STUDIA UNIVERSITATIS BABEŞ-BOLYAI, CHEMIA, LIV, Sp.Iss. 2, 2009

TEMPERATURE DEPENDENCE OF ENANTIOMER SEPARATION PARAMETERS BY GAS-CHROMATOGRAFIC AND SUPERCRITICAL FLUID CHROMATOGRAPHIC METHODS[*]

BÉLA TŐKÉS[a,], ZOLTÁN JUVANCZ[b], ROBERT IVÁNYI[c],**
SZENDE VANCEA[a], GABRIELLA DONÁTH-NAGY[a],
VOLKER SCHURIG[d], ANCA-GABRIELA CÂRJE[a]

ABSTRACT. Although the temperature is a decisive factor of the separation methods, its systematic study requires solvation of numerous problems. The present results frequently are contradictory. In this paper is discussed in detail the temperature dependence of enantiomer separation by GC and SFC methods of some chiral compounds having essentially different chemical structures, are calculated and interpreted characteristic thermodynamic parameters of the processes, especially the differences of the normal (standard) free enthalpy, enthalpy and entropy changes, $\Delta_{BA}\Delta G$, $\Delta_{BA}\Delta H$ and $\Delta_{BA}\Delta S$.

Keywords: separation methods, temperature effect, thermodynamic parameters

INTRODUCTION

The temperature dependence of selectivity can be studied by following idea: the partition of a given component *i* between two phases (1 and 2) suppose the equality of the component's chemical potential in the two phases. It has to be mentioned that the partition phenomenon is considered being a general equilibrium, not only a particular one between two liquid phases.

$$\mu_{i1} = \mu_{i2}$$

so:

$$\frac{C_{i1}}{C_{i2}} = \exp\left[\frac{\left(\mu_{i2}^0 - \mu_{i1}^0\right)}{RT}\right] = K_i$$

---

[*] This paper was presented at the 15th International Conference on Chemistry, EMT, Tg. Mures, November 12-15, 2009

[a] University of Medicine and Pharmacy, Tg. Mureş, Physical Chemistry Laboratory, Romania, ** belatokes@yahoo.com

[b] Dep. of Environmental Engineering, Tech. Polytechnical Institution, Budapest, Hungary

[c] Cyclolab Cyclodextrin R & D. Laboratory Ltd., Budapest, Hungary

[d] University of Tübingen, Institute of Organic Chemistry, Germany

For simplicity suppose that the ratio of activities can be expressed as the concentrations' ratio, which is the partition coefficient of the given component. In the other hand, the selectivity factor of two components (A and B) – i.e. the degree of their separation – can be expressed as follows:

$$\alpha = \frac{K_B}{K_A} = \frac{k'_B}{k'_A} = \frac{(t'_R)_B}{(t'_R)_A}$$

where k'_A and k'_B, $(t'_R)_A$ and $(t'_R)_B$ are the corresponding capacity factors and reduced retention times. Therefore:

$$\ln\alpha = \ln\frac{K_B}{K_A} = -\frac{\Delta(\Delta\mu^0_{BA})}{RT} = -\frac{\Delta_{BA}\Delta G^0}{RT} = -\frac{\Delta_{BA}\Delta H^0}{RT} + \frac{\Delta_{BA}\Delta S^0}{R}$$

or:

$$\alpha = \text{Const.}e^{-\frac{\Delta_{BA}\Delta H^0}{RT}}$$

which can be considered as a special case of the Gibbs-Helmholtz equation:

$$\frac{\partial}{\partial T}\left(\frac{\Delta\mu^0}{T}\right) = -\frac{\Delta H}{T^2}$$

In case of chiral separation the R and S indexes can be used instead of A and B. Sometimes the entropic factor may be the dominant one in the steric mechanism of the separation, so the separation will not depend much on the temperature. The temperature dependence is reflected by the enthalpic factor, which is shows the interactions between the solute and the phases. Chromatography deals at the same time with several interactions (adsorption, dissolution, thermal effects), which depend differently on the temperature. ΔG^0, ΔH^0 and ΔS^0 represent the standard free energy, enthalpy and entropy changes of component transition between the mobile and stationary phases [1]. The relative selectivity ($\Delta\alpha/\alpha$) is often used as well, which is:

$$\frac{\Delta\alpha}{\alpha} = 2\mathrm{sh}\left(\frac{\Delta_{BA}\Delta G^0}{2RT}\right)$$

