



Study on retention factor and resolution of tocopherols by supercritical fluid chromatography

Chongwen Jiang*, Qilong Ren, Pingdong Wu

National Laboratory of Secondary Resources Chemical Engineering, Zhejiang University, Hangzhou 310027, China

Received 18 December 2002; received in revised form 29 April 2003; accepted 29 April 2003

Abstract

To develop a process for separation of natural α -tocopherol from γ - and δ -tocopherols by supercritical fluid chromatography, the effects of pressure, temperature and the ethanol concentration in the mobile phase on the retention factor and resolution of tocopherols were studied comprehensively. The ranges of the studied pressure, temperature and ethanol concentration were 12 to 20 MPa, 30 to 90 °C and 0 to 6.54 wt% respectively. It was found that the retention factors of the tocopherols decrease as the ethanol concentration in the mobile phase increases. The resolution of tocopherols increases as the temperature increases and as the pressure decreases, and there is a maximum of the ethanol concentration curve at a definite temperature and pressure. A simplified model of the retention factor $\ln k' = A + B/T + C\rho - D(\rho/T) + E(\rho^2/T)$ based on the unified molecular theory was proposed. The experimental data were correlated by the model with the AARD% less than 14.70%.

© 2003 Elsevier B.V. All rights reserved.

Keywords: Retention factor; Resolution; Supercritical fluid chromatography; Tocopherols

1. Introduction

Supercritical fluid chromatography (SFC) has attracted much attention due to its ability to separate nonvolatile and thermally labile compounds. Tocopherols (α , β , γ and δ -tocopherol) are the main constituents of natural Vitamin E extracted from deodorization distillate of vegetable oil. Because α -tocopherol exhibits much higher biological activity than the other constituents, the commercial natural Vitamin E product is mainly composed of α -tocopherol. Since it is well known that separation by

supercritical fluid has advantages over other operations (solvent extraction, molecular distillation, etc.), it is of significance to study SFC as a potential operation for separation of α -tocopherol from the other homologues.

There are a few publications concerning SFC of tocopherols as a method of analysis. Upmooor and Brunner [1] described the retention behavior of α -tocopherol on various stationary phases modified with methanol. Perrin and Prevot [2] separated tocopherols by SFC on silica gel by using dichloromethane and methanol as modifier. Yarita et al. [3] investigated the effect of methanol concentration on the retention and resolution of tocopherols on ODS-silica column. Grzegorz [4] studied SFC as a method

*Corresponding author. Tel.: +86-571-8795-1225.

E-mail address: yes@zju.edu.cn (C. Jiang).

for assay of tocopherols in some natural antioxidant. As regards separation of tocopherols, Saito et al. [5,6] have employed supercritical fluid extraction (SFE) coupled to semi-preparative SFC for enrichment of tocopherols from wheat-germ oil, purity of 85% α -tocopherol and 70% (wt%) β -tocopherol were obtained. A similar system was employed by King et al. [7] to enrich tocopherols from soybean flakes, the enrichment factors of tocopherols ranged from 30.8 for δ -tocopherol to 2.41 for β -tocopherol. The recoveries were 76% for γ -tocopherol and 87% for δ -tocopherol, but the recovery of α - and β -tocopherol was not reported because of their extremely low content in soybean oil.

Up to now, there has been no report concerning the influence of temperature, pressure and modifier concentration on retention factor and resolution. The present work intends to study the effect of pressure, temperature and concentration of ethanol (as modifier of mobile phase) on the retention factor and resolution of tocopherol homologues comprehensively. In addition, a model for correlation of experimental results is to be developed. It is expected that the results will provide fundamental data for optimi-

zation of operation conditions for separation of natural d- α -tocopherol by using SFC.

2. Experimental

2.1. Instrumentation

The scheme of the SFC apparatus is shown in Fig. 1. After being liquified in a cooling coil, carbon dioxide was compressed, mixed with ethanol and then flowed through the preheater, the injector and the column, all of which were immersed in a water thermostat bath. The size of the chromatographic column is 250×3.9 mm I.D., packed with Waters Spherisorb $5 \mu\text{m}$ silica gel. The peaks were detected by an UV detector at 205 nm and the signal from the detector was processed by a microcomputer.

