Adsorption Equilibria of Fatty Acids Between Methanol/Water and Reversed-Phase Chromatographic Adsorbents

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ABSTRACT: The adsorption equilibria are discussed for fatty acids 16:1n-7, 16:2n-4, and 20:5n-3 (eicosapentaenoic acid, EPA). These fatty acids are major components of a polyunsaturated fatty acid concentrate from the microalga *Phaeodactylum tricornutum*. The solvents used were methanol/water (1% acetic acid) mixtures of different compositions, and the adsorbents used were chromatographic reversed-phases octylsilyl C_8, octadecylsilyl C_{18}, and dodecylsilyl C_{22} of different particle and pore sizes. The kinetic studies showed that equilibrium was attained instantaneously, suggesting an absence of mass transfer limitations. The equilibrium data were fitted by the Freundlich isotherm. The separation efficiency of EPA from 16:1n-7 and 16:2n-4 in all the adsorbent–solvent systems was compared in terms of the separation factors $\alpha_{EPA/16:2n-4} = K_{EPA}/K_{16:2n-4}$ and $\alpha_{16:1n-7/EPA} = K_{16:1n-7}/K_{EPA}$, where K_i is the fatty acid distribution ratio between the stationary and the liquid phases. The EPA separation from 16:1n-7 and 16:2n-4 by liquid chromatography could be predicted using the Craig model for the various solvent–adsorbent combinations. The best adsorbents for purifying EPA were: C_{18}, PEP, 8 µm, 100 Å, and C_{22}, 10 µm, 100 Å, and the best solvent was methanol/water (1% acetic acid) 75:25, w/w.

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KEY WORDS: Adsorption isotherms, Craig model, eicosapentaenoic acid, reversed-phase chromatographic adsorbents.

The n-3 polyunsaturated fatty acid (PUFA) eicosapentaenoic acid (EPA, 20:5n-3) has an important role in human health. EPA has potential uses for prevention or treatment of medical disorders such as heart and circulatory diseases (1,2), inflammation (3), and cancer (4). Pharmaceutical and clinical applications require highly pure EPA. The lack of adequate amounts of pure material for nutritional and clinical trials seriously affects systematic investigation of preventive and therapeutic roles of n-3 PUFA (5).

We recently developed a three-step process to obtain highly pure EPA from fish oil and microalgae (6,7). The three steps are: (i) fatty acid extraction by direct saponification of wet biomass, (ii) enrichment of PUFA by urea fractionation, and (iii) isolation of EPA through reversed-phase preparative high-performance liquid chromatography (HPLC). In the final step 635 mg of PUFA concentrate from the microalga *Phaeodactylum tricornutum* was loaded in a Waters (Milford, MA) reversed-phase compression radial cartridge (Bondapak; C_{18}, 5 µm particle size, 8 nm pore, 4.7 cm i.d. × 30 cm), obtaining 326 mg of 95.8% pure EPA; a flow rate of 66 mL·min^{-1} of methanol/water (1% acetic acid) 80:20 (w/w) was used. Also EPA purities of 96 and 94% (8,9) were attained from microalgae *Isochrysis galbana* and *Porphyridium cruentum*, respectively. These investigations demonstrated that reversed-phase chromatography is a good technique to purify fatty acids. This paper attempts to improve this technique. Purification of fatty acid can be achieved also by using other stationary phases such as argentated silica gel. This approach has been described for recovering EPA from *P. tricornutum* and *Monodus subterraneus* (10). The maximum purity obtained was 96% using hexane/acetone 90:10 (vol/vol), and in these conditions fatty acids were contaminated by silver. Contamination occurred when the percentage of acetone in the mobile phase (hexane/acetone) was higher than 5% (10). In general, a purification scheme for food and pharmaceutical products should use only low or nontoxic solvents (11,12). Methanol is less toxic than other solvents such as acetonitrile. In addition, use of methanol is preferable because it is easier (less expensive) to remove by evaporation than is acetonitrile (boiling points of 65 and 82°C, respectively).

EPA purity depends on the composition of the PUFA concentrate. The above-mentioned 94–96% pure EPA fractions obtained by reversed-phase chromatography were mainly contaminated with the fatty acids 16:1n-7 and 16:2n-4 (6,8,9), because the system stationary–mobile phases used did not completely separate EPA from 16:1n-7 and 16:2n-4. To improve this separation, this work undertook batch-adsorption studies to determine the kinetics and the adsorption equilibria of the fatty acids 16:2n-4, EPA, and 16:1n-7, in different reversed-phase chromatographic adsorbents, using methanol/water of different compositions as solvents.

