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Determination of Partial Molar Volumes of EPA and DHA Ethyl Esters in Supercritical Carbon Dioxide

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Abstract: The use of supercritical-fluid chromatography for determining partial molar volumes of ethyl esters of *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA) and *cis* -4,7,10,13,16,19-docosa-hexaenoic acid (DHA) in supercritical carbon dioxide is presented and discussed. Partial molar volumes of EPA and DHA esters are obtained from the variation of the retention properties with the density of mobile phase at 313.15 K, 323.15 K, 333.15 K and in the pressure range from 9 MPa to 21 MPa.

Keywords: EPA and DHA, capacity factor, partial molar volume, supercritical-fluid chromatography.

The use of supercritical fluids (SCFs) as solvents has been attracting widespread interest in the research and commercial applications. The study of partial molar volumes of solutes at infinite dilution in the near-critical region is of considerable fundamental importance, as these data reflects the interactions occurring between the solute and the solvent.

Experimental measurements of partial molar volumes may be divided into two categories: direct and indirect methods. The direct methods are normally based on density or volume determinations and the partial molar properties are derived directly from their definitions. Indirect methods are all based on measuring the variation of a given equilibrium property (*e.g.* solubility, capacity factor) with pressure.

EPA and DHA are constituents of marine lipids, which is a rich source of long-chain ω-3 polyunsaturated fatty acids. Clinical studies indicate that EPA and DHA have beneficial effects on human health[1]. In this work, supercritical-fluid chromatography has been used to measure the variation with pressure of retention properties of EPA and DHA in supercritical carbon dioxide.

According to the standard chromatographic terminology, the capacity factor k_i is defined as

$$k_i = \frac{t_i - t_0}{t_0} \tag{1}$$

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Where t_i is the retention time of component i and t_0 is the retention time of an unretained solute.

Based on the relationship of the equilibrium compositions in the mobile phase and stationary phase, partial molar quantities are expressed by the following form under the assumption that the k_i factor only varies with temperature, pressure and density.

$$\left(\frac{\partial \ln k_i}{\partial P}\right)_T = \frac{\bar{v}_{im}^{\infty} - \bar{v}_{is}^{\infty}}{RT} - k \tag{2}$$

Where $\bar{v}_{im}^{\infty}$ and $\bar{v}_{is}^{\infty}$ are the partial molar volumes at infinite dilution of component i in the mobile and stationary phase, respectively, and k is the mobile phase isothermal compressibility that is defines as

$$k = -\frac{1}{V}\left(\frac{\partial V}{\partial P}\right)_T \tag{3}$$

From equation (2), the derivative of the capacity factor with respect to the density is expressed by the following relationship

$$\left(\frac{\partial \ln k_i}{\partial r}\right)_T = \left(\frac{\partial P}{\partial r}\right)_T \left(\frac{\bar{v}_{im}^{\infty} - \bar{v}_{is}^{\infty}}{RT} - k\right) \tag{4}$$

Transforming the above expression we achieve

$$\left(\frac{\partial \ln k_i}{\partial \ln r}\right)_T = \left(\frac{\bar{v}_{im}^{\infty} - \bar{v}_{is}^{\infty}}{RT\,k} - 1\right) \tag{5}$$

Since $\bar{v}_{im}^{\infty}$ is much larger than $\bar{v}_{is}^{\infty}$ near the solvent critical point, the latter can be neglected. Thus the following relationship can be obtained :

$$\left(\frac{\partial \ln k_i}{\partial \ln r}\right)_T = \frac{\bar{v}_{im}^{\infty}}{RTk} - 1 \tag{6}$$

Because $\frac{\bar{v}_{im}^{\infty}}{k}$ is relatively constant in the vicinity of the solvent critical point, the partial molar volume can be easily obtained by rearranging this relationship.

Experimental

The marine lipids were purchased from Harbing Heilong Pharmaceutical Co. The concentration of ethyl esters of *cis*-5,8,11,14,17-eicosapentaenoic acid and *cis*-4,7,10,13, 16,19-docosahexaenoic acid in marine lipids is 70%. CO_2 (purity 99.99%) was obtained from the Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences.

The apparatus used was described in detail elsewhere[2]. Briefly, it consisted of a gas cylinder, a cooling bath, one HPLC-pump, a separator (50×4.6 mm ODS column), two digital pressure gauge, a high pressure ultra-violet detector, and a constant temperature bath. The experimental procedure employed in this study was based on the measurement of solute retention times using SFC. The determinations were carried out at 313.15 K, 323.15 K, 333.15 K and in the pressure range from 9 MPa to 21 MPa. A constant flow rate was used at each pressure and was measured using a bubble soap meter. Injection was made with a 20-μL loop. The experimental data were collected and analyzed by chromatographic workstation.