2.2. Reagent

Carbon dioxide used was of 99.99% purity obtained from Mingxing Gas Co. Ltd. (Hangzhou, China). Ethanol (AR) was from Changzheng Chemi-

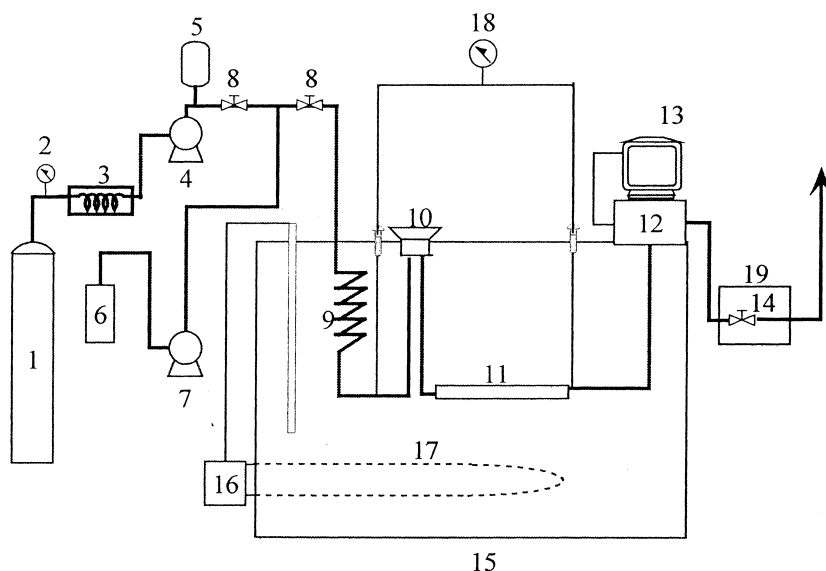


Fig. 1. Schematic diagram of supercritical fluid chromatography equipment. (1) CO_2 cylinder, (2) pressure gauge, (3) cooling coil, (4) CO_2 pump, (5) surge tank, (6) modifier container, (7) modifier pump, (8) stop valve, (9) preheater, (10) sample injector, (11) chromatographic column, (12) UV detector, (13) microcomputer, (14) micrometer valve, (15) water bath, (16) temperature controller, (17) heater, (18) pressure transducer, (19) heating oven.

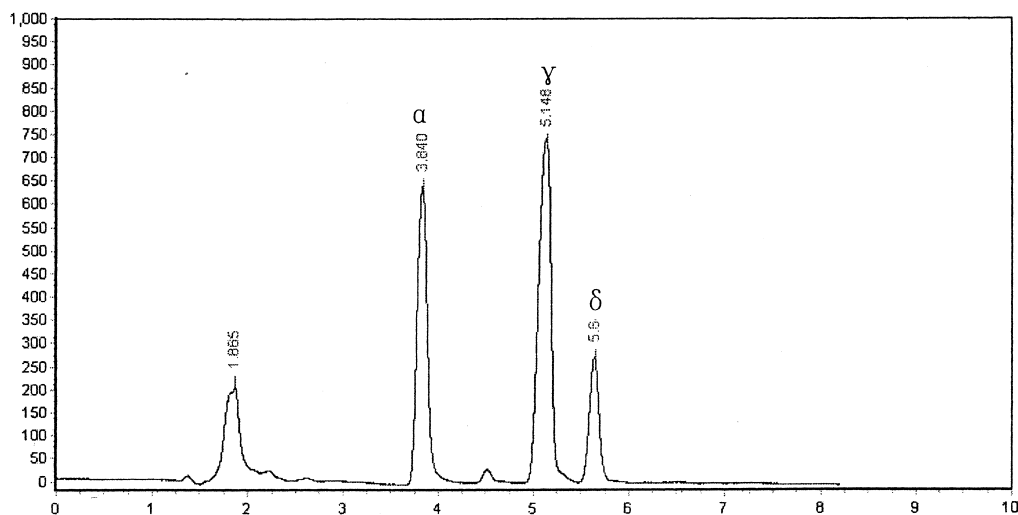


Fig. 2. Chromatogram of tocopherols. Conditions: 3.98% ethanol, 18 MPa, 40 °C, Waters Spherisorb 5 μ m silica column (250 \times 3.9 mm I.D.).

cal Plant (Hangzhou, China). *n*-Hexane (AR) was from Hangzhou Petroleum Refinery (Hangzhou, China). The tocopherol concentrate (with 13.72% α -tocopherol, 30.38% γ -tocopherol and 6.06% δ -tocopherol determined by HPLC) was purchased from Hunan Kingkang Biologics Co. Ltd. (Changsha, Hunan, China).

3. Results and discussion

A typical SFC chromatogram of the mixed tocopherols is shown in Fig. 2. The first peak is some contaminate in the sample, then are the peaks of α -, γ - and δ -tocopherol in sequence. As the mixed tocopherols were of soybean origin, the content of

β -tocopherol in the sample was very low, and its data were not treated. *n*-Hexane was used to obtain t_0 for calculating retention factor k' .

