Partition Coefficients for Fatty Acid Esters in Supercritical Fluid CO_2 with and Without Ethanol

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The complex nature of fish oils was exploited to study the dependence of structural factors upon fatty acid ester solubility in supercritical fluid carbon dioxide (SCF-CO_2). Partition coefficients were determined for a number of components present in two mixtures of fatty acid ethyl esters derived from menhaden oil in SCF-CO_2 at 60°C and 125 bar. Analogous data also were obtained for SCF-CO_2 with 5% (w/w) ethanol added. The addition of ethanol was found to increase partition coefficients for all species, but resulted in a decrease of fluid selectivity. Aside from the chain length of a component, both the degree and position of unsaturation were found to be structural factors that affect the value of the partition coefficient.

KEY WORDS: Cosolvent, ethanol, fatty acid esters, fish oil, partition coefficients, supercritical fluid carbon dioxide.

Interest in the biochemical properties of fatty acids of the ω3 class continues to be rather widespread. Recent reports have suggested that ω3 fatty acids may be essential for the development of the retinal and brain tissues of preterm infants and newborns (1). Interest in the use of these fatty acids for prevention and/or treatment of cardiovascular (2) and autoimmune (3) diseases, as well as some kinds of cancer (4), remains strong. A review of activities in these areas has been published (5). Such research has led to increasing demand for concentrates of the two major ω3 fatty acids—all *cis*-5,8,11,14,17 eicosapentaenoic acid (EPA or 20:5ω3) and all *cis*-4,7,10,13,16,19 docosahexaenoic acid (DHA or 22:6ω3). Presently, the most plentiful source for these two compounds is fish oil.

The National Marine Fisheries Service Laboratory in Charleston, South Carolina, is currently producing the ethyl esters of EPA and DHA in purities of 95% or better (6). Briefly, the process involves conversion of purified menhaden oil triglycerides to ethyl esters followed by urea adduction (7) at 5°C, which yields a concentrate composed primarily of ω3 polyunsaturates. This ω3 concentrate is then fractionated with supercritical fluid carbon dioxide (SCF-CO_2) in a batch process (8,9) to produce two new concentrates, one of which is primarily EPA and the other containing DHA as the major component. Finally, these two concentrates are used as feedstock in the final preparatory high-performance liquid chromatography (HPLC) step (10) to produce the EPA and DHA end products. Predicated upon a desire to use solvents of low toxicity, the mobile phase used in the final step is ethanol (EtOH)/water.

The batch supercritical fluid fractionation step is one of the most labor-intensive of the overall process. This is due to the fact that the conditions under which the selectivity of the pure fluid is sufficient to fractionate the feed material are also those at which the solubilities of components present in the feed are rather low, thus leading to a reduce throughput. It has been suggested that modification of the pure fluid with some appropriate cosolvent can accomplish an increased throughput in such processes (11). In the present case we were limited to selecting a cosolvent that would introduce no additional solvent residues that would be difficult to remove completely from the final products. Given that EtOH is already being utilized in the mobile phase of the prep-HPLC step, the effect of its addition to supercritical fluid CO_2 was investigated. The hope was that the addition of a few weight percent EtOH would increase loading of the solvent and thus decrease the time necessary for production of the EPA and DHA concentrates used as the feedstock in the final HPLC step.

To test the feasibility of this notion, partition coefficients (defined below) were determined for a number of fatty acid ethyl esters in mixtures derived from menhaden oil. Components in these mixtures contain anywhere from 14 to 22 carbons, with esters of equal chain length containing anywhere from 0 to 6 double bonds. Several isomeric polyunsaturates are also present. The inherent complexity of fish oils has been cited often as problematic in efforts to isolate potentially valuable EPA and DHA. In this study, the diverse series of fatty acids occurring in fish oils presents a unique opportunity to investigate the influence of structural characteristics upon the solubility of ethyl esters in both pure and ethanol-modified supercritical fluid CO_2.

EXPERIMENTAL PROCEDURES

Materials. Initial experiments were performed with ethyl esters derived from light, cold-pressed menhaden oil. Triglycerides were converted to ethyl esters (12), vacuum bleached and molecularly distilled. The composition of the clear product is given in Table 1. Part of this same material was fractionated with supercritical fluid carbon dioxide to produce a concentrate of 20-carbon esters (*ca.* 86%), the composition of which is also given in Table 1. The SCF-CO_2 fractionation procedure has been thoroughly described elsewhere (8,9). The C_{20}-rich fraction was isolated to concentrate 20-carbon components present at only trace levels in the whole ester mixture to levels sufficient to give reliable partition coefficient data.