MATERIALS AND METHODS

Table 1 lists the adsorbent–solvent systems used and the characteristics of adsorbents. Methanol and acetic acid were HPLC quality, and water was purified with a Milli-Q system (Millipore Co., Bedford, MA). Table 2 shows the fatty acid composition of the PUFA concentrate from *P. tricornutum* used to study the adsorption of the fatty acids 16:2n-4, 16:1n-7, and EPA. The preparation of the PUFA concentrate from *P. tricor-*

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TABLE 1
Adsorbents and Solvents Used in the Studies of Adsorption of 16:1n-7, 16:2n-4, and EPA. Physical Characteristics of Adsorbents

	Physical characteristics of adsorbent					
Adsorbent	Particle size (μm)	Pore size (Å)	Pore volume (cm^3/g)	Surface area (m^2/g)	Carbon load (%)	Solvent: methanol/water (1% AcH) (w/w)
Hyperprep C_8[a]	8	120	0.70	200	7.0	80:20
Hyperprep C_{18}[a]	8	120	0.70	200	10.5	80:20
Hyperprep C_{18}[a]	12	120	0.70	200	10.5	80:20
Hyperprep C_{18}[a]	30	120	0.70	200	10.5	80:20
Hyperprep C_{18}, HS[a]	8	100	0.70	300	15.0	80:20
Hyperprep C_{18}, PEP[a]	8	100	0.70	300	15.0	80:20
C_{22}[b]	10	100	—	—	—	80:20
Hyperprep C_{18}[a]	30	120	0.70	200	10.5	75:25
C_{22}[b]	10	100	—	—	—	75:25
Hyperprep C_{18}[a]	30	120	0.70	200	10.5	70:30
C_{22}[b]	10	100	—	—	—	70:30

[a]Provided by Shandon HPLC (Life Sciences International GmbH, Frankfurt, Germany). Hyperprep PEP has more narrow particle-size distribution than Hyperprep HS.
[b]Provided by INC Biomedicals GmbH (Schwege, Germany).

nutum biomass has been described elsewhere (6).

The kinetic trials were carried out using solutions of PUFA concentrate with a concentration of 4 $mg \cdot mL^{-1}$ (except the trials with the system C_{18}, 30 μm, 120 Å–methanol/water (1% acetic acid) 80:20, w/w, for which the concentration was 6 $mg \cdot mL^{-1}$). The equilibrium trials were carried out with solutions in which the PUFA concentration range was 0.5–31.4 $mg \cdot mL^{-1}$. PUFA solution (1.5 mL) contained in a glass tube was placed in an incubator chamber (Hotcold-M. Selecta, Barcelona, Spain) at 25°C. Added was 150 mg of adsorbent and the mixture was agitated with a magnetic stirrer. In the kinetic trials, the mixtures were agitated for 0.25, 1, 5, and 60 min but for only 20 min in the equilibrium trials. Then the liquid and solid phases were separated by filtering through a 0.45-μm filter (Lida, Kenosha, WI).

TABLE 2
Fatty Acids Composition (% of total fatty acid) of the *Phaeodactylum tricornutum* PUFA Concentrate Where 16:1n-7, 16:2n-4, and EPA Are Contained[a]

Fatty acid	PUFA concentrate
14:0	0.3
16:0	—
16:1n-7	6.4
16:2n-4	6.4
16:3n-4	16.8
16:4n-1	5.5
18:0	—
18:1n-9	—
18:1n-7	—
18:2n-6	1.6
18:3n-3	0.3
18:4n-3 (SA)	0.7
20:4n-6 (AA)	3.4
20:5n-3 (EPA)	50.2
22:6n-3 (DHA)	3.0
Others	5.5

[a]PUFA, polyunsaturated fatty acid; EPA, eicosapentaenoic acid; AA, arachidonic acid; DHA, docosahexaenoic acid; SA, stearidonic acid.

The amounts of 16:2n-4, EPA, and 16:1n-7 adsorbed per mg of adsorbent (q) were calculated by Equation 1:

$$q = \frac{(c_0 - c) \cdot V_0}{W} \quad [1]$$

where c_0 and c are, respectively, the concentrations of 16:2n-4, EPA, or 16:1n-7 in the liquid phase before adding the adsorbent and at the end of the adsorption experiment; V_0 is the PUFA solution volume; and W is the weight of adsorbent. To determine the values of c and c_0, fatty acids were analyzed by gas chromatography (GC). The necessary methylation and methyl ester analyses have been described elsewhere (13). The deviations and errors in c are first due to the analysis of fatty acids by GC. Regarding reproducibility, only some experiments were checked for repeatability. The maximal deviation was 5%. The experiments in which the standard deviation exceeded 5% were repeated. These experiments are also those in which a clear difference with respect to the general trend was observed in Figures 1 and 2.

RESULTS AND DISCUSSION

Experimental observations show that adsorption of fatty acids depends on the purity of the fatty acids in the mixture from which they are adsorbed. That is, adsorption of EPA, 16:1n-7, and 16:2n-4 from pure solutions is different from PUFA concentrate (Table 2) containing various fatty acids. This work focuses on fatty acid adsorption studies from a PUFA concentrate (Table 2), because in practical situations EPA is generally purified from such concentrates.