3.1. Effect of modifier concentration

The k' and resolution (R) values determined at 18 MPa, 40 °C and at various ethanol concentrations are listed in Tables 1 and 2. Test by carbon dioxide (without ethanol) as mobile phase showed that tocopherols were not eluted up to 100 min.

The plots of the data in Tables 1 and 2 are shown in Figs. 3 and 4. It can be seen from the figures, at 40 °C and 18 MPa, as the ethanol concentration increases, the retention factor decreases rapidly in the low concentration range, then the curve gradually

Table 1
 k' of tocopherols at various ethanol concentration

Ethanol (wt%)	k'			Ethanol (wt%)	k'		
	α	γ	δ		α	γ	δ
1.57	3.975	6.938	8.268	3.98	1.786	2.727	3.09
1.66	3.832	6.673	8.145	4.60	1.546	2.227	2.476
1.95	3.258	5.581	6.729	5.43	1.215	1.659	1.811
2.18	3.047	5.130	6.107	6.22	1.007	1.253	1.322
2.75	2.225	3.633	4.264	6.54	0.944	1.162	1.224
3.43	2.054	3.209	3.684				

Table 2
R of tocopherols at various ethanol concentration

Ethanol (wt%)	R		Ethanol (wt%)	R	
	α - γ	γ - δ		α - γ	γ - δ
1.57	7.146	2.627	3.98	4.906	1.935
1.66	8.318	3.180	4.60	4.193	1.623
1.95	9.105	3.789	5.43	3.000	1.105
2.18	8.592	3.378	6.22	1.709	Not
2.75	6.599	2.807	6.54	1.640	Not
3.43	5.992	2.374			

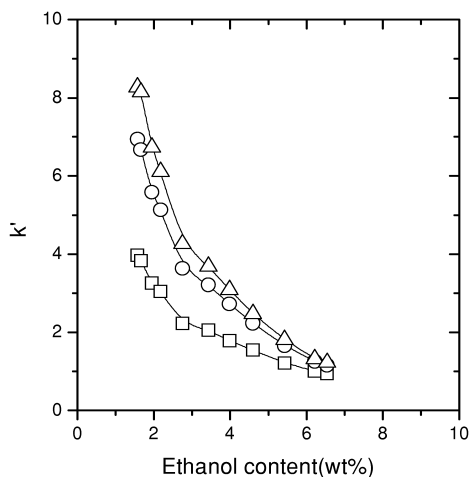


Fig. 3. Effect of the ethanol content on k' . (\square) α -tocopherol; (Δ) δ -tocopherol, (\circ) γ -tocopherol.

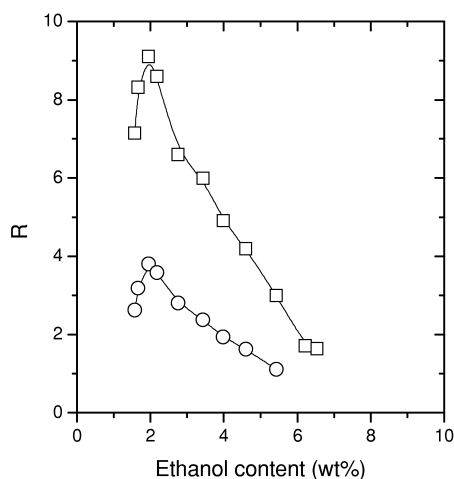


Fig. 4. Effect of the ethanol content on R . (\square) α and γ -tocopherol; (\circ) γ and δ -tocopherol.

levels off. In addition, the difference between the retention factors of the homologues becomes less with increasing pressure as well. The k' values of all three tocopherol homologues approach to 1. On the other hand, as the ethanol concentration increases, the R of the adjacent peaks increases initially, reaches a maximum around 2% of ethanol, then decreases monotonically. By compromise of retention time and resolution, the appropriate ethanol concentration for separation of tocopherols would be around 5% (wt). Under this condition, the k' values are quite small (the retention times are short), while the resolution values are still acceptable (R is 3.6 and 1.4, respectively).

3.2. Effect of pressure

By keeping the ethanol concentration at $4.9 \pm 0.2\%$ (wt), the retention factor and resolution of tocopherols are determined in the temperature range of 30–90 °C and the pressure range of 12–20 MPa. The results are listed in Tables 3 and 4.

The effect of pressure on k' is shown in Figs. 5–7, and that on R is shown in Figs. 8 and 9. It can be seen that both the k' and R values of tocopherols decrease as the pressure increases; in addition, the higher the temperature, the greater the effect of the pressure.

Here again, to make a compromise between k' and R , a pressure range of 18–20 MPa would be appropriate for separation of tocopherols, particularly in the case of separating α - from other tocopherols because R between α - and γ - is much greater than R between γ - and δ -tocopherol.

