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Adsorption Equilibria of *cis*-5,8,11,14,17-Eicosapentaenoic Acid Ethyl Ester and *cis*-4,7,10,13,16,19-Docosahexaenoic Acid Ethyl Ester from Supercritical Carbon Dioxide on Silica Gel

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Adsorption data of *cis*-5,8,11,14,17-eicosapentaenoic acid ethyl ester (EPA-EE) and *cis*-4,7,10,13,16,19-docosahexaenoic acid ethyl ester (DHA-EE) from supercritical carbon dioxide on silica gel were determined at temperatures from (319.15 to 340.35) K and carbon dioxide densities from (0.668 to 0.734) $g \cdot mL^{-1}$ by using the elution by characteristic point (ECP) method. The equilibrium isotherm data were fitted using a Langmuir model. The monolayer adsorption capacity of EPA-EE is 0.130 $mmol \cdot g^{-1}$, and that of DHA-EE is 0.119 $mmol \cdot g^{-1}$. The adsorption enthalpy change of EPA-EE is in the range of (34.4 to 38.0) $kJ \cdot mol^{-1}$, and that of DHA-EE is in the range of (34.4 to 40.6) $kJ \cdot mol^{-1}$.

Introduction

In recent years, *cis*-5,8,11,14,17-eicosapentaenoic acid (EPA) and *cis*-4,7,10,13,16,19-docosahexaenoic acid (DHA) have attracted great attention because of their beneficial role in human health. These two ω-3 fatty acids are vital for the retina of human eyes and for the nervous system and reduce the risk of cardiovascular and inflammatory disease. Up to now, they have usually been processed into the form of their ethyl ester (EE) or methyl ester (ME) and used as their mixtures. The functions of EPA-EE and DHA-EE are different; i.e., DHA-EE especially benefits mental capacity and is usually added in infant foods to improve baby's brains, whereas EPA-EE is harmful to children's eyesight. So, it is of significance to separate them into their pure form.

Over the past ten years, some studies related to the separation of EPA-EE from DHA-EE by supercritical fluid chromatography (SFC) using supercritical CO_2 as the mobile phase have been carried out. Alkio et al.[1] prepared DHA-EE of 95 % assay and EPA-EE of 50 % assay at 338.15 K and 145 bar by using C18-bonded silica as the stationary phase. Yamaguchi et al.[2] obtained DHA-EE of 96 % assay by using argentated silica gel as the stationary phase and acetone or acetonitrile as the modifier. Pettinello et al.[3] prepared EPA-EE of 90 % assay with silica gel. Yang et al.[4] compared silica gel and C18-bonded silica and found that they both can separate EPA-EE from DHA-EE. However, other impurities were not well separated, and combination of the two stationary phases could improve the quality and productivity of EPA-EE and DHA-EE. However, to date, investigations on the separation of EPA-EE and DHA-EE by SFC have focused mainly on process technology. For the optimization of process and engineering design, it is essential to describe the SFC process by an appropriate mathematical model. As a consequence, basic data such as adsorption isotherms, diffusion coefficients and mass transfer coefficients are necessary. On the other hand, as a problem of physical chemistry, the adsorption of solutes from a supercritical fluid is unique in comparison with the adsorption from the gas phase or ordinary liquid solutions. Only a few relevant studies are found in the literature. For example, Tan and his co-workers[5,6] studied the adsorption of toluene and ethyl acetate on activated carbon from supercritical carbon dioxide. In addition, adsorption of some solutes such as α-tocopherol,[7–9] vitamin D_3,[7,8] terpene,[10,11] furfural,[12] salicylic acid,[13] and eicosane[14] from supercritical CO_2 have also been studied. Thus, it is of great value to undertake some research in this field. The present work is intended to determine the adsorption isotherms of EPA-EE and DHA-EE from supercritical carbon dioxide on silica gel.

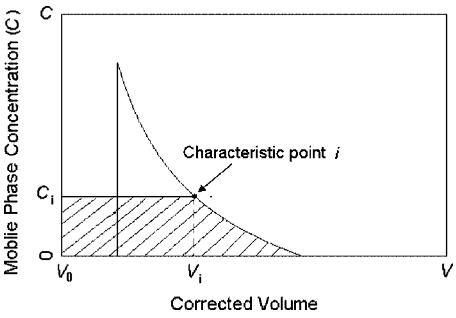

Figure 1. Elution by characteristic point (ECP).

