was prepared according to a modified procedure [12].

R-Methyl lactate is eluted as the second peak on Lipodex E. Therefore, $\Delta_{R,S}(\Delta G^0)$ is a negative quantity.

4. Results and discussion

According to the literature, thermodynamic parameters responsible for the formation of diastereomeric complexes between the enantiomers of a selectand and a chiral selector have been obtained both by Method A [10] and by Method B [11,12].

4.1. Method A

By this method the $-\Delta_{R,S}(\Delta G^0)$ values are calculated from Eq. (2) using the separation factor α obtained for the enantiomers on a column containing the chiral selector at a given separation temperature. In fact, Eq. (2) is derived for the calculation of $-\Delta_{R,S}(\Delta G^0)$ for columns coated with the pure (undiluted) chiral selector. There are problems to use Eq. (2) for chromatographic systems with diluted CSPs, because α_{dil} is rendered concentration dependent, whereas the thermodynamic quantity $-\Delta_{R,S}(\Delta G^0)$ should be strictly independent on the concentration. Since the solvent S (achiral part of the stationary phase) is clearly non-enantioselective, a non-linear dependence of the selectivity factor α on the concentration of the CSP (chiral part of the stationary phase) should be expected. The overall retention factors (k) of the enantiomers *R* and *S* in a diluted chiral stationary phase (*dil*) depend on an achiral (*a*) and a chiral (*ch*) contribution to retention according to the equations:

$$k_{R,\mathrm{dil}} = k_{R,a}x_a + k_{R,\mathrm{ch}}x_{\mathrm{ch}} \tag{6}$$

$$k_{S,\mathrm{dil}} = k_{S,a}x_a + k_{S,\mathrm{ch}}x_{\mathrm{ch}} \tag{7}$$

where x represents the mol fraction of the components of a diluted stationary phase. It is obvious that $k_{R,a}=k_{S,a}$ and $x_a+x_{\mathrm{ch}}=1$. It follows from Eq. (6) and Eq. (7) that are linear if the properties of both phases in the mixed phase are additive:

$$k_{R,\mathrm{dil}} = k_{R,a} + x_{\mathrm{ch}}(k_{R,\mathrm{ch}} - k_{R,a}) \tag{8}$$

$$k_{S,\mathrm{dil}} = k_{S,a} + x_{\mathrm{ch}}(k_{S,\mathrm{ch}} - k_{S,a}) \tag{9}$$

The separation factor α of the enantiomers in a diluted stationary phase can be expressed by the equation:

$$\alpha = \frac{k_{R,\mathrm{dil}}}{k_{S,\mathrm{dil}}} = \frac{k_{R,a} + x_{\mathrm{ch}}(k_{R,\mathrm{ch}} - k_{R,a})}{k_{S,a} + x_{\mathrm{ch}}(k_{S,\mathrm{ch}} - k_{S,a})} \tag{10}$$

and it increases non-linearly with an increase of a chiral selector concentration x_{ch} as was previously shown by Jung and Schurig [13].

Table 1 lists $-\Delta_{R,S}(\Delta G^0)$ values calculated by Eq. (2) from selectivity factors α of the enantiomers of methyl lactate on column I ($x_{\mathrm{ch}}=0.0537$) and II

Table 1
Thermodynamic data determined by Method A [$-\Delta_{R,S}(\Delta G^0)$*] and Method B [$-\Delta_{R,S}(\Delta G^0)$] for columns I and II

Temp. (°C)	Column I				Column II			
	$-\Delta_{R,S}(\Delta G^0)$			$-\Delta_{R,S}(\Delta G^0)^a$	$-\Delta_{R,S}(\Delta G^0)$			$-\Delta_{R,S}(\Delta G^0)^a$
	n-Heptane	*n*-Octane	*n*-Nonane		*n*-Heptane	*n*-Octane	*n*-Nonane	
50	1.16	1.16	1.16	0.86	1.19	1.19	1.19	0.99
55	1.11	1.11	1.11	0.77	1.11	1.11	1.11	0.88
60	1.02	1.02	1.03	0.66	1.04	1.04	1.04	0.78
65	0.95	0.95	0.95	0.56	0.95	0.96	0.96	0.67
70	0.86	0.86	0.86	0.46	0.89	0.89	0.89	0.59
75	0.79	0.79	0.79	0.38	0.82	0.82	0.82	0.50
80	0.73	0.74	0.75	0.32	0.75	0.74	0.75	0.42
85	0.65	0.64	0.64	0.24	0.67	0.68	0.68	0.35
90	0.59	0.61	0.60	0.20	0.59	0.59	0.59	0.28
95	0.54	0.53	0.54	0.16	0.54	0.54	0.55	0.23

The values $-\Delta_{R,S}(\Delta G^0)$ and $-\Delta_{R,S}(\Delta G^0)^a$ are in kJ mol^{-1}. $-\Delta_{R,S}(\Delta G^0)^a$ was calculated from Eq. (2).