3.3. Effect of temperature

The effect of temperature on retention factor is shown in Figs. 10–12. Under 14 MPa, the retention factor increases as the temperature increases with a steep slope of the curve. Under the other studied pressures, there is a maximum of the retention factor–temperature curve, similar to the results of polycycloaromatics by Bartle [8]. In the mean time, the effect of temperature becomes less and the maximum of the curve moves to higher temperature as the pressure increases. Figs. 13 and 14 show the relation between resolution and temperature. The

Table 3
k' of tocopherols at various temperature and pressure

Temperature	Tocopherol	12 MPa	14 MPa	16 MPa	18 MPa	20 MPa
30 °C	α		1.451	1.223	1.079	1.018
	γ		1.977	1.666	1.461	1.380
	δ		2.11	1.788	1.567	1.482
40 °C	α	2.086	1.684	1.41	1.134	1.042
	γ	2.801	2.264	1.892	1.557	1.416
	δ	3.006	2.445	2.051	1.696	1.540
50 °C	α		2.473	1.845	1.318	1.225
	γ		3.373	2.571	1.850	1.647
	δ		3.671	2.821	2.04	1.806
60 °C	α		4.601	2.848	1.761	1.552
	γ		5.256	4.029	2.608	2.117
	δ		6.823	4.513	2.968	2.35
80 °C	α		–	1.874	1.618	1.109
	γ		–	2.738	2.496	1.640
	δ		–	3.072	2.872	1.988
90 °C	α		–	–	1.281	1.265
	γ		–	–	1.962	1.932
	δ		–	–	2.280	2.248

resolution increases as the temperature increases. It can be concluded that the appropriate temperature for separation would be around 80 °C since the resolution is much greater than that at low temperature (around 40 °C) while the retention factors at both temperatures are almost the same.

3.4. Correlation of the experimental data

The general relationship between the retention

factor and temperature has been well established based on thermodynamics of chromatography as [9]:

$$\ln k' = -\Delta H^0/RT + \Delta S^0/R + \ln \beta \quad (1)$$

Ikushima et al. [10] modified the Kamlet–Taft equation [11] and correlated extraction yield and separation efficiency by a solubility parameter and multi-parameter approach.

In the case of SFC, in addition to temperature, the

Table 4
R of the adjacent tocopherol peaks at various temperature and pressure

Temperature	Tocopherol	12 MPa	14 MPa	16 MPa	18 MPa	20 MPa
30 °C	α–γ	–	3.040	2.732	2.451	2.109
	γ–δ	–	0.811	0.788	0.729	0.654
40 °C	α–γ	4.009	3.402	3.053	2.795	2.39
	γ–δ	1.238	1.078	1.057	1	0.944
50 °C	α–γ		4.117	3.828	3.341	2.573
	γ–δ		1.445	1.374	1.244	1.058
60 °C	α–γ		6.063	5.915	5.434	3.809
	γ–δ		2.356	2.332	2.198	1.738
80 °C	α–γ		–	1.513	7.323	5.486
	γ–δ		–	×	2.768	2.530
90 °C	α–γ		–	–	7.107	7.281
	γ–δ		–	–	2.968	2.968

Note: “–” peak not eluted; “×” the adjacent peaks are not separated.

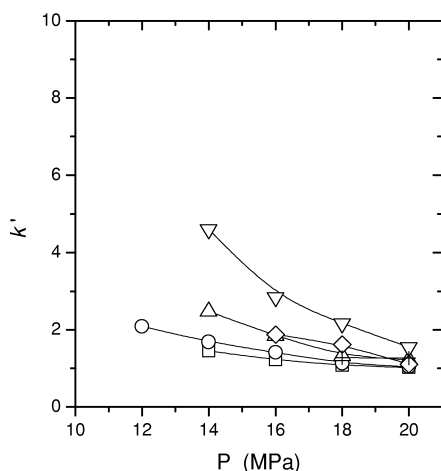


Fig. 5. Effect of pressure on k' of α -tocopherol. (\square) 30 °C; (\circ) 40 °C; (\triangle) 50 °C; (∇) 60 °C; (\diamond) 80 °C; (+) 90 °C.

effect of the pressure and the concentration of the modifier of mobile phase should be considered as variables as well. Bartle et al. [8] derived an expression including terms related to temperature, pressure and density of carbon dioxide; the concentration of modifier was not included. Martire and Boehm [12] developed a general model of chromatography based on a lattice–fluid model and applied it to the case of SFC of n -alkanes. This model is simplified and applied to correlate the data of the