Methods. All partition coefficients were obtained in a flow-through apparatus that has been described elsewhere (8,9). Briefly, the heart of the system is a double-ended, diaphragm-type compressor rated to 680 bar (Newport Scientific, Jessup, MD). System pressure was controlled by a black pressure regulator (Tescom, Elk River, MN) and measured with a pressure transducer (Validyne, Northridge, CA). Pressure fluctuations in the extractor were damped to about ± 1 bar by means of a surge vessel. Carbon dioxide was heated to the desired temperature prior to entering the extractor, which, like the preheater, was wrapped with silicon rubber heating tapes. These tapes were wired to digital PID temperature controllers (Syscon International, Elkhart, IN) which regulated the current supplied based upon input from internal thermocouple probes (Newport Scientific). The extraction vessel consisted of a 10″ length of pipe nipple with an internal

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TABLE 1

Fatty Acid Profiles of Fatty Acid Ethyl Ester (FAEE) Test Materials

FAEE component	Whole esters[a]	C_{20} concentrate[a]
14:0	7.5	n.d.[c]
16:0	15.6	n.d.
16:1ω7	11.0	n.d.
18:0	3.1	3.7
18:1ω9	7.8	2.6
20:0	0.2	0.5
20:1ω11	0.1	0.7
20:1ω9	1.2	4.9
20:1ω7	0.2	0.8
20:2ω9[b]	0.2	1.0
20:2ω6	0.2	0.9
20:3ω6	0.3	1.1
20:3ω3	0.2	0.7
20:4ω6	1.0	3.9
20:4ω3	1.6	5.9
20:5ω3	17.1	64.6
22:6ω3	11.3	3.1

[a] GC peak area percent.
[b] Tentative identification.
[c] n.d., Not detected.

diameter of 11/16″ (Autoclave Engineers, Erie, PA). Extracted material was expanded across a heated valve and extract was collected in a U-Tube immersed in an ice bath. Flow rate was monitored with a digital mass flow meter (Sierra Instruments, Inc., Monterey, CA) and total gas volume was measured a dry test meter (American Meter Co., Philadelphia, PA). In runs in which the SCF-CO_2 was modified, ethanol was introduced between the compressor and the preheater through an HPLC pump (Shimadzu, Columbia, MD). The pumping rate was adjusted to give CO_2 containing 5% ethanol (w/w). The phase behavior of the CO_2-ester systems was observed visually with a Jurgeson gauge (Clark-Reliance, Strongsville, OH).

In a typical experiment, 7.5–10 g of test material was suspended on Pyrex wool in the extractor and the remaining volume was filled with 0.16″ Propak, a stainless steel packing material (Scientific Development, State College, PA). The system was then pressurized and equilibrated at the desired temperature. Pure or ethanol-modified SCF-CO_2 was then passed through the extractor at *ca.* 3 standard liters/min, a flow rate that has been shown previously to be low enough to result in near-equilibrium conditions for triolein (13). Gas chromatographic (GC) analyses were performed in a Shimadzu GC-6A with flame ionization detection (FID) and by methodology described elsewhere (8). Peak areas were not corrected for FID response (14,15), as sample calculations showed that introduction of response factors had no effect on the data. Component identifications were made by GC-mass spectroscopy on a Hewlett-Packard (Palo Alto, CA) model 5890A with a 5970 Series mass selective detector. Separation was achieved on a 30 M × 0.25 mm-ID Supelcowax 10 fused silica column (Supelco, Bellefonte, PA). All spectra were recorded at an ionization energy of 70 eV. Double bond positions for methylene-interrupted polyenoic esters were assigned based upon knowledge of the elution order of positional isomers and by comparison of ion intensities at m/e = 108, 150 and 192, as described by Fellenberg *et al.* (16).