Adsorption kinetics. Table 3 shows the variation of the ratio c/c_0 (ratio between the concentrations of 16:2n-4, EPA, or 16:1n-7 in the liquid phase after and before contacting with the adsorbent) vs. time for the adsorption of fatty acid on three different adsorbents. As shown, the fatty acid concen-

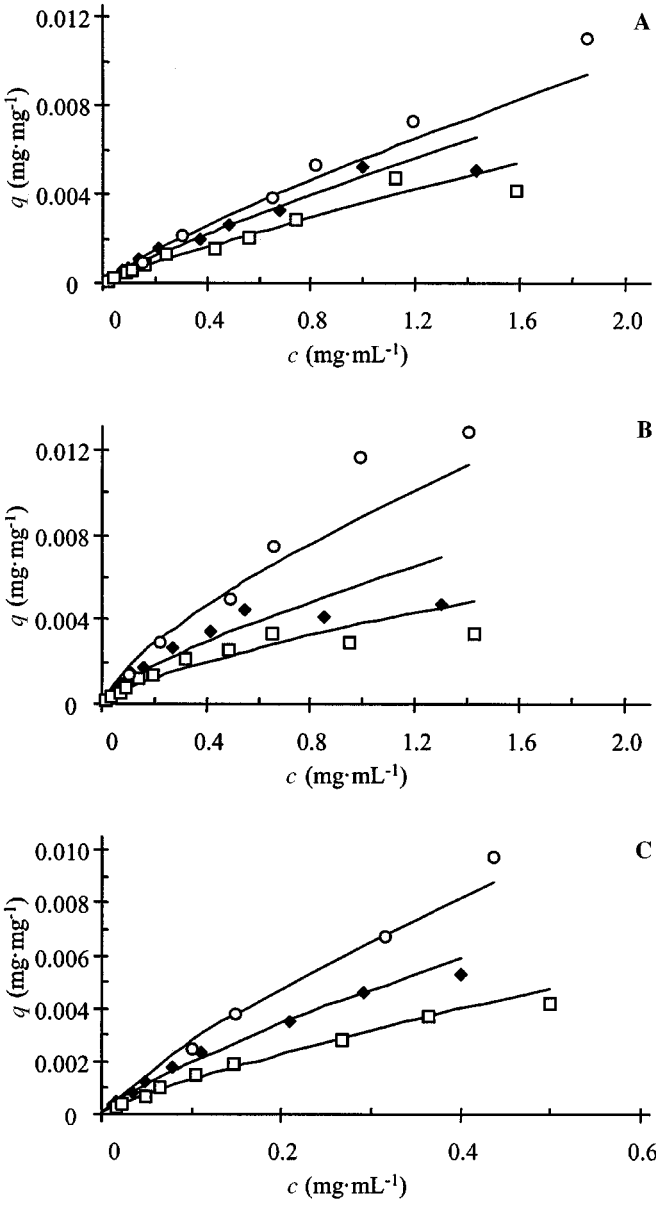

FIG. 1. Adsorption equilibria of fatty acids ◆ 16:1n-7, □ 16:2n-4, and ○ 20:5n-3. Adsorbent: octadecylsilyl (C_{18}, 30 µm, 120 Å). Solvent: methanol/water (1% acetic acid) (A) 80:20, (B) 75:25, and (C) 70:30, w/w.