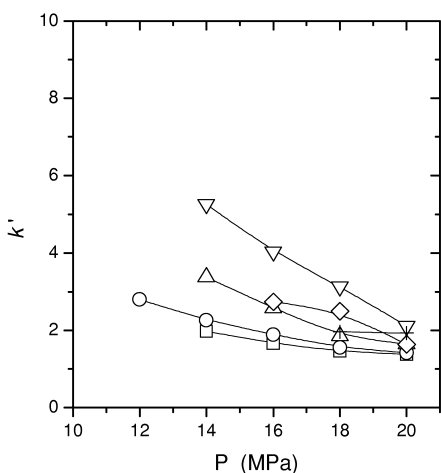


Fig. 6. Effect of pressure on k' of γ -tocopherol. (\square) 30 °C; (\circ) 40 °C; (\triangle) 50 °C; (∇) 60 °C; (\diamond) 80 °C; (+) 90 °C.

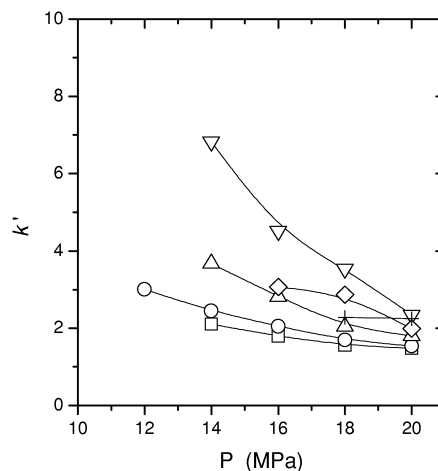


Fig. 7. Effect of pressure on k' of δ -tocopherol. (\square) 30 °C; (\circ) 40 °C; (\triangle) 50 °C; (∇) 60 °C; (\diamond) 80 °C; (+) 90 °C.

present work. The original expression of solute participation coefficient is as follows:

$$\ln K = \ln K^0 + F(T_r, \rho_r) + \Delta \quad (2)$$

where

$$\ln K^0 = \left(\frac{r_a}{r_b} \right) \left\{ \left[r_b (r_d^{-1} - 1) \right] + \left[\frac{(1 + r_b^{1/2})^2}{T_r} \right] \left(\frac{\varepsilon_{ad}}{\varepsilon_{bb}} - \frac{\varepsilon_{dd}}{2\varepsilon_{bb}} \right) \right\} \quad (3)$$

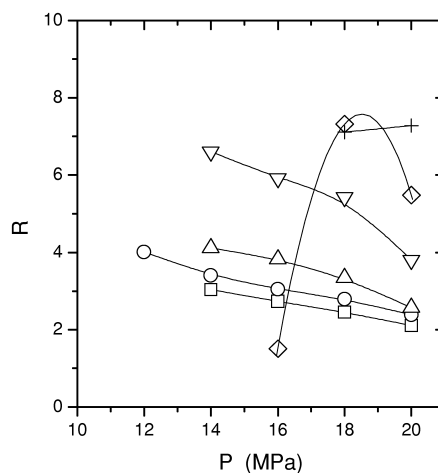


Fig. 8. Effect of pressure on R of α - and γ -tocopherol. (\square) 30 °C; (\circ) 40 °C; (\triangle) 50 °C; (∇) 60 °C; (\diamond) 80 °C; (+) 90 °C.

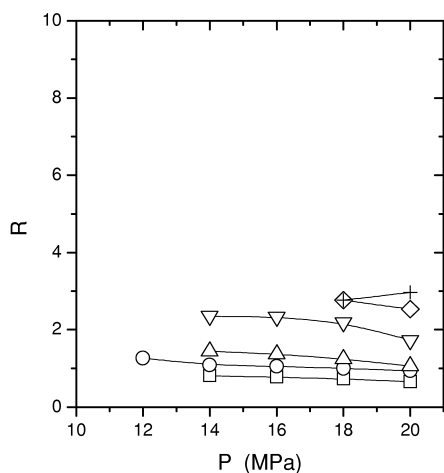


Fig. 9. Effect of pressure on R of γ - and δ -tocopherol. (\square) 30 °C; (\circ) 40 °C; (\triangle) 50 °C; (∇) 60 °C; (\diamond) 80 °C; (+) 90 °C.

$$F(T_r, \rho_r) = \left(\frac{r_a}{r_b}\right) \left[(r_b^{1/2} - 1) \rho_r - T_r^{-1} \left[(1 + r_b^{1/2}) \left(\frac{\varepsilon_{ab}}{\varepsilon_{bb}}\right) \rho_r - \frac{\rho_r^2}{2} \right] \right] \quad (4)$$

and Δ is the correction term due to uptake of supercritical fluid by the stationary phase.