RESULTS AND DISCUSSION

To evaluate the effect of addition of EtOH to supercritical fluid CO_2, the partition (or distribution) coefficients for various components present in complex ethyl ester mixtures at 60°C and 125 bar were determined in CO_2 modified by the addition of 5% EtOH by weight on a solute-free basis. These data were compared with partition coefficients obtained in pure CO_2 for the same mixtures and the same temperature and pressure. From sight glass observations, it was determined that under these experimental conditions, the CO_2-ester system consists of two phases—a denser lipid-rich phase, in which some CO_2 is dissolved and a lighter CO_2-rich phase in which some esters are dissolved. The partition coefficient of any given component *i*, PC(*i*), is defined as:

$$PC(i) = Y_i/X_i \qquad [1]$$

where Y_i is the concentration of component *i* in the CO_2-rich phase, and X_i is the concentration of component *i* in the lipid-rich phase.

Weight concentration units are used throughout this work. From the GC analysis and weight of the fraction, as well as from the gas volume, Y_i in Equation [1] was determined for all components of interest. The value of X_i in Equation [1] was inferred from mass balance considerations on a CO_2-free basis. (In separate experiments, the CO_2 content of the lower phase was found to be *ca.* 30% by weight at the pressure and temperature used in this study. Since neglecting the CO_2 content of this phase introduces a systematic error, which does not effect any conclusions discussed below, data have not been corrected.)

Partition coefficients at 60°C and 125 bar for the major components in the whole ester mixture in CO_2 and CO_2 modified with 5% ethanol (w/w) are given in Table 2. Each value represents the mean of 20–30 determinations, and the numbers in parentheses are standard deviations. The data, presented in graphical form in Figure 1, demonstrate the well-known fact that esters of shorter chain length are relatively more soluble than those of longer chain length (17,18). The data also demonstrate that addition of ethanol at the 5% level increases the partition coefficients for all components. In Table 2, values are also give for the selectivities (χ) of CO_2 for 14:0 relative to the other major components. The selectivities are calculated by taking the ratio of the partition coefficient of 14:0 to those of the other components. Generally, the larger the value for χ, the greater the ease with which 14:0 can be separated from the component of interest. By comparison of the value for χ in pure CO_2 *vs.* those values in ethanol-modified CO_2, it is apparent that, at least for longer chain length species, the modified solvent is less selective. For example, while the selectivity of the pure fluid for 14:0 relative to 22:6ω3 is 28.8, the addition of ethanol results in the reduction of this selectivity value to 12.8. Although the general increase of the partition coefficients of all components has the desired effect of greater loading of the solvent, in practical terms the decreased selectivity values will be reflected by a less successful fractionation.

Enhancement of solubilities or partition coefficients by addition of cosolvents to supercritical fluids has been of interest to several groups in recent years (11,19,20). The "entrainer effect" has been defined as an increase in both

TABLE 2

Partition Coefficients (PC) and Selectivities (χ) for Selected Fatty Acid Ethyl Ester (FAEE) Components of the Whole Ester Mixture in Pure CO_2 and CO_2 Modified with 5% Ethanol at 60°C and 125 Bar (figures in parentheses represent standard deviation from the mean)

FAEE component	PC × 10^3 (pure CO_2)	χ^a	PC × 10^3 (CO_2 + EtOH)	χ^b
14:0	28.0 (2.7)	—	39.6 (3.9)	—
16:0	13.1 (2.1)	2.2	20.4 (2.4)	1.9
16:1ω7	15.1 (2.3)	1.9	22.6 (2.5)	1.8
18:0	5.0 (1.0)	5.6	8.8 (2.4)	4.5
18:1ω9	6.3 (1.0)	4.4	10.9 (1.5)	3.6
20:1ω9	2.0 (0.5)	14.0	5.4 (1.4)	7.3
20:5ω3	2.9 (0.6)	9.7	6.2 (1.1)	6.3
22:6ω3	1.0 (0.3)	28.0	3.1 (0.8)	12.8

[a] Selectivity of pure CO_2 for 14:0 relative to the stated component.

[b] Selectivity of ethanol-modified CO_2 for 14:0 relative to the stated component.