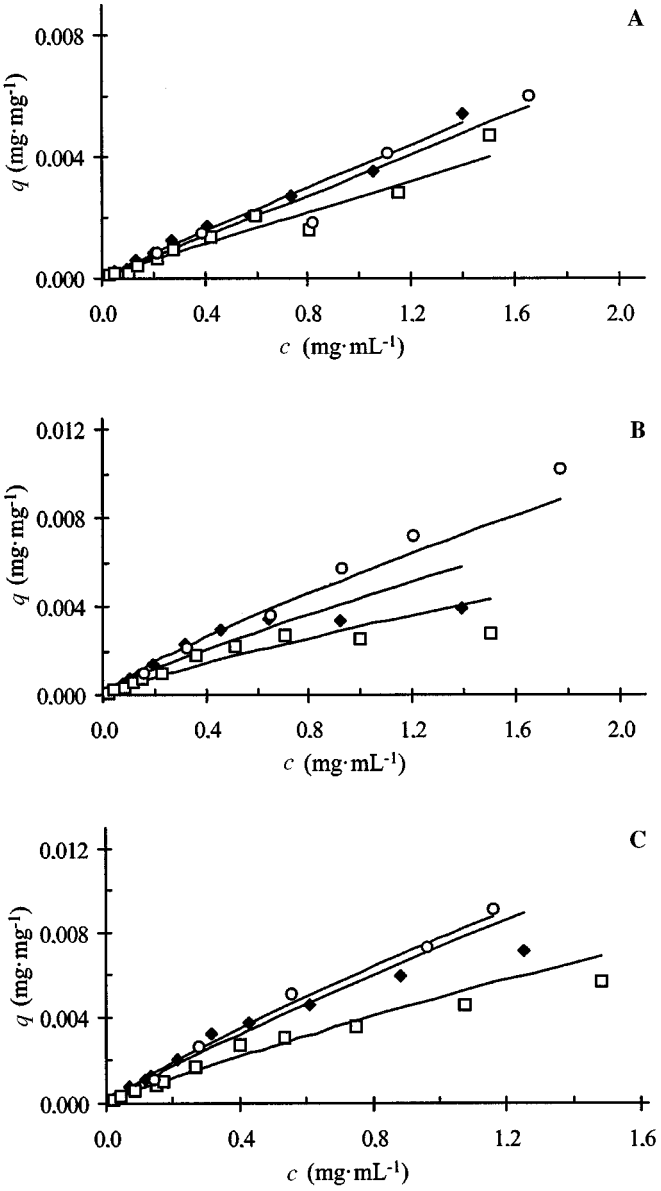

FIG. 2. Adsorption equilibria of fatty acids ◆ 16:1n-7, □ 16:2n-4, and ○ 20:5n-3. Adsorbent: (A) octylsilyl (C_8, 8 µm, 120 Å), (B) octadecylsilyl (C_{18}, 8 µm, 120 Å) and (C) dodecylsilyl (C_{22}, 10 µm, 100 Å). Solvent: methanol/water (1% acetic acid) 80:20, w/w.

tration in the liquid phase remains fairly constant; the standard deviations were lower than 5%, as is shown in Table 3. This indicates that the equilibrium between the adsorbent and liquid phase is reached instantaneously. Similar results were obtained for the other kinetic experiments. Therefore, the assumption of an instantaneous equilibrium between the mobile and the stationary phase in a hypothetical chromatographic fractionation using these adsorbent–solvent systems is reasonable, and phenomena such as adsorption kinetics and mass transfer can be neglected. With the assumption of local equilibrium, q is determined from the equilibrium isotherm as a function of the concentration in the liquid phase. These results are in agreement with the ones obtained by Golshan-Shirazi and Guiochon (14); these authors obtained a good agreement when comparing experimental data for chromatographic adsorption of phenol on C_{18}, eluted with methanol/water (80:20, vol/vol), and the profiles calculated with an equilibrium-dispersive model of chromatography. This model assumed that the components were in dynamic equilibrium between the mobile and the stationary phases and that all the contributions to band broadening could be lumped into a single apparent dispersion coefficient.

Table 3 also shows that the c/c_0 values decrease in the order 16:2n-4, EPA, and 16:1n-7, which is consistent with the chromatographic elution order of the fatty acids observed in a previous work (6). Table 3 shows that there are no important differences among the adsorption of the fatty acids on the C_{18}

TABLE 3
Time Course Variation and Average Values of the Ratio Between the Concentrations of 16:1n-7, 20:5n-3, and 16:2n-4[a]

	c/c_0		
Time (min)	16:1n-7	20:5n-3	16:2n-4
	C_8, 8 µm, 120 Å		
0.25	0.693	0.720	0.756
1.00	0.711	0.743	0.778
5.00	0.707	0.731	0.771
60.00	0.707	0.733	0.771
Average value	0.705 ± 0.008	0.732 ± 0.009	0.769 ± 0.009
	C_{18}, 8 µm, 120 Å		
0.25	0.545	0.595	0.651
1.00	0.534	0.576	0.623
5.00	0.538	0.577	0.634
60.00	0.548	0.584	0.627
Average value	0.541 ± 0.006	0.583 ± 0.009	0.634 ± 0.012
	C_{22}, 10 µm, 100 Å		
0.25	0.527	0.577	0.635
1.00	0.500	0.553	0.609
5.00	0.535	0.585	0.642
60.00	0.559	0.610	0.672
Average value	0.530 ± 0.024	0.581 ± 0.024	0.640 ± 0.026

[a]In the liquid phase after and before contacting with the adsorbent, c/c_0, in octylsilyl (C_8, 8 µm, 120 Å), octadecylsilyl (C_{18}, 8 µm, 120 Å), and dodecylsilyl (C_{22}, 10 µm, 100 Å). Solvent: methanol/water (1% acetic acid) 80:20, w/w.

and C_{22} adsorbents, whereas the adsorption on C_8 is less.

Adsorption equilibria. Freundlich isotherm. Figures 1 and 2 show the adsorption isotherms (25°C) of the three fatty acids on different adsorbent–solvent systems. Each isotherm is made up of 11 experimental data, some of which are not shown because of the scale used. Similar figures were obtained in the rest of the experiments aimed to study the adsorption equilibrium (data not shown). All the equilibrium data were acceptably fitted to the Freundlich isotherm:

$$q = K_{IF} c^z \quad [2]$$

The values of the parameters K_{IF} and z and the correlation coefficients corresponding to the linearized form of Equation 2 are shown in Table 4. The parameter z is fairly similar for the three fatty acids when the adsorption is in a given adsorbent–solvent system, i.e., z did not depend on the nature of the fatty acid. Moreover, the z values are not much affected by the adsorbent particle size (for example, z values for 16.2n-4 were 0.82, 0.85, and 0.86 for the experiments with the C_{18} adsorbent of 8-, 12-, and 30-µm particle size, respectively). However, K_{IF} depended on the nature of the fatty acids and determined their separation. An acceptable separation could be obtained using the mobile phase methanol/water (1% acetic acid) 75:25, w/w, as shown in Figure 1.