Fundamentals. Adsorption isotherms are usually determined by either the static method or the frontal analysis method (FA). These methods are very tedious and time-consuming, particularly when used for a system composed of a supercritical fluid and viscous solutes like EPA-EE and DHA-EE. The method of elution by characteristic point (ECP), which was proposed by Glueckauf[15] and then well developed by Cremer and Huber,[16] is more convenient for the system studied in the present work. By ECP, an appropriate amount of an adsorbate sample is injected into a chromatographic column packed with an adsorbent and a chromatogram with a steep front, and a diffuse rear profile is obtained by elution. Assuming instant adsorption equilibrium is reached between the adsorption phase and the fluid phase, it was derived that at a characteristic point *i* (Figure 1) on the diffuse profile which corresponds to a concentration

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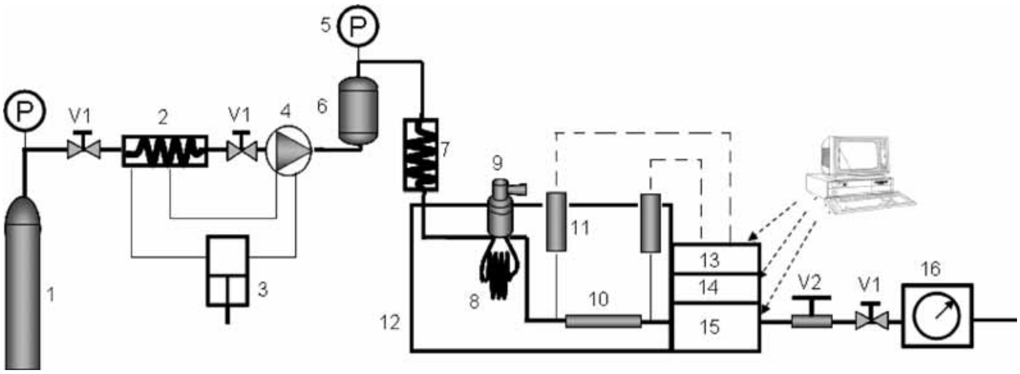

Figure 2. Schematic diagram of the experimental apparatus. 1, CO_2 cylinder; 2, CO_2 condenser; 3, cooling water pump; 4, CO_2 pump; 5, pressure gauge; 6, surge tank; 7, preheater; 8, injection loop; 9, injector; 10, chromatographic column; 11, pressure transducer; 12, thermostat; 13, pressure display; 14, temperature controller; 15, UV detector; 16, flow meter; V1, stop valve; V2, pressure regulator.

Table 1. Physical Properties of Silica Gel

mean particle size	$5 \cdot 10^{-6}$ m
surface area	$358.3\ m^2 \cdot g^{-1}$
pore volume	
< 30 Å	$0.054\ cm^3 \cdot g^{-1}$
30 to 200 Å	$0.397\ cm^3 \cdot g^{-1}$
> 200 Å	$0.044\ cm^3 \cdot g^{-1}$
average pore diameter	115.3 Å

of C_i in the fluid phase, the adsorption amount q_i is expressed by

$$q_i = \frac{1}{M}\int_0^{C_i} V_R(C)dC \tag{1}$$

$$V_R = V_i - V_0 \tag{2}$$

where M is the mass of adsorbent in the column; V_i is the retention volume of the characteristic point i; and V_0 is the dead volume of the column.

Though ECP is based on the instant equilibrium model which neglects all peak broadening factors, some studies have demonstrated that ECP is of high accuracy and precision when the column efficiency is close to or exceeds 5000 theoretical plates.[17,18]

Experimental

The experimental apparatus is illustrated in Figure 2. The volume of the two injection loops were (200 and 5) μL. The column of size of 250 × 4.6 mm i.d. was filled with silica gel purchased from EKA Chemicals, Bohus, Sweden (the physical properties are listed in Table 1). The bed void and particle porosity were 0.46 and 0.45, respectively. Carbon dioxide was purchased from Hangzhou Mingxing Ind. & Mer. Ltd., Hangzhou, China (purity > 99.995 %). EPA-EE and DHA-EE were prepared in this laboratory (purity > 99 %), and *n*-hexane was purchased from Eedia Company Inc., USA (spectroscopic grade).