Assuming that

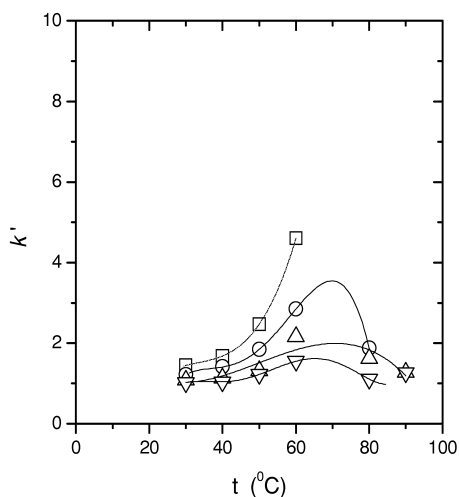


Fig. 10. Effect of temperature on k' of α -tocopherol. (\square) 14 MPa; (\circ) 16 MPa; (\triangle) 18 MPa; (∇) 20 MPa.

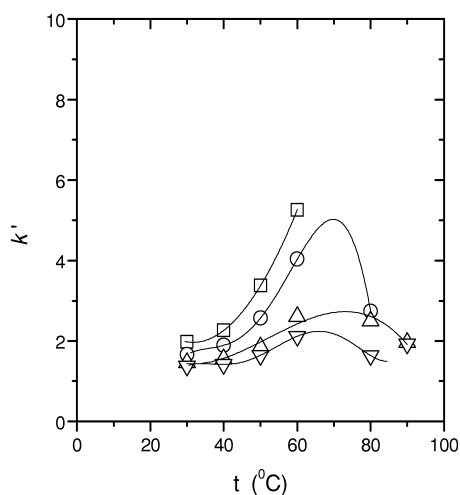


Fig. 11. Effect of temperature on k' of γ -tocopherol. (\square) 14 MPa; (\circ) 16 MPa; (\triangle) 18 MPa; (∇) 20 MPa.

(1) Δ is sufficiently small compared to $F(T_r, \rho_r)$ and could be neglected;

(2) ε_{ij} is the dimensionless total interaction energy depending on the nature of solute and the supercritical fluid, it can be regarded as a constant in the narrow range of density;

(3) the participation coefficient K is related to the k' by $K = k'(V_m/V_s)$, where V_s does not change with temperature and pressure;

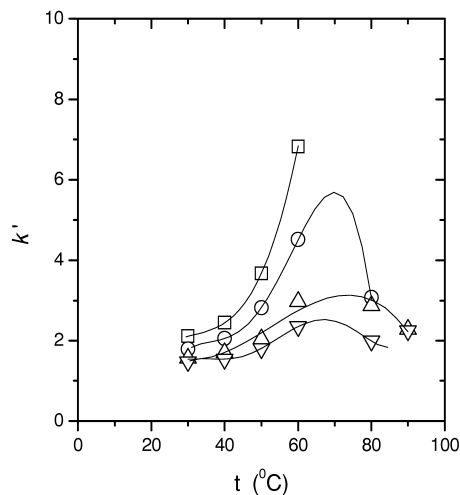


Fig. 12. Effect of temperature on k' of δ -tocopherol. (\square) 14 MPa; (\circ) 16 MPa; (\triangle) 18 MPa; (∇) 20 MPa.

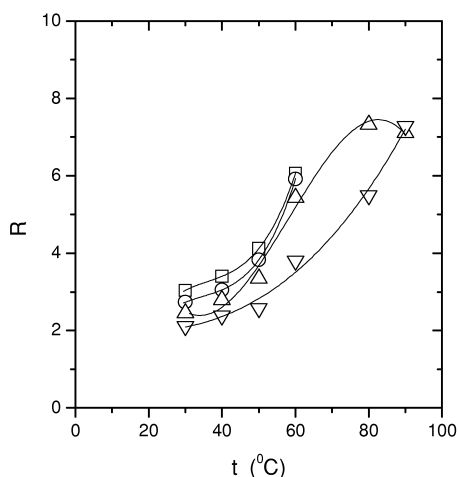


Fig. 13. Effect of temperature on R of α - and γ -tocopherol. (\square) 14 MPa; (\circ) 16 MPa; (\triangle) 18 MPa; (∇) 20 MPa.