Results and Discussion

For each experimental run, four quantities were obtained: the retention time of the solute, temperature, pressure, and flow rate of the mobile phase. Data of the retention time represent an average of 2 or 3 determinations. And pure carbon dioxide density and the isothermal compressibility calculations were performed using the data from the IUPAC tables[3]. Thus from the slope of the capacity factor *vs*. density curve partial molar volume can be calculated.

Capacity factor(k_i) of EPA and DHA esters

Table 1 Capacity ratio k_i of EPA and DHA ethyl esters at infinite dilution

Temp. (K)	Press. (MPa)	k_i		Temp. (K)	Press. (MPa)	k_i	
		EPA	DHA			EPA	DHA
313.15	9.94	3.965	5.177	323.15	12.22	3.624	4.766
	10.96	3.086	3.947		14.57	2.239	2.845
	11.63	2.904	3.679		17.10	1.898	2.332
	12.25	2.238	2.828		20.77	1.297	1.550
	14.74	1.756	2.158	333.15	12.21	10.664	14.716
	17.06	1.466	1.788		14.63	4.170	5.434
	18.93	1.227	1.495		17.00	2.648	3.333
	20.78	1.161	1.378		18.76	1.974	2.456
323.15	11.71	5.842	7.774		20.59	1.627	1.994

The results of the experiments for the carbon dioxide-ester systems are presented in **Table 1** and in **Figure 1**. It is found that the natural logarithm of the capacity factors related approximately linearly to $\ln\rho$, which is similar to the theoretical relationship.

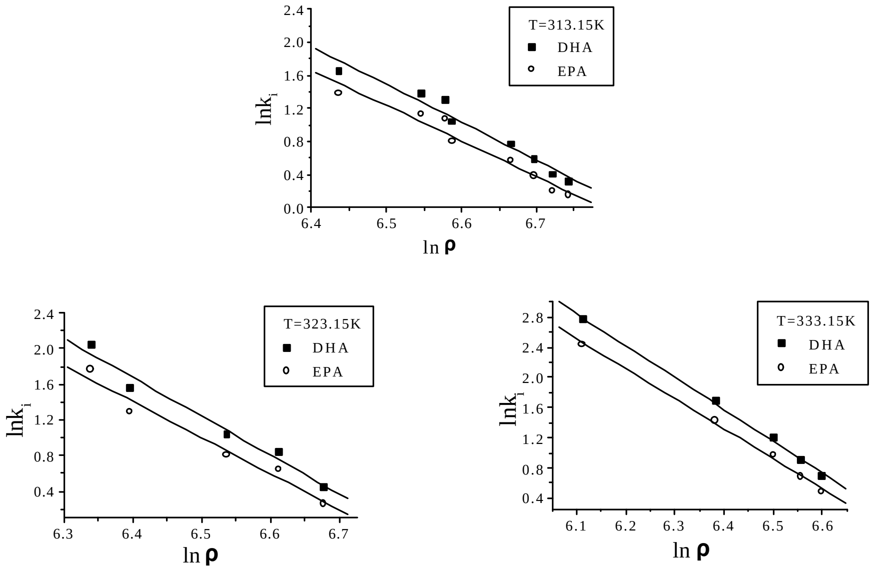
Determination of partial molar volumes

The experimentally determined partial molar volumes for the EPA and DHA ethyl esters at infinite dilution in supercritical carbon dioxide are presented in **Table 2**. It can be seen that the partial molar volumes of the ester-carbon dioxide systems are large and negative. This result indicates that a large number of solvent molecules collapse around a single solute molecule.

Table 2 Partial molar volumes of EPA and DHA ethyl esters at infinite dilution

Temp. (K)	Press. (MPa)	$\bar{V}_{im}^{\infty}$ (cm^3/mol)		Temp. (K)	Press. (MPa)	$\bar{V}_{im}^{\infty}$ (cm^3/mol)	
		EPA	DHA			EPA	DHA
313.15	9.94	-1110	-1210	323.15	12.22	-780	-861
	10.96	-473	-515		14.57	-320	-353
	11.63	-421	-458		17.10	-188	-207
	12.25	-318	-347		20.77	-116	-128
	14.74	-170	-185	333.15	12.21	-1330	-1350
	17.06	-128	-140		14.63	-557	-566
	18.93	-105	-114		17.00	-297	-302
	20.78	-89	-97		18.76	-214	-217
323.15	11.71	-1040	-1150		20.59	-164	-166

Figure 1 Plot of Ink_i *vs.* Inr for EPA and DHA ethyl esters



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