Calibration of the solute concentration in supercritical CO_2 vs the detector signal was carried out by the static method described in Tan's work.[5] The detector will be applied to the method of ECP in this paper. It was found that up to the concentration of EPA-EE or DHA-EE of 8 $mg \cdot mL^{-1}$, at the detection wavelength of 220 nm, the detector signal (S) was linear with respect to the solute concentration (C) in supercritical CO_2, i.e., $S = hC$. The value of h depended on temperature and the density of CO_2, while R^2 values of regression were almost the same. For example, the regression for EPA-EE at 329.15 K and 0.700 $g \cdot mL^{-1}$ results in $S = 303.4C$ with an R^2 of 0.9987. Measurement of. the adsorption isotherms was conducted by injecting 200 μL of EPA-EE or DHA-EE solution with a concentration of 200 $mg \cdot mL^{-1}$ in *n*-hexane (about 132 mg of *n*-hexane was injected) at a defined temperature, pressure, and flow rate. The flow rate was kept constant for each run, and for all the runs, the flow rates were in the range of (2.9 to 3.5) $mL \cdot min^{-1}$. Before a sample was injected, 5 μL of EPA-EE or DHA-EE solution (0.5 $mg \cdot mL^{-1}$ in *n*-hexane) was injected to check the column efficiency under each condition.

Results and Discussion

Typical diffuse profiles of the chromatograph of EPA-EE at 329.15 K of three densities are shown in Figure 3. It can be seen that the front of the chromatogram is almost vertical to the abscissa which is a typical result of a favorable adsorption isotherm under the instant equilibrium condition. Furthermore, a peak of Gaussian distribution was obtained when a sample of 5 μL was injected, and the column efficiency determined was greater than 10 000. This ensures that the instant equilibrium

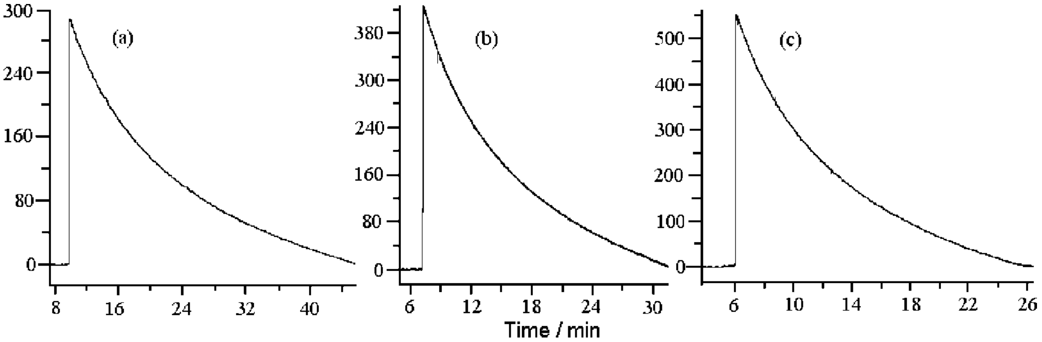

Figure 3. Elution peaks of EPA-EE with large injection obtained at 329.15 K: (a) 0.668 $g \cdot mL^{-1}$, (b) 0.700 $g \cdot mL^{-1}$, (c) 0.734 $g \cdot mL^{-1}$.

