Fluorous affinity chromatography for enrichment and determination of perfluoroalkyl substances

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Supporting Information

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Note. In the following paragraphs, reference to equations of the main text is denoted by the usual writing “Eq.” followed by the number of the equation included between round brackets (i.e., “Eq. (X)” means equation X of the main text). For equations appearing only in Supplementary Information, the writing “Eq. (S-)” has been adopted (i.e., Eq. (S-Y) refers to the equation S-Y of Supporting Information).

Analogously, bibliographic references cited only in Supplementary Material are indicated by a superscript number preceded by the letter “S” (i.e., S1 indicates the first entry of Supplementary Information bibliography). The list of S-references is given at the end of Supplementary Information.

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Theory

In this section, a detailed discussion regarding the meaning of the equations used in the main text and how they can be obtained is presented.

Tracer pulse chromatography

The integral mass balance equation for tracer pulse (TP) chromatography with binary eluents is

\[ V^*_{R,i} = \frac{n^\text{tot}_i}{C^M_i} \]  \hspace{1cm} (S-1)

where \( V^*_{R,i} \) is the retention volume of the isotopically labeled \( i \) compound, and \( n^\text{tot}_i \) and \( C^M_i \) are the total amount of unlabeled species \( i \) in the column (taken as a whole) and its molar concentration in the mobile phase, respectively. This equation is the base for isotherm determination through TP method\(^{39-45}\). By assuming the same partial molar volumes of the organic modifier upon adsorption or mixing to form the binary eluent, from Eq. (S-1) one obtains\(^{47}\):

\[ V^*_{R,i} = \frac{V^\text{tot}_i}{\theta^M_i} \] \hspace{1cm} (S-2)

being \( V^\text{tot}_i \) and \( \theta^M_i \) the total volume of \( i \) in the column and its volume fraction in the bulk eluent, respectively. Owing to the complexity of the interfacial region in liquid chromatography (LC) with stationary phases consisting of carbon moieties, the exact volume of adsorbed and bulk-liquid phases cannot be unambiguously delineated.\(^{51}\) This problem involves the specification of the hypothetical Gibbs Dividing Surface (GDS) which delineates the boundary between the stationary phase and the mobile phase. Two conventions are commonly used in LC for establishing the position of GDS: either the GDS is assumed to coincide with the physical surface of the solid adsorbent (so-called “nothing is adsorbed in terms of volume”, or /vNA, convention\(^{S2}\)) or the GDS is established at the interface between an adsorbed film (that constitutes the stationary phase volume, \( V_S \)) and the bulk fluid (this convention is said “component J not adsorbed”, or /JNA, convention\(^{S2}\)).
With /vNA convention, the void volume of the column (also referred to as “thermodynamic dead volume”\textsuperscript{40,47,68} is given by the total volume of the eluent in the column, herein denoted by the symbol $V_0$. If the /JNA convention is adopted, instead, the pertinent void volume is the so-defined “kinetic void volume”, $V_M$. The relationship between $V_M$ and $V_0$ is given by Eq. (5). Literature is replete with discussions concerning the difficulties involved in the accurate estimation of $V_M$ (primarily due to the problem of finding a truly unretained and unexcluded compound). On the other hand, $V_0$ can be easily estimated for instance, through pycnometry, perturbation on the plateau technique or TP experiments.\textsuperscript{46,47,53} With TP technique, for a binary system ($i, j$), $V_0$ is given by\textsuperscript{47} Eq. (2). $V_0$ is accordingly obtained by injecting small samples of eluent with isotopically labeled solute components and determining the elution volume ($V_{R,i}^{*}$ and $V_{R,j}^{*}$) for each labeled component. Obviously, the method can be used with a pure eluent ($\theta_i^M$ or $\theta_j^M = 1$) and this is by far the simplest way of determining $V_0$.\textsuperscript{47} The convention adopted for positioning the GDS, also determines if an excess or an absolute volume of a component in the stationary phase is obtained. With /vNA convention, $V_i^{tot}$ (Eq. (S-2)) is composed of an excess term, $V_i^{exc}$, and a bulk one:

\[
V_i^{tot} = V_i^{exc} + V_0 \theta_i^M \quad \text{convention /vNA} \quad (S-3)
\]

where the bulk term, $V_0 \theta_i^M$, represents the volume (hypothetical) of $i$ that would be present in the system with a totally inert adsorbent. With /JNA convention, on the other hand, the corresponding equation writes:\textsuperscript{54}

\[
V_i^{tot} = V_i^S + V_M \theta_i^M \quad \text{convention /JNA} \quad (S-4)
\]

where $V_i^S$ and $V_M \theta_i^M$ are the volumes of $i$ in the stationary and in the bulk mobile phase, respectively.