TABLE 3

Partition Coefficients (PC) and Selectivities (χ) for Selected Fatty Acid Ethyl Ester (FAEE) Components of the C_{20} Concentrate in Pure CO_2 and CO_2 Modified with 5% Ethanol at 60°C and 125 Bar (figures in parentheses represent standard deviation from the mean)

FAEE component	PC × 10^3 (pure CO_2)	χ^a	PC × 10^3 (CO_2 + EtOH)	χ^b
20:0	2.4 (0.4)	1.8	8.1 (1.0)	1.3
20:1ω11	3.3 (0.5)	1.3	8.6 (1.5)	1.3
20:1ω9	3.3 (0.4)	1.3	9.2 (0.6)	1.2
20:1ω7	3.3 (0.5)	1.3	9.1 (1.2)	1.2
20:2ω[c]	3.7 (0.4)	1.1	10.0 (0.7)	1.1
20:2ω6	3.4 (0.5)	1.2	9.3 (0.6)	1.2
20:3ω6	3.7 (0.4)	1.1	10.2 (0.6)	1.1
20:3ω3	3.1 (0.5)	1.4	8.6 (0.6)	1.3
20:4ω6	4.2 (0.5)	—	10.9 (0.6)	—
20:4ω3	3.5 (0.5)	1.2	9.7 (0.7)	1.1
20:5ω	3.9 (0.5)	1.1	10.3 (0.6)	1.1

[a] Selectivity of pure CO_2 for 20:4ω6 relative to the stated component.

[b] Selectivity of ethanol-modified CO_2 for 20:4ω6 relative to the stated component.

[c] Tentative identification.

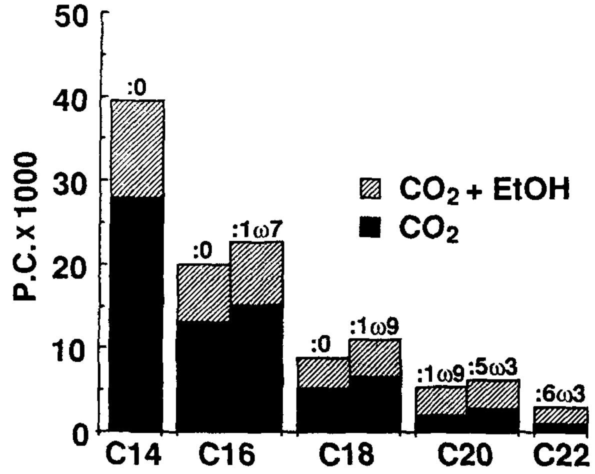

FIG. 1. Partition coefficients for selected components of the whole ester mixture for pure CO_2 and CO_2 + 5% ethanol (w/w) at P = 125 atm and T = 60°C.

solvent power and selectivity of a supercritical fluid upon addition of a small amount of certain cosolvents (20). In many systems, the observed solubility enhancements have been attributed to chemical interactions, such as hydrogen bonding between solute molecules and the cosolvent. Solubility enhancements due to such chemical interactions are distinguished from those that are merely due to an increase in the density of the solvent attributable to addition of the cosolvent. Such an increase in density would be expected to also lead to a decrease in selectivity similar to that observed for the data in Table 2. In fact, a decrease in the molal volume has been noted when a few wt.% ethanol is mixed with CO_2 at elevated pressures (21). If the increase of partition coefficients found upon addition of EtOH is in fact due to a resultant increase in solvent density, similar values should be found in pure CO_2 at the same temperature, but at a somewhat higher pressure. To test this hypothesis, partition coefficients for the whole esters in pure CO_2 were measured at slightly higher pressures. Partition coefficients quite similar to those of Table 2 for the EtOH-modified system were found for all components in pure CO_2 at 60°C and 131 bar. It is therefore apparent that, as defined above, ethanol does not exhibit an entrainer effect in this system. Again, the practical consequence of these findings is that EtOH serves no useful purpose as a cosolvent with CO_2 in SCF fractionation of fatty acid esters to concentrate EPA and DHA.

Table 3 summarizes partition coefficient data for components of the C_{20} concentrate in CO_2, both with and without 5% added EtOH. It is immediately obvious that addition of EtOH has little effect upon the selectivity of the fluid for components of this mixture, but increases the value of partition coefficients by a factor of *ca.* 2–3. Since all components are of equal chain length, the PC values are similar and the postulated increase of fluid density upon addition of EtOH is not expected to lead to notable changes in selectivity.