($x_{ch}=0.1008$) at different temperatures. In agreement with Eq. (10), α values increase non-linearly with an increase of x_{ch}. Since the dependence of $-\Delta_{R,S}(\Delta G^0)/T$ on $1/T$, is not linear (Fig. 1, part A) for diluted CSPs, the van't Hoff dependence:

$$\ln \alpha = -\frac{\Delta(\Delta H^0_{R,S})}{RT} + \frac{\Delta(\Delta S^0_{R,S})}{R} \quad (11)$$

cannot be used for the calculation of $-\Delta_{R,S}(\Delta H^0)$ and $-\Delta_{R,S}(\Delta G^0)$ parameters in the present system, as expected.

4.2. Method B

In the present investigation three volatile compounds (*n*-heptane, *n*-octane and *n*-nonane) were selected as reference standards. It should be noted that these reference standards are not truly inert as they are believed to interact weakly with the modi-

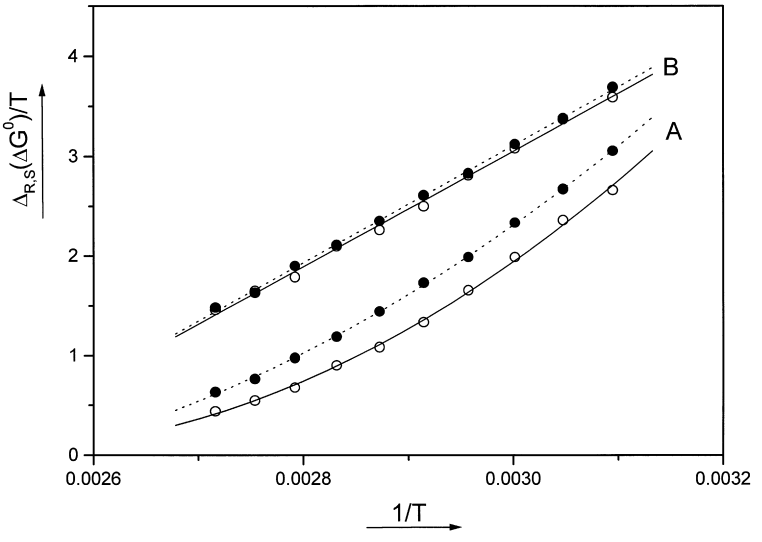

Fig. 1. Planck function $-\Delta_{R,S}(\Delta G^0)/T$ versus $1/T$ for methyl lactate with the 10.08% (●) (Column II) and 5.37% (○) (Column I) concentrations of Lipodex E. For the construction of this figure data calculated by the Method A, and Method B were used.

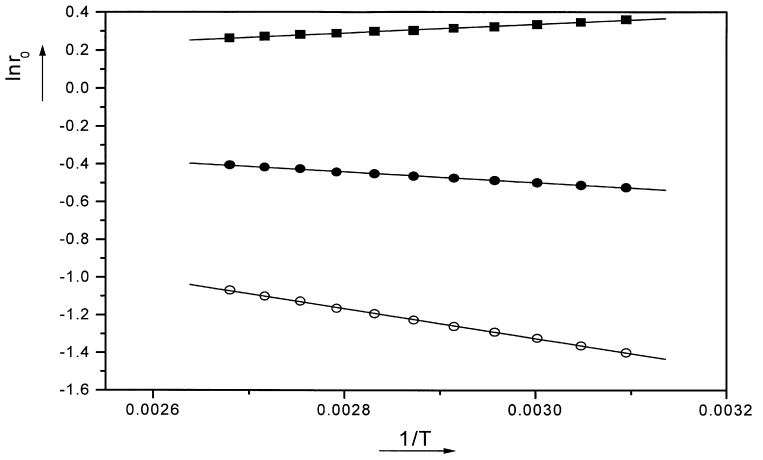

Fig. 2. Calibration of $ln\ r_0$ vs. $1/T$ for methyl lactate on the reference column coated with SE 54 for three reference compounds (*n*-heptane (■), *n*-octane (●) and *n*-nonane (○)).