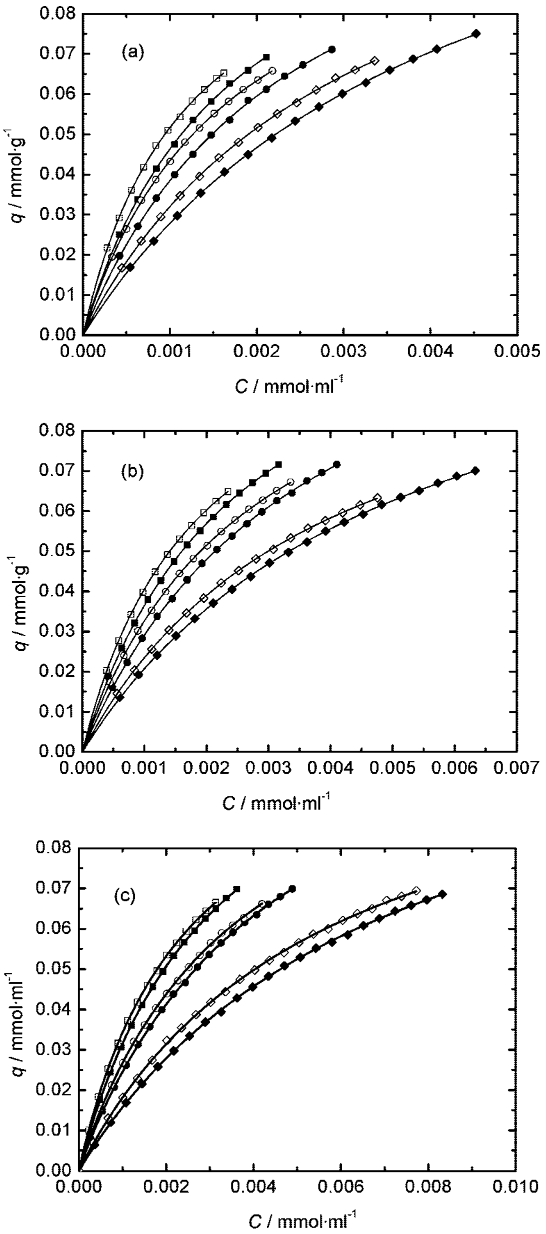

Figure 4. Adsorption data at densities of (a) 0.668 g·mL^{-1}, (b) 0.700 g·mL^{-1}, and (c) 0.734 g·mL^{-1} fitted by the Langmuir equation: ■ and □, $T = 319.15$ K; • and ○, $T = 329.15$ K; ◇ and ◆, $T = 340.35$ K ($T = 337.15$ K at 0.700 g·mL^{-1}); closed symbols, EPA-EE (1); open symbols, DHA-EE (2); solid lines, Langmuir model.

model of the ECP method is satisfied. The adsorption data of EPA-EE and DHA-EE at (319.15, 329.15, and 340.35) K under densities of (0.668, 0.700, and 0.734) g·mL^{-1} were obtained from the diffuse profiles and are plotted in Figure 4. Densities in this study were calculated by the Span and Wagner equation of state.[19]

Experimental data in Figure 4 indicate that the adsorption amount decreases with increasing temperature at a fixed CO_2 density. This trend is in accord with the basic principle of thermodynamics. In comparison to adsorption from ordinary liquid solutions, the adsorption amount depends not only on the temperature and the concentration but also on a new variable, i.e., the density of the supercritical fluid. The interaction between the molecules of the adsorbate and carbon dioxide increases as the CO_2 density increases. In addition, the competition adsorption of carbon dioxide on silica gel is enhanced by a higher CO_2 density as well. As a result, the adsorption amount of EPA-EE or DHA-EE decreases significantly with increasing CO_2 density. For example, at 329.15 K and a fluid concentration of 0.002 mmol·mL^{-1}, the adsorption amount of EPA-EE under 0.668 g·mL^{-1} is about 0.06 mmol·g^{-1}, whereas under 0.734 g·mL^{-1}, it is about 0.04 mmol·g^{-1}.

Under the same conditions, the adsorption amount of DHA-EE is more than that of EPA-EE at the same equilibrium concentration in CO_2. This is mainly due to the oxygen atom of the hydroxyl on the surface of the silica gel which tends to adsorb electron donors such as double bonds, and there is one more double bond in DHA-EE than in EPA-EE.

Modeling of the Adsorption Data. The Langmuir model (eq 3) is applied to represent adsorption data

$$q = \frac{q_s K C}{1 + KC} \tag{3}$$

where q_s is the monolayer adsorption capacity (mmol·g^{-1}) and K is the adsorption coefficient (mL·mmol^{-1}).

The solid lines in Figure 4 are the fitted results using the Langmuir model. It is apparent that the Langmuir model achieves a satisfactory fit with an average absolute deviation (AAD %) of about 0.6 %. The fitting parameters are listed in Table 2. As shown in Table 2, under all conditions, the monolayer adsorption capacity (q_s) of EPA-EE is in the range of (0.125 to 0.144) mmol·g^{-1}, and there is no relationship between q_s and temperature or CO_2 density. The calculated average q_s of all the conditions is 0.130 mmol·g^{-1}, with an average deviation of 0.004 mmol·g^{-1}. This makes sense because the adsorption amount of a monolayer depends mainly on the way the adsorbed molecules are packed on the surface and thus would not change with adsorption conditions. The same is observed for q_s of DHA-EE which is in the range of (0.113 to 0.131) mmol·g^{-1}, with an average of 0.119 mmol·g^{-1}.