If the selectivity factors do not differ very much from each other, the above relation can be written:

$$\frac{\Delta\alpha}{\alpha} = \frac{\Delta_{BA}\Delta G^0}{2RT}$$

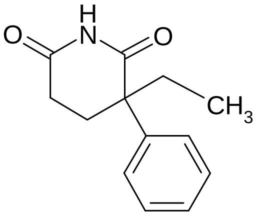
This term emphasizes again that the severability of components increases with the difference between the free energy changes. Based on this fact the temperature dependence of selectivity factor can be discussed.

So, the logarithm of selectivity (lnα) should depend linearly on the inverse of temperature (1/T), in case of only one separation mechanism plays a decisive role.

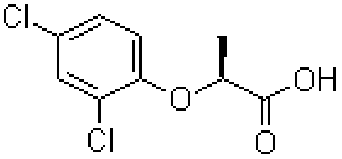
Considering the (lnα, 1/T) regression equations' coefficients – besides the equilibrium constants' ratio – the differences of standard free energy, enthalpy and entropy changes ($\Delta_{BA}\Delta G$, $\Delta_{BA}\Delta H$ and $\Delta_{BA}\Delta S$) of the separation mechanism (adsorption, dissolution, evaporation) can be determined in case of given enantiomers. Obviously the determinations would be more precise if – considering the Kirchoff law – the temperature dependence of enthalpy change would have been considered.

In this paper the temperature dependence of chiral separation selectivity [2] in case of three compounds with totally different chemical structure was studied. The three compounds were:

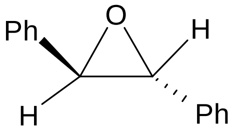
1. Glutethimide (synonyms: 2-ethyl-2-phenylglutarimide, or 3-ethyl-3-phenyl-2,6-pyperidinedione, or alpha-ethyl-alpha-phenyl glutarimide) is a sedative with a barbiturate-like effect [3,4]. It has been proved that it has toxic side effects like hallucinations, muscle contractions, fever.



2. Methyl ester of 2-(2,4-dichloro-phenyl)propionic acid (2,4-DPM). The chloro-derivatives of phenoxy-acids, among them 2,4-DPM are well known pesticides, but also environmental pollutants with long effect [5]. They are polar compounds, with low volatility. Their strong adsorption on the stationary phases makes the gas-chromatographic separation difficult. A solution of this problem is their derivatization to methyl esters.



3. Trans-stilbene-oxyde (TSO) (Synonyms: 1,2-diphenyl-ethylene-oxyde, α,α'-diphenyl-epoxyde) is a strong-effect inductor of epoxy-hydratase enzyme, and also has proestrogene effect [6]. Its chiral enantiomers have been separated by preparative HPLC methods. It is a good model compound because of its rigid structure and affinity to π-π and p-π interactions.



RESULTS AND DISCUSSION

In the this chapter will be presented the most significant experimental data and results obtained by different separation methods for some practically important coumpounds. Their analysis were performed on the basis of the theoretical considerations discussed.

The statistical evaluation of experimental data shows that the chromatographic method's selectivity linearly decreases with the increase of temperature. The representative curves are running slightly concave which is in concordance with the exponential nature of the theoretical context. From the same law was to be expected that the (lnα, 1/T) are linear correlations. The calculated regression equation linearity really proved to be excellent, which confirms assumptions are correct.