Separation factors. For applying these equilibrium data to the separation of these fatty acids from a PUFA concentrate, it seems reasonable to compare the concentration equilibrium ratios of the three fatty acids at their concentrations in the PUFA concentrate. The concentration equilibrium ratio for a fatty acid i is given by Equation 3:

$$K_i = \frac{q_i}{c_i} \quad [3]$$

The comparison between K_i of two fatty acids (i and j) can be made by the separation factor α_{ij}, which is given by Equation 4:

$$\alpha_{ij} = \frac{K_i}{K_j} \quad [4]$$

Taking into account the elution order of these fatty acids—16:2n-4, EPA, and 16:1n-7—we must evaluate the separation factors $\alpha_{EPA/16:2n-4}$ and $\alpha_{16:1n-7/EPA}$ for all the adsorbent–solvent systems:

$$\alpha_{EPA/16:2n-4} = \frac{K_{EPA}}{K_{16:2n-4}} \quad [5]$$

$$\alpha_{16:1n-7/EPA} = \frac{K_{16:1n-7}}{K_{EPA}} \quad [6]$$

Table 5 shows the values of these separation factors, which have been calculated using the mean values of the concentration ratios at all the equilibrium concentrations obtained in the experiments carried out in this work (examples of equilibrium data are depicted in Figs. 1 and 2). With the stationary phases C_{18} and C_{22}, an increase in the water content of the liquid-phase diminished $\alpha_{16:1n-7/EPA}$ and increased $\alpha_{EPA/16:2n-4}$ (Table 5). This was due to the fact that the EPA solubility in the liquid phase diminished more than the solubilities of 16:1n-7 and 16:2n-4 when the water content increased. Therefore, of the three liquid phases tested, the best one to separate EPA from 16:1n-7 and 16:2n-4 seemed to be methanol/water (1% acetic acid) 75:25 w/w, as also shown in Figure 1B. There were slight differences between the separation factors of the stationary phases C_{18} and C_{22} (for example the $\alpha_{EPA/16:2n-4}$ values for the systems C_{18}, 12 µm, 120 Å-methanol/water 75:25 and C_{22}, 10 µm, 100 Å-methanol/water 75:25 were, respectively, 1.29 and 1.32, but the $\alpha_{16:1n-7/EPA}$ values for these systems were the same), although both were greater than the separation factor of C_8. The adsorbent particle size did not affect the separation factors significantly.

The separation factor $\alpha_{EPA/16:2n-4}$ of the adsorbent C_{18} PEP was greater than the ones for the adsorbents C_{18} HS and C_{18}, 8 µm, 120 Å (Table 5). The stationary phases C_{18} PEP and C_{18} HS had higher surface areas and carbon load than the other stationary phases (Table 1), which implied a higher adsorption of low-polarity molecules.

Craig model. Because the kinetic factors do not affect the separation of the three fatty acids as shown before (Table 3), the equilibrium data obtained in this work could be used to predict the chromatograms for separation of 16:2n-4, EPA, and 16:1n-7. This prediction was made using the Craig model (15), which considers the chromatographic column as a series of well-mixed plates where the solute concentrations in the

TABLE 4
Freundlich Isotherm Constants (K_{IF} and z), Linear Isotherm Constants (K), and Regression Coefficients for Adsorption of 16:1n-7, 16:2n-4, and EPA from a PUFA Concentrate on Different Adsorbent—Solvent Systems[a]