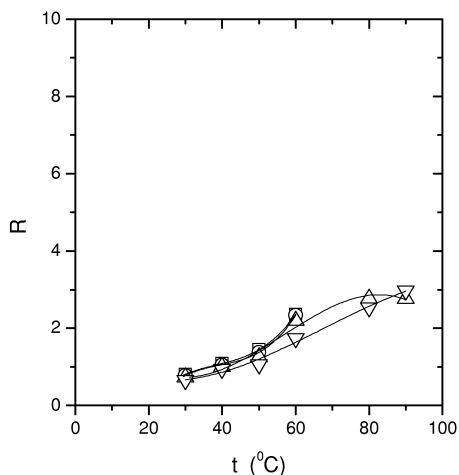


Fig. 14. Effect of temperature on R of γ - and δ -tocopherol. (\square) 14 MPa; (\circ) 16 MPa; (\triangle) 18 MPa; (∇) 20 MPa.

based on the original equation and the relation between K and k' the following simplified equation is obtained:

$$\ln k' = A + \frac{B}{T} + C\rho - D\left(\frac{\rho}{T}\right) + E\left(\frac{\rho^2}{T}\right) \quad (5)$$

where

$$A = \ln\left(\frac{V_s}{V_m}\right) + r_a(r_d^{-1} - 1) \quad (6)$$

$$B = \left(\frac{r_a}{r_d}\right)(1 + r_b^{1/2})^2 \left(\frac{\varepsilon_{ad}}{\varepsilon_{bb}} - \frac{\varepsilon_{dd}}{2\varepsilon_{bb}}\right) \left(\frac{1}{T_c}\right) \quad (7)$$

$$C = \left(\frac{r_a}{r_b}\right)(r_b^{1/2} - 1) \left(\frac{1}{\rho_c}\right) \quad (8)$$

$$D = \left(\frac{r_a}{r_b}\right) \left(\frac{T_c}{\rho_c}\right) (1 + r_b^{1/2}) \left(\frac{\varepsilon_{ab}}{\varepsilon_{bb}}\right) \quad (9)$$

$$E = \left(\frac{r_a}{r_b}\right) \left(\frac{1}{2\rho_c^2}\right) \quad (10)$$

$$\rho_r = \frac{\rho}{\rho_c}, \quad T_r = \frac{T}{T_c} \quad (11)$$

The density of supercritical CO_2 is calculated by an equation of state for carbon dioxide developed by F.H. Huang [13].

The parameters obtained by regression of experimental data are given in Table 5. It can be seen that the value of ARR%D for α -tocopherol is 14.70%, and that of other tocopherols are much smaller. Figs. 15–17 compare the plots and the curves by regression.

4. Conclusion

The retention factor and the resolution between α -

Table 5
Correlation of retention factor data with temperature and density

tocopherol	Points	A	B	C	D	E	AARD
α	17	11.18	-2037	-23.52	-5981	-412	14.70%
γ	17	21.32	-5845	-35.89	-11 731	-1613	3.75%
δ	17	25.22	-7324	-40.12	-13 754	-2046	3.09%

$$\text{AARD}(\%) = \frac{1}{N} \sum \frac{|k_i^{\text{exp}} - k_i^{\text{cal}}|}{k_i^{\text{exp}}} \times 100$$

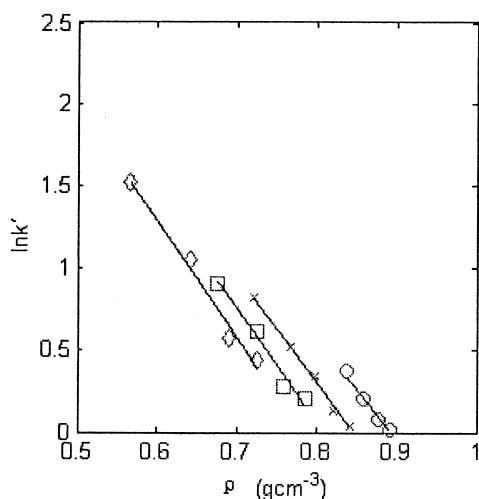


Fig. 15. Correlation of $\ln k'$ of α -tocopherol with temperature and density in supercritical CO_2 modified with ethanol. (○) 30 °C; (×) 40 °C; (□) 50 °C; (◇) 60 °C.

and γ -tocopherols and between γ - and δ -tocopherols were determined in the temperature range of 30 to 90 °C and pressure range of 12 to 20 MPa by SFC, the mobile phase was supercritical fluid carbon dioxide with 0 to 6.54% ethanol as modifier. It was found that the appropriate condition for the separation of α -tocopherol from γ - and δ -tocopherols is at 18 MPa, 80 °C, with 5% ethanol in carbon dioxide