Table 1. Temperature dependence of selectivity (linear regression)

Method		GC			SFC		
Parameters		N	R	SD	N	R	SD
Glutethimide	α, T ^oC	5	- 0,993	0,0030	7	- 0,992	0,0034
		$\alpha = -(1,40 \pm 0,09)10^{-3}(^OC)\ t + (1,312 \pm 0,017)$			$\alpha = -(1,15 \pm 0,07)10^{-3}\ t(^OC) + (1,186 \pm 0,005)$		
	lnα, 1/T K^{-1}	5	0,996	0,0021	7	0,996	0,0024
		$\ln\alpha = (0,272 \pm 0,014)\ 10^3(1/T) - (0,542 \pm 0,031)$			$\ln\alpha = (\ 0,130 \pm 0,005\ )\ 10^3 1/T - (\ 0,281 \pm 0,015\ )$		
DPM	α, T ^oC	5	- 0,976	0,0044	4	- 0,996	0,0007
		$\alpha = -(1,07 \pm 0,14)10^{-3}\ t(^OC) + (1,210 \pm 0,021)$			$\alpha = -(0,540 \pm 0,030)10^{-3}\ t(^OC) + (1,0739 \pm 0,0019)$		
	lnα, 1/T K^{-1}	5	0,980	0,0037	4	0,997	0,0006
		$\ln\alpha = (0,183 \pm 0,021)\ .10^3(1/T) - (0,384 \pm 0,050)$			$\ln\alpha = (0,059 \pm 0,003)\ 10^3(1/T) - (0,137 \pm 0,010)$		
TSO MMBC 5 SE-54	α, T ^oC	7	- 0,986	0,0043			
		$\alpha = -(2,00 \pm 0,08).10^{-3}\ t\ (^OC) + (1,213 \pm 0,013)$					
	lnα, 1/T K^{-1}	7	0,990	0,0034			
		$\ln\alpha = (0,193 \pm 0,012)10^3\ (1/T) - (0,408 \pm 0,028)$					
TSO MMBC 10 OV-1701	α, T ^oC	6	- 0,982	0,0039			
		α = – (1,00 ± 0,09).10-3 t (ºC) + (1,264 ± 0,015)					
	lnα, 1/T K^{-1}	6	0,994	0,0028			
		$\alpha = (0,228 \pm 0,012)10^3\ 1/T - (0,465 \pm 0,028)$					

Table 2. Calculated thermodynamic parameters

	Glutethimide		DPM		TSO- MMBC 5 SE-54	TSO- MMBC 10 OV-1701
	GC	SFC	GC	SFC	GC	GC
$A = \Delta_{BA}\Delta S^0/R$	0,542	0,281	0,384	0,137	0,408	0,465
$\Delta_{BA}\Delta S^0$(J/mol K)	4,506	2,33	3,20	1,139	3,39	3,86
$B = \Delta_{BA}\,\Delta H^0/R$	272	130	183	59	408	228
$\Delta_{BA}\Delta H^0$ (kJ/mol)	2,269	1,0808	1,52	0,49	1,60	1,89
$\Delta_{BA}\Delta G^0$ (kJ/mol)	1,038	0,44	0,64	0,17	0,67	0,83