fied cyclodextrin selector [13]. However, the association constants as compared to the selectand methyl lactate are considered to be negligible. Both the relative retention of the enantiomers of methyl lactate in respect to the reference standards on the reference column (r_0) as well as that on the reactor columns (r) were determined. Highly confident r_0 values were obtained by a linear regression analysis of the relative retention data measured on the reference column using the dependence of $\ln r_0$ on $1/T$ (Fig. 2), according to a published procedure [12]. The relative retention data r obtained on the two reactor columns containing the chiral selector in the solvent S were used for the calculation of the retention increments R' according to Eq. (5), and consequently, the parameters $-\Delta_{R,S}(\Delta G^0)$ were calculated according to Eq. (4) at the whole temperature range employing three reference standards (Table 1). Fig. 1 part B shows a plot of the Planck function $-\Delta_{R,S}(\Delta G^0)/T$ on $1/T$, averaged over three reference standards. Table 2 lists the Gibbs–Helmholtz parameters $[-\Delta_{R,S}(\Delta H^0)$ and $-\Delta_{R,S}(\Delta G^0)]$ obtained by a linear regression analysis of the calculated data from the slopes and intercepts on the Y axis both for column I and column II. Thermodynamic parameters (ΔH^0 and ΔS^0) for both enantiomers on columns I and II obtained from linear regression analysis of calculated data are also shown in Table 2.

Fig. 3 shows the dependence of $R \ln K$ on $1/T$ for column I(A) and column II(B). In all cases the expected linear Van't Hoff plots are obtained and the thermodynamic data obtained are strictly concentration-independent. Thus, Method B represents a reliable method to determine thermodynamic parameters in enantioselective gas chromatography utilizing diluted CSPs [14].

5. Conclusions

$-\Delta_{R,S}(\Delta G^0)$ values listed in Table 1 shows that the data calculated by the Method A are too small and are dependent on the concentration of the chiral

Table 2
modynamic data for *S*- and *R*-enantiomers of methyl lactate obtained for columns I and II from linear regression analysis of calculated data

	$-\Delta_{R,S}(\Delta H^0)$ (kJ mol^{-1})	$-\Delta_{R,S}(\Delta S^0)$ (J mol^{-1} K^{-1})	$-\Delta H^0_S$ (kJ mol^{-1})	$-\Delta S^0_S$ (J mol^{-1} K^{-1})	$-\Delta H^0_R$ (kJ mol^{-1})	$-\Delta S^0_R$ (J mol^{-1} K^{-1})
Column I	5.79	14.32	39.67	25.28	45.47	39.61
Column II	5.87	14.51	38.00	21.23	43.87	35.75

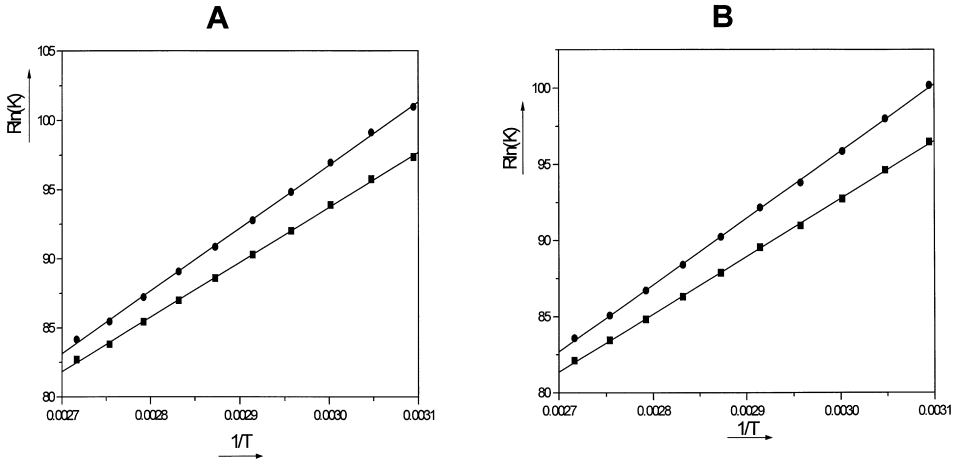

Fig. 3. Plot of $R \ln K$ versus $1/T$ for methyl lactate with the 10.08% (A) (Column I) and 5.37% (B) (Column II) concentrations of Lipodex E for *S*- (■) and *R*-enantiomers (●).

selector in the diluted stationary phase. Since the dependence of ln α on $1/T$ for methyl lactate enantiomers on Lipodex E columns was nonlinear, it was not possible to calculate $-\Delta_{R,S}(\Delta H^0)$ and $\Delta_{R,S}(\Delta S^0)$ values by this method.

Comparison of $-\Delta_{R,S}(\Delta G^0)$ values listed in Table 1 shows that Method B yields values essentially independent on the choice of reference compound and the concentration of the chiral selector in the mixed stationary phase, as required.

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