Each PC value in Table 3 represents the mean of 18 determinations with numbers in parentheses giving the standard deviations from the means. It might be argued that within the spread of the values, the PC of, for example, 20:5ω3 is not statistically different from that of 20:4ω6. It is, however, important to note that in all 18 determinations, the PC for 20:4ω6 was greater than that of 20:5ω3. With the exception of the monoenoic isomers, which have PC values that are essentially identical, the same is true when comparing the values of any pair of components in Table 3. A couple of conclusions regarding the dependence of PC values upon certain structural factors arise. First of all, the higher the degree of unsaturation, the higher the value of the partition coefficient, as is apparent from Figure 2. This observation is consistent with previous studies on the solubilities of fatty acids in "conventional" solvents, such as acetone (22). It is also worth noting that in the distillation of similarly complex mixtures of esters which, like SCF-CO_2, separates

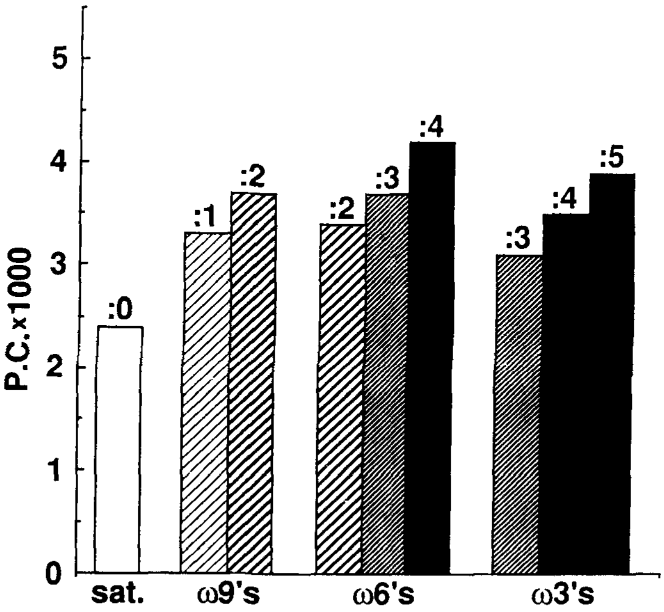

FIG. 2. Dependence of partition coefficients for selected components in the C_{20} concentrate upon degree of unsaturation; P = 125 bar, T = 60°C.

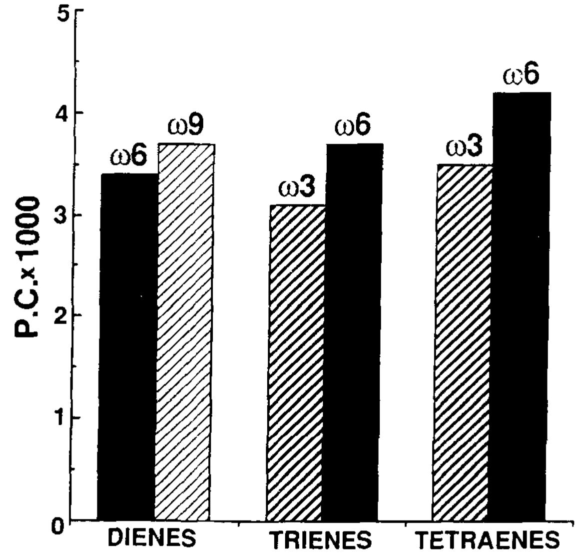

FIG. 3. Dependence of partition coefficients for isomeric components in the C_{20} concentrate upon position of unsaturation; P = 125 bar, T = 60°C.

primarily by chain length, the more highly unsaturated esters of a given chain length distill prior to less unsaturated components of equal chain length (23).

Figure 3 illustrates the dependence of the PC for isomers upon the location of unsaturation along the chain. For all three pairs of isomeric polyenes, the higher the "ω" designation, the larger the partition coefficient. In comparing the structures of 20:4ω3 and 20:4ω6, it may be relevant to note that for the latter isomer, the center of unsaturation more closely corresponds to the center of the 20-carbon chain. Based on molecular symmetry considerations, it may therefore be reasonable to expect that the ω6 isomer has a slightly higher vapor pressure than the ω3 isomer. Similarly, for the other two pairs of isomers illustrated in Figure 3, the isomeric species with the higher PC value also corresponds to that in which the center of unsaturation more closely corresponds to the center of the chain. We therefore speculate that the observed (small) differences in relative solubilities are due to differences in the vapor pressures of these isomeric species. Unfortunately, due to a shortage of vapor pressure data for isomers of fatty acid esters, this hypothesis cannot be tested.

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