1. Glutethimide

1.1. Separation of glutethimide enantiomers by GC analysis

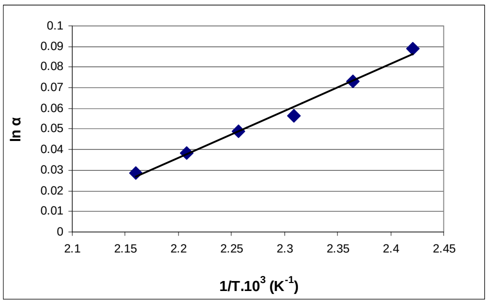


Figure 1. Temperature dependence of selectivity (α, T ^{0}C) by GC data

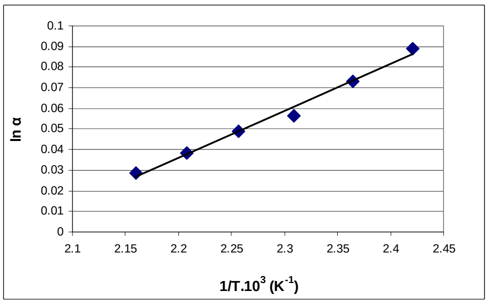


Figure 2. Temperature dependence of selectivity (lnα, 1/T K^{-1}) by GC data

1.2. Separation of glutethimide enantiomers by SFC analysis [3]

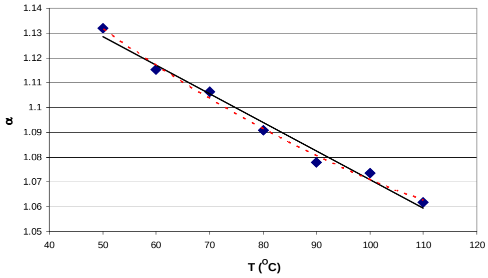


Figure 3. Temperature dependence of selectivity (α, T ^{o}C) by SCF data

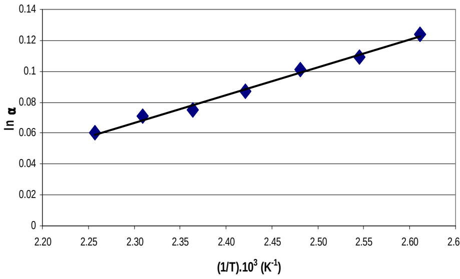


Figure 4. Temperature dependence of selectivity (lnα, 1/T K^{-1}) by SCF data

2. (2, 4-dichlorophenyl) propionic acid methyl ester (DPM)