Fatty acid	Adsorbent	Solvent methanol/water (w/w)	Freundlich isotherm			Linear isotherm	
			K_{IF}	z	r^2	K	r^2
16:2n-4	C_8, 8 µm, 120 Å	80:20	0.0037	0.97	0.989	0.0028	0.948
20:5n-3			0.0027	0.94	0.961	0.0034	0.984
16:1n-7			0.0034	0.99	0.983	0.0037	0.988
16:2n-4	C_{18}, 8 µm, 120 Å	80:20	0.0044	0.82	0.966	0.0042	0.971
20:5n-3			0.0031	0.83	0.960	0.0050	0.959
16:1n-7			0.0055	0.82	0.966	0.0060	0.966
16:2n-4	C_{18}, 12 µm, 120 Å	80:20	0.0044	0.85	0.963	0.0042	0.903
20:5n-3			0.0029	0.82	0.945	0.0049	0.977
16:1n-7			0.0050	0.85	0.966	0.0060	0.966
16:2n-4	C_{18}, 30 µm, 120 Å	80:20	0.0048	0.86	0.986	0.0039	0.970
20:5n-3			0.0036	0.89	0.987	0.0043	0.956
16:1n-7			0.0056	0.84	0.991	0.0052	0.959
16:2n-4	C_{18}, HS, 8 µm, 100 Å	80:20	0.0045	0.76	0.965	0.0046	0.958
20:5n-3			0.0028	0.71	0.952	0.0058	0.962
16:1n-7			0.0064	0.74	0.967	0.0068	0.955
16:2n-4	C_{18}, PEP, 8 µm, 100 Å	80:20	0.0045	0.76	0.965	0.0045	0.957
20:5n-3			0.0028	0.71	0.952	0.0064	0.976
16:1n-7			0.0064	0.74	0.967	0.0075	0.957
16:2n-4	C_{22}, 10 µm, 100 Å	80:20	0.0073	0.90	0.986	0.0055	0.949
20:5n-3			0.0049	0.88	0.985	0.0069	0.950
16:1n-7			0.0077	0.87	0.985	0.0085	0.967
16:2n-4	C_{18}, 30 µm, 120 Å	75:25	0.0057	0.73	0.962	0.0056	0.941
20:5n-3			0.0038	0.71	0.959	0.0072	0.938
16:1n-7			0.0089	0.71	0.960	0.0087	0.959
16:2n-4	C_{22}, 10 µm, 100 Å	75:25	0.0119	0.83	0.980	0.0096	0.945
20:5n-3			0.0078	0.84	0.978	0.0127	0.929
16:1n-7			0.0143	0.82	0.978	0.0154	0.948
16:2n-4	C_{18}, 30 µm, 120 Å	70:30	0.0122	0.79	0.992	0.0109	0.954
20:5n-3			0.0082	0.79	0.993	0.0146	0.939
16:1n-7			0.0167	0.78	0.992	0.0170	0.947
16:2n-4	C_{22}, 10 µm, 100 Å	70:30	0.0309	0.93	0.994	0.0204	0.968
20:5n-3			0.0193	0.92	0.995	0.0289	0.965
16:1n-7			0.0315	0.91	0.994	0.0331	0.972

[a]See Table 2 for abbreviations.

solid and liquid phases are in equilibrium. Solvent flows from one plate segment to the next, but adsorbent remains in the same plate. The result of these transfers is that each solute develops a concentration profile across the series of plates, which leads to the separation and isolation of solutes. As the number of total transfer n becomes large, the fraction of the original solute that is in plate r after n total transfers, $f(r, n)$, is given by Equation 7 (13):

$$f(r,n) = \frac{1}{\sqrt{2\pi np(1-p)}} \exp\left(-\frac{(r-np)^2}{2np(1-p)}\right) \quad [7]$$

where

$$p = \frac{G}{1+G} = \frac{\frac{KL}{A}}{1+\frac{KL}{A}} \quad [8]$$

G is the adsorption factor, K is the linear equilibrium constant,

TABLE 5
Separation Factors $\alpha_{EPA/16:2n-4}$ and $\alpha_{16:1n-7/EPA}$ for All the Adsorbent—Solvent Systems[a]

Adsorbent	Solvent methanol/water (1% acetic acid) (w/w)	$\alpha_{20:5n-3/16:2n-4}$	$\alpha_{16:1n-7/20:5n-3}$
C_8, 8 µm, 120 Å	80:20	1.21	1.09
C_{18}, 8 µm, 120 Å	80:20	1.19	1.20
C_{18}, 12 µm, 120 Å	80:20	1.17	1.22
C_{18}, 30 µm, 120 Å	80:20	1.10	1.21
C_{18}, HS, 8 µm, 100 Å	80:20	1.26	1.17
C_{18}, PEP, 8 µm, 100 Å	80:20	1.42	1.17
C_{22}, 10 µm, 100 Å	80:20	1.25	1.23
C_{18}, 30 µm, 120 Å	75:25	1.29	1.21
C_{22}, 10 µm, 100 Å	75:25	1.32	1.21
C_{18}, 30 µm, 120 Å	70:30	1.34	1.16
C_{22}, 10 µm, 100 Å	70:30	1.42	1.15

[a]See Table 2 for abbreviation.

and L and A are the volume of solvent and the amount of adsorbent, respectively (1.5 mL and 150 mg, respectively; see the Materials and Methods section). To apply this model, it is necessary to use linear isotherms (Eq. 3). Thus, the Freundlich isotherm was approximated to a linear distribution. This simplification did not introduce any appreciable divergence from the experimental data. Indeed, the approximation was fairly close to the experimental results for diluted solutions because the z values were between 0.71 and 0.99 (Table 4). Table 4 shows the distribution constants (K) for linear isotherms obtained by linear regression from the experimental equilibrium concentrations corresponding to the most diluted solutions. The plate on which the fatty acid fraction, $f(r,n)$, is maximal is shown in Equation 9 (13):

$$r_{max} = np \quad [9]$$

The distribution of fatty acids between the liquid and solid phases, $f(r,n)$, was calculated by Equation 7 for n = 1000 transfers. Hence the p values of the three fatty acids were known, and we could also calculate the r_{max} of each one. We can give r values around the r_{max} of EPA to obtain the $f(r,n)$ corresponding to the three fatty acids (Eq. 7). To compare the different adsorbent–solvent systems, the EPA purity was calculated taking the number of plates around the EPA maximum so that the EPA yield was about 95%. Yield, Y(EPA), and purity, P(EPA), were calculated, respectively, by Equations 10