The constant K decreases with increasing temperature or increasing density. Under the same conditions, the K value of

Table 2. Fitting Parameters of the Langmuir Model and Selectivity Factors for EPA-EE (1) and DHA-EE (2)

		EPA-EE			DHA-EE			
ρ / g·mL^{-1}	T / K	q_s / mmol·g^{-1}	K / mL·mmol^{-1}	AAD %	q_s / mmol·g^{-1}	K / mL·mmol^{-1}	AAD %	α
0.668	319.15	0.125	588	0.5	0.113	839	0.6	1.29
	329.15	0.131	414	0.6	0.117	591	0.6	1.28
	340.45	0.144	240	0.5	0.131	323	0.5	1.22
0.700	319.15	0.129	394	0.4	0.117	531	0.4	1.22
	329.15	0.135	276	0.5	0.122	367	0.5	1.20
	337.15	0.126	196	0.6	0.115	257	0.3	1.19
0.734	319.15	0.130	319	0.4	0.120	401	0.5	1.16
	329.15	0.134	223	0.5	0.124	275	0.6	1.14
	340.45	0.130	140	1.2	0.116	178	2.1	1.13

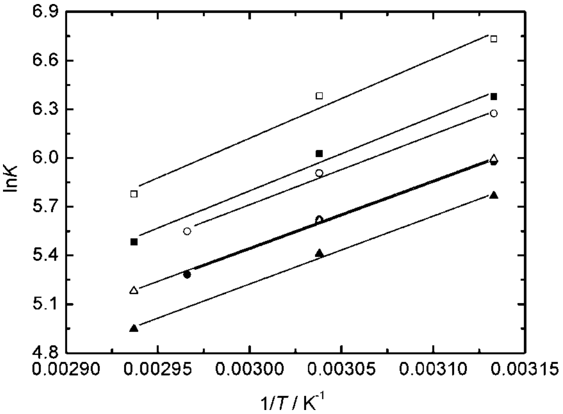

Figure 5. Plots of ln K vs 1/T at different densities: ■ and □, 0.668 g·mL^{-1}; • and ○, 0.700 g·mL^{-1}; ◇ and ◆, 0.734 g·mL^{-1}; closed symbols, EPA-EE (1); open symbols, DHA-EE (2).

Table 3. Enthalpy Change of Adsorption

	$-\Delta H$/kJ·mol^{-1}	
ρ/g·mL^{-1}	EPA-EE	DHA-EE
0.668	38.0	40.6
0.700	34.4	35.9
0.734	34.8	34.4

DHA-EE is greater than that of EPA-EE. The selectivity of DHA-EE and EPA-EE on silica gel can be defined as

$$\alpha = \frac{K_{\text{DHA-EE}}}{K_{\text{EPA-EE}}} \tag{4}$$

It can be seen from Table 2 that α decreases as CO_2 density or temperature increases.

Enthalpy Change of Adsorption. Suppose the Langmuir equilibrium constant K obeys the following equation[20]

$$K = k_0 e^{\frac{-\Delta H}{RT}} \tag{5}$$

where k_0 is an adsorption energy independent constant (mL·mmol^{-1}); ΔH is the enthalpy change of adsorption (kJ·mol^{-1}); and R is the universal ideal gas constant (8.314 J·mol^{-1}). ΔH can be determined from the slope of the line of the plot of ln K vs 1/T. Figure 5 shows the plots, and the estimated ΔH is given in Table 3. It can be seen that ΔH at 0.668 g·mL^{-1} is greater than at 0.700 g·mL^{-1}, while those at (0.700 and 0.734) g·mL^{-1} are almost the same.

Conclusion

Using the method of ECP, the adsorption isotherms of EPA-EE and DHA-EE from supercritical carbon dioxide on silica gel have been measured. The selectivity factor α for EPA-EE and DHA-EE is greater at lower temperature and density. Density is the main factor that affects the adsorption isotherm. The equilibrium isotherm data were fitted well by the Langmuir model with an average deviation of about 0.6 %. The monolayer adsorption capacity of EPA-EE and DHA-EE are similar. The enthalpy change of adsorption of EPA-EE and DHA-EE are in the range of (34.4 to 40.6) kJ·mol^{-1}.

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