2.1. Separation of DPM enantiomers by GC analysis

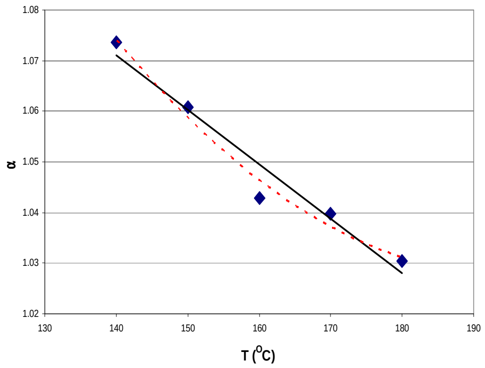


Figure 5. Temperature dependence of selectivity (α, T °C) by GC data

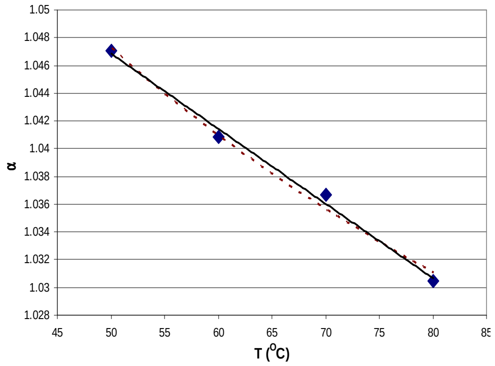


Figure 6. Temperature dependence of selectivity (lnα, 1/T K^{-1}) by GC data

2.2. Separation DPM enantiomers by SFC analysis

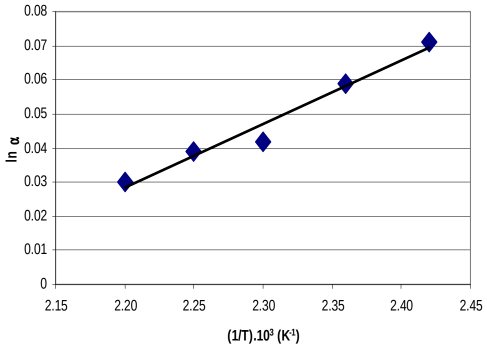


Figure 7. Temperature dependence of selectivity (α, T °C) by SCF data

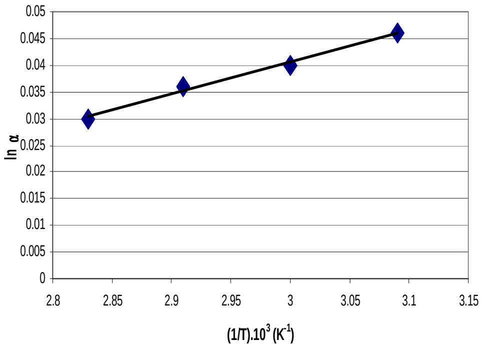


Figure 8. Temperature dependence of selectivity (lnα, 1/T K^{-1}) by SCF data

3. Trans-stilbene-oxide (TSO)

3.1. Separation of TSO (MMBC 5 SE-54) enantiomers by GC analysis

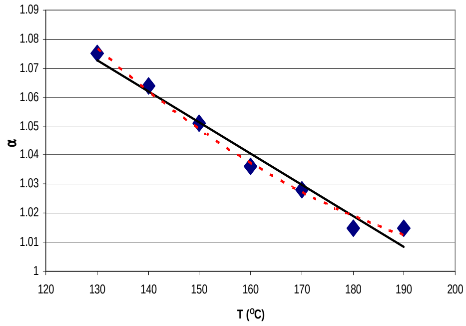


Figure 9. Temperature dependence of selectivity (α, T °C) by GC data

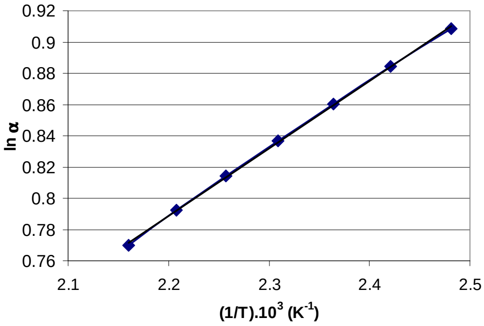


Figure 10. Temperature dependence of selectivity (lnα, 1/T K^{-1}) by GC data

3.2. Separation of TSO (MMBC 10 OV-1701) enantiomers by GC analysis

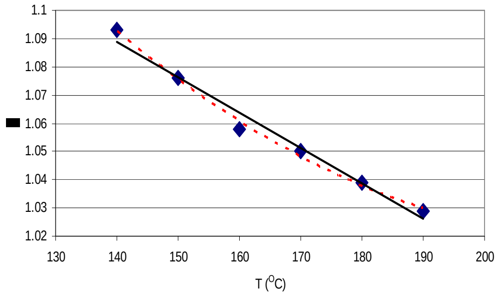


Figure 11. Temperature dependence of selectivity (α, T °C) by GC data

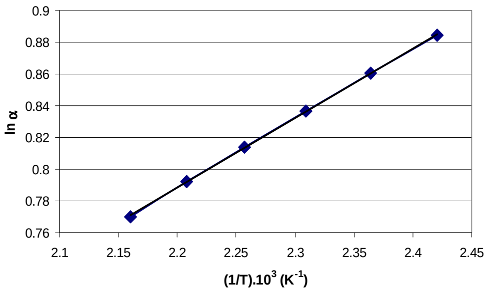


Figure 12. Temperature dependence of selectivity (ln α, 1/T K^{-1}) by GC data

CONCLUSIONS

We calculated the thermodynamic parameters (standard free enthalpy, enthalpy and entropy changes, $\Delta_{BA}\Delta G$, $\Delta_{BA}\Delta H$ and $\Delta_{BA}\Delta S$ differences) from the (lnα, 1/T) linear regression equation's coefficients for the enantiomers of the studied chiral substances, which characterize the separation in the above specified conditions.

Of course, the greater these differences (differences in selectivity or differences in resolution) the more effective is the separation of isomers. The selectivity itself is also the ratio of two similar thermodynamic parameters (ratio of distribution coefficients).

From the comparison of slopes of the regression equations (i.e., the found susceptibility of values of lnα 1/T against the independent variables) relies that the sensitivity of gas chromatographic procedure is significantly greater (approximately twice) than the supercritical fluid chromatography's sensitivity (Table 3).

Table 3. Slopes of the regression equations'

	GC	SFC
Glutethimide	0,277	0,130
2,4-DPM	0,183	0,059
TSO (MMBC 5 SE-54)	0,193	
TSO (MMBC 10 OV-1701)	0,228	

As it can be seen, of course, attributed to the same method, the pitch depends on the column filling, and other additives (chiral selector, etc.). Similar findings are made in the regression equation's intercepts, which reflect the differences of the entropic factors (Table 4).

Table 4. Intercepts of the regression equations'

	GC	SFC
Glutethimide	-0,542	-0,281
2,4-DPM	-0,384	-0,137
TSO (MMBC 5 SE-54)	-0,408	
TSO (MMBC 10 OV-1701)	- 0,465	

The appropriate (corresponding) thermodynamic data, due to their physico-chemical content, describe more expressively the occurred thermal effects (Table 5).