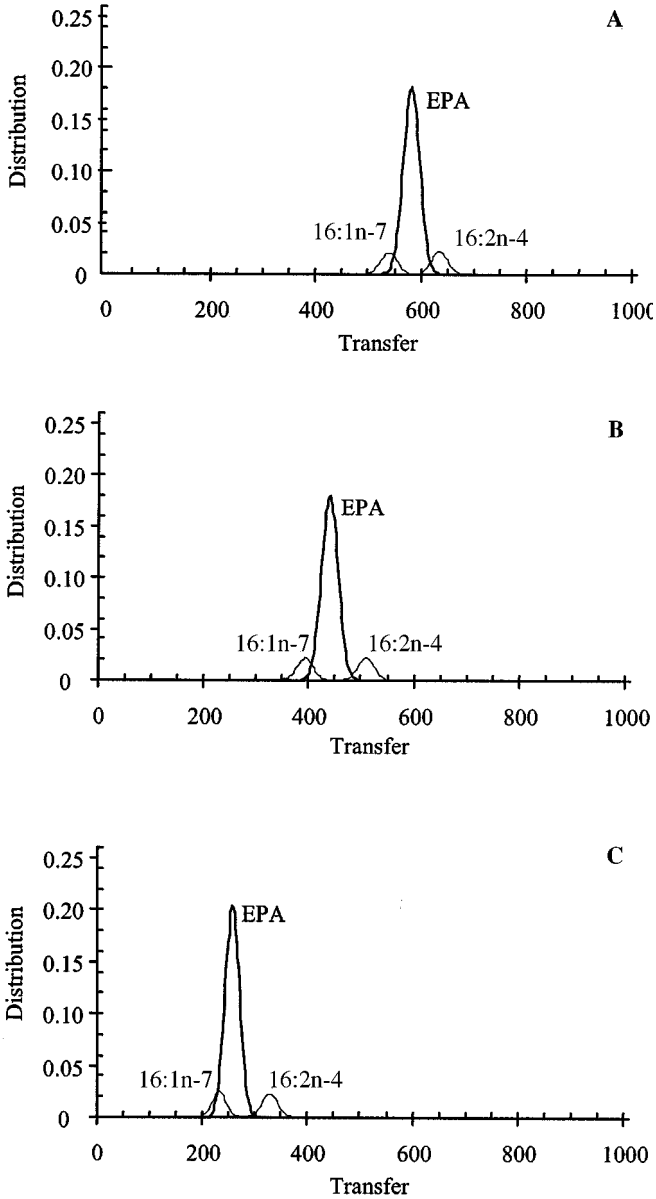

FIG. 3. Concentration profiles of fatty acids 16:1n-7, 20:5n-3, and 16:2n-4, applying the Craig model. Adsorbent: octadecylsilyl (C_{18}, 30 µm, 120 Å). Solvent: methanol/water (1% acetic acid, (A) 80:20, w/w; (B) 75:25, w/w; and (C) 70:30, w/w. EPA, eicosapentaenoic acid = 20:5n-3.