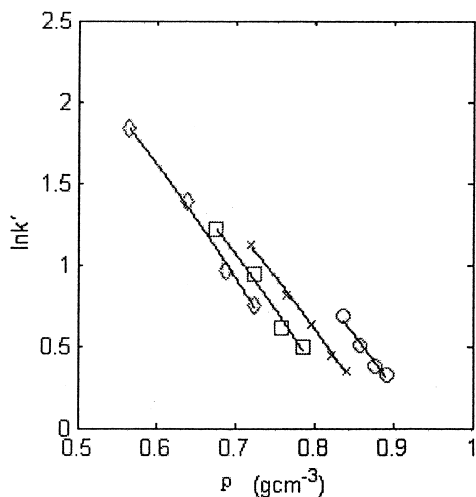


Fig. 16. Correlation of $\ln k'$ of γ -tocopherol with temperature and density in supercritical CO_2 modified with ethanol. (○) 30 °C; (×) 40 °C; (□) 50 °C; (◇) 60 °C.

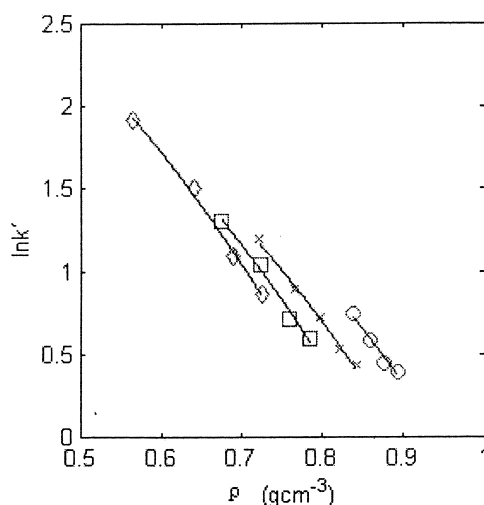


Fig. 17. Correlation of $\ln k'$ of δ -tocopherol with temperature and density in supercritical CO_2 modified with ethanol. (○) 30 °C; (×) 40 °C; (□) 50 °C; (◇) 60 °C.

as mobile phase. More accurate conditions are to be studied further by optimization.

The lattice model of Martire was applied to correlate the data of SFC of the studied system, including retention factor, density and temperature. The results are quite satisfactory.

5. Nomenclature

K	participation coefficient
K^0	participation coefficient $\rho_r \rightarrow 0$
k'	retention factor
R	resolution
T_r	reduced temperature
ρ_r	reduced density
ρ	density of binary mixture
r_i	lattice sizes of i molecular
Δ	the correction item to $\ln K$
ε_{ij}	the dimensionless total interaction energy of i and j molecular
V_m	volume of mobile phase
V_s	volume of stationary phase
ΔH^0	Standard enthalpy change
ΔS^0	Standard entropy change
A, B, C, D, E	the fitting parameters

Subscript

<i>a</i>	solute
<i>b</i>	mobile molecular
<i>c</i>	critical state
<i>d</i>	stationary molecular

Acknowledgements

This work was supported from Zhejiang Natural Science Foundation, China. Project Number: 201092.

References

- [1] D. Upomoor, G. Brunner, *Ber Bunsenges Phys. Chem.* 93 (1989) 1009.
- [2] J.L. Perrin, A. Prevot, *Rev. Fr. Corps Gras.* 35 (1988) 329.
- [3] T. Yarita, A. Nomura, K. Abe, Y. Takeshita, *J. Chromatogr. A* 679 (1989) 329.
- [4] G. Grzegorz, M. Gogolewski, *Chem. Anal. (Warsaw)* 41 (1996) 737.
- [5] M. Saito, Y. Yamauchi, *J. Chromatogr. Sci.* 27 (1989) 79.
- [6] M. Saito, Y. Yamauchi, *J. Chromatogr.* 505 (1990) 257.
- [7] J.W. King, F. Favati, S.L. Taylor, *Separat. Sci. Technol.* 31 (13) (1996) 1843.
- [8] K.D. Bartle, A.A. Clifford, J.Pa. Kithinji, D.F. Shilstone, *J. Chem. Soc. Faraday Trans. 1* 84 (12) (1998) 4487.
- [9] K. Yaku, K. Aoe, N. Nishimura, F. Marishita, *J. Chromatogr. A* 848 (1999) 337.
- [10] Y. Ikushima, N. Saito, K. Hatakeda, S. Ito, M. Arai, K. Arai, *Ind. Eng. Chem. Res.* 31 (1992) 568.
- [11] R.W. Taft, M.J. Kamlet, *J. Am. Chem. Soc.* 98 (1976) 2886.
- [12] D.E. Martire, R.E. Boehm, *J. Phys. Chem.* 91 (1987) 2433.
- [13] F.H. Huang, M.H. Li, L.L. Lee, K.E. Starling, F.T.H. Chung, *J. Chem. Eng. Jpn.* 18 (6) (1985) 490.