Table 5. Calculated thermodynamic parameters

Thermodynamic parameter	$\Delta_{BA}\Delta G^o$ (kJ/mol)	$\Delta_{BA}\Delta H^o$ (kJ/mol)	$\Delta_{BA}\Delta S^o$ (J/mol)
Glutethimide GC	1,04	2,26	4,51
Glutethimide SFC	0.44	1.08	2.33
2,4-DPM GC	0,64	1,52	3,20
2,4-DPM SFC	0,17	0,49	1,14
TSO (MMBC 5 SE-54)	0,67	1,60	3,39
TSO (MMBC 10 OV-1701)	0,83	1,89	3,86

If we analyze from any point of view the experimental and calculated values, they indicate that the gas chromatographic method gives a better separation. Preliminary information about this can provide the simple (α, t^oC)

curves and equations themselves to. Of course, for the method development, beyond the temperature dependence, has to be taken into account other technical specificities as well.

In accordance with the foregoing theoretical facts which are supported by the experimental data, the heat effect ΔH, which means the algebraic sum or rather the average of distribution heat exchanges (in gas chromatography the dissolution and absorption heat, in supercritical fluid chromatography, because of its "hybrid" nature, the vaporization and dissolution heats), for the GC is generally higher than for the SFC separations.

This means that in the first case the growth of selectivity should be larger than the latter. Since compounds with different structure were studied, it appears that this conclusion does not depend on the quality of any of the compounds tested, nor the chiral selector.

Therefore, it follows that the knowledge of temperature dependence on separation helps to choose the optimum conditions for analytical methods.

Experimental section

Separation of enantiomers of studied chiral compounds was performed by gas chromatography (GC) and supercritical fluid chromatography (SFC), in the CIB-Geigy Corporation (Basel, Switzerland) laboratories.

1. Chromatographic columns used for *glutethimide* and *2,4-DPM* determination:
   In GC analysis: Quartz capillary 10 m x 0,1 mm, with Chirasil-Dex stationary phase (15% chemically bonded permethyl-β-cyclodextrin) humified at 0,15 mm thickness.
   In SFC analysis: Quartz capillary 7,5 m x 0,1 mm, with Chirasil-Dex stationary phase (15% chemically bonded permethyl-β-cyclodextrin) humified at 0, 15 mm thickness [7].
2. Chromatographic columns used for *trans-stilbene oxide* determination:
   In GC analysis: Quartz capillary 10 m x 0.1 mm, 95% SE-54 + 5% MMBC (meta-methyl-benzoyl-cellulose) humified at 0, 2 mm thickness.
   In SFC analysis: Quartz capillary 10 m x 0.1 mm, 90 % OV1701Vi + 10% MMBC (meta-methyl-benzoyl-cellulose) humified at 0, 2 mm thickness [8,9].

ACKNOWLEDGEMENTS

This work has been supported in part by the research projects OTKA K72861 and NKTH OTKA 68863.

REFERENCES

1. T. Kremmer, L. Boross – *Gélkromatográfia*. Műszaki Könyvkiadó, Budapest, **1974**
2. Z. Juvancz, K.E. Markides. *LC-GC International,* **1992**, *5*, 44
3. A. Overbeke, H.Y. Aboul-Enein, W. Baeyens, G. Guido Weken, C. Dewaele, *Analytica Chimica Acta,* **1992**, *346*, 183
4. C. Weinz, G. Blaschke, H-M Schiebel, *J. Chromatography, B: Biomedical Sciences and Applications*, **1997**, *690*, 233
5. L. Ferencz – *Pesticides.* University Press, Tg. Mureş, **2006**
6. T.K. Tachibana; A. Ichida, *J Liquid Chromatography and Related Technologies*, **2003**: 3235 – 3248
7. Z. Juvancz, K. Grolimund, V. Schurig, *J. Microcol. Separation,* **1993**, *5*, 459
8. E. Francotte, K. Grolimund, Z. Juvancz, *Chirality,* **1993**, *5,* 232
9. Z. Juvancz, K. Grolimund, E. Francotte, *Chirality*, **1992**, *4,* 459