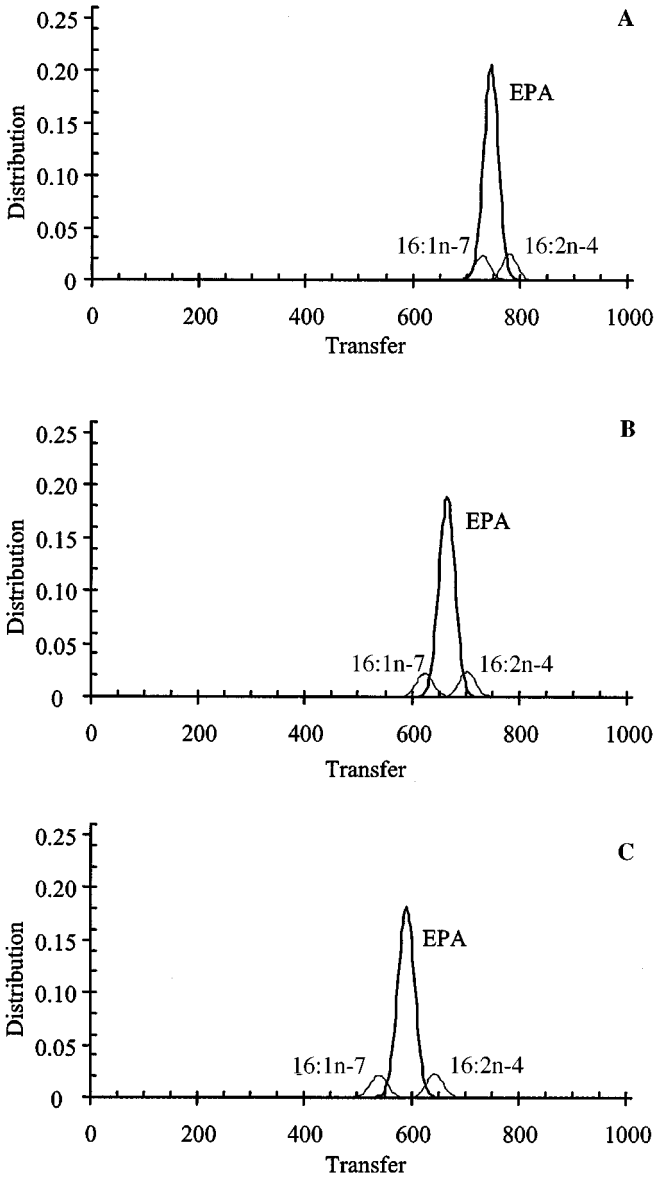

FIG. 4. Concentration profiles of fatty acids 16:1n-7, 20:5n-3, and 16:2n-4, applying the Craig model. Adsorbent: (A) octylsilyl (C_8, 8 µm, 120 Å), (B) octadecylsilyl (C_{18}, 8 µm, 120 Å), and (C) dodecylsilyl (C_{22}, 10 µm, 100 Å). Solvent: methanol/water (1% acetic acid) 80:20, w/w. See Figure 3 for abbreviation.

TABLE 6
Estimations of the EPA Yields and Purity for 1,000 Transfers in the Range –30 Tubes[a]

Adsorbent	Solvent methanol/water (w/w)	r_{max}	EPA yield (%)	EPA purity (%)
C_{18}, 30 µm, 120 Å	80:20	699	96.46	89.02
	75:25	581	94.94	97.76
	70:30	407	95.03	95.86
C_{22}, 10 µm, 100 Å	80:20	592	95.03	98.06
	75:25	441	94.79	98.22
	70:30	257	97.27	92.58
C_8, 8 µm, 120 Å	80:20	746	97.33	87.24
C_{18}, 8 µm, 120 Å		667	95.92	93.67
C_{22}, 10 µm, 100 Å		592	95.03	98.06
C_{18}, 8 µm, 120 Å	80:20	667	95.92	93.67
C_{18}, 12 µm, 120 Å		649	95.67	94.55
C_{18}, 30 µm, 120 Å		699	96.46	89.02
C_{18}, HS, 8 µm, 100 Å	80:20	633	95.46	95.38
C_{18}, PEP, 8 µm, 100Å		610	95.20	96.39
C_{18}, 8 µm, 120 Å		667	95.92	93.67

[a]With respect to r_{max}, by application of the Craig model to the adsorption of 16:1n-7, 16:2n-4, and EPA to different adsorbent–solvent systems. See Table 2 for abbreviations.

and 11:

$$Y(EPA) = \frac{\sum_{-a}^{a} m_{0\text{EPA}} f(r,n)_{\text{EPA}}}{m_{0\text{EPA}}} \quad [10]$$

$$P(\text{EPA}) = \left[\sum_{-a}^{a} m_{0\text{EPA}} f(r,n)_{\text{EPA}}\right] \Bigg/ \left\{\sum_{-a}^{a}\left[m_{0\text{EPA}} f(r,n)_{\text{EPA}} + m_{016:1n\text{-}7} f(r,n)_{16:1n\text{-}7} + m_{016:2n\text{-}4} f(r,n)_{16:2n\text{-}4}\right]\right\} \quad [11]$$

where $m_{0\text{EPA}}$, $m_{016:1n\text{-}7}$, and $m_{016:2n\text{-}4}$ are the initial amount of fatty acids on the first plate (fatty acid amounts in the PUFA concentrate, 7.07, 0.83, and 0.84 mg, respectively) and a is the number of plates that need to be considered above and below the r_{max}, which constitutes the EPA fraction. Figures 3 and 4 show the profile concentrations of the three fatty acids when using the model. The overlapping of the EPA peak with the 16:2n-4 and 16:1n-7 peaks is similar to the ones observed when the separation was carried out in a preparative compression radial cartridge (6). Table 6 shows the EPA yields and purities obtained using Equations 10 and 11 with the different adsorbent–solvent systems. The estimated purities obtained with the model (Table 6) agreed reasonably well with the ones obtained (94–96%) from algae by our group using a reversed-phase, C_{18}, as stationary phase and methanol/water (1% acetic acid) 80:20 (w/w) as mobile phase (6,8,9). Table 6 and Figure 3 again show that the best separation (maximal purity) is attained with methanol/water (1% acetic acid) 75:25, w/w. On the other hand, among the stationary phases C_8, C_{18}, and C_{22} (Fig. 4, Table 6), the best separation was obtained using the reversed-phase C_{22}, although also the stationary phase C_{18} PEP gave a high EPA purity (Table 6).

Therefore, we can conclude that, with the adsorbent–solvent systems tested, the adsorption equilibrium depends more on the liquid phase than on the adsorbent phase. The best adsorbents to purify EPA are C_{18}, PEP, 8 µm, 100 Å, and C_{22}, 10 µm, 100 Å, and the best solvent is methanol/water (1% acetic acid) 75:25 (w/w), within the adsorbent–solvent systems used in this work. These simulations confirm the results obtained in a previous work (6), which shows that this methodology can be useful for testing several adsorbent–solvent systems before using them in chromatographic separations.

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