Substitution of Eq. (S-3) in Eq. (S-2) leads to:

\[
V_{R,i}^* = \frac{V_i^{exc}}{\theta_i^M} + V_0 \quad (S-5)
\]
from which

\[ V_{i}^{\text{exc}} = (V_{R,i}^* - V_0)\theta_i^M \]  \hspace{1cm} (S-6)

that is the operational equation for calculating excess volumes from retention data. By introducing Eq. (2) in Eq. (S-6) and using the equality \((1 - \theta_i) = \theta_j\), Eq. (S-6) can be recast in the form given by Eq. (1), which is particularly useful as it allows for excess volume calculation from experimental data without knowledge of either \(V_0\) or extra-column volume present in the chromatographic system. Different approaches are available to obtain information about capacity and thickness of the surface phase (absolute adsorption) from excess adsorption data. In this work, we will limit our attention to the method described by Schay and Nagy based on the analysis of the linear region of the excess adsorption isotherm. By substituting \(V_0 = V_{M} + V_{S}\) (by Eq. (5)) in Eq. (S-3) and explicitating \(V_{i}^{\text{exc}}\), one obtains:

\[ V_{i}^{\text{exc}} = V_{i}^{\text{tot}} - (V_{S} + V_{M})\theta_i^M \]  \hspace{1cm} (S-7)

from which, by simple math, one gets Eq. (3), inasmuch as:

\[ V_{i}^{S} = V_{i}^{\text{tot}} - V_{M}\theta_i^M \]  \hspace{1cm} (S-8)

Eq. (3) provides estimates of the stationary phase volume and the volume of \(i\) in the stationary phase, respectively from the slope and the intercept of \(V_{i}^{\text{exc}}\) vs \(\theta_i^M\) plot. It is important to stress that this equation holds only in the linear decreasing range of excess isotherm.

**Thermodynamic quantities in linear chromatography**

The classical retention factor, \(k\), for a solute is defined by Eq. (4). For \(k\) to have a defined thermodynamic meaning, the kinetic void volume has to be used in Eq. (4) and not the thermodynamic volume \(V_0\). \(k\), in fact, represents the ratio between the time spent by a component in the stationary phase with respect to that spent in the mobile phase or, in virtue of the ergodic hypothesis,
the ratio of the amounts of solute in stationary, \( n^S \), and mobile, \( n^M \), phases:

\[
k = \frac{n^S}{n^M} \tag{S-9}
\]

Eq. (S-9) can be recast as:

\[
k = KF \tag{S-10}
\]

where \( K \) is the equilibrium constant for the transfer of a solute from the mobile to the stationary phase (adsorption or partition coefficient, also said Henry’s constant of adsorption) and \( F \) the phase ratio given by Eq. (6). The relationship to phase transfer thermodynamics, finally, is established through the classical equation between the equilibrium constant and the Gibbs free energy of transfer (\( \Delta G^0 \)):

\[
\ln K = -\frac{\Delta G^\circ}{RT} = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \tag{S-11}
\]

where \( R \) is the universal gas constant, \( T \) the temperature in Kelvin and \( \Delta H^\circ \) and \( \Delta S^\circ \) are the enthalpy and entropy change of the transfer of solute from the mobile to the stationary phase (assumed to be \( T \)-independent), respectively. Herefrom, by means of Eq. (S-10), the well known van’t Hoff equation is achieved (Eq. (7)), which allows for the estimation of \( \Delta H^\circ \) and \( \Delta S^\circ \) through the slope \( (-\Delta H^\circ / R) \) and the intercept \( (\Delta S^\circ / R + \ln F) \) of \( \ln k \) versus \( 1/T \) plots.

**Selectivity**

Selectivity, \( \alpha \), is an important quantity often used to study nonspecific and specific interactions in chromatographic systems\(^{51}\). It is defined as the ratio of the retention factors of two solutes (1 and 2) as indicated in Eq. (8). When calculated for adjacent members of homologous series, \( \alpha \) is called nonspecific selectivity or, in the case of alkyl homologues, methylene selectivity (in opposition to the specific selectivity for two solutes differing only in a functional group). \( \ln \alpha \) multiplied by the factor \(-RT\) gives the change of Gibbs free energy for the transfer of a methylene group from the mobile to the stationary phase. Let \( \Delta G_1^\circ \) and \( \Delta G_2^\circ \) be the changes of Gibbs free energies for the transfer of the two adjacent alkyl homologues 1 and 2, by Eq. (8) and Eq. (7), after simple math,
one obtains:

\[ \Delta G_2^0 - \Delta G_1^0 = -RT \ln \alpha = \Delta G_{\text{CH}_2}^0. \]  

(S-12)

that corresponds to Eq. (9).

**Experimental Section**

**Equipment and MS parameters**

ESI (negative polarity) operational conditions were: source voltage −4.5 kV; capillary temperature 250°C; capillary voltage -30 V; tube lens -100 V. All perfluoroalkyl acids were detected as dissociated acid ions \([\text{M-H}]^-\) and confirmed by their most intense MS/MS transition \([\text{M-COOH}]^-\) (263 → 219, 313 → 269, 363 → 319 and 413 → 369 for PFPeA, PFHxA, PFHpA and PFOA, respectively). APCI (positive polarity) operational conditions were: source current 5 µA; source voltage 6 kV; capillary temperature 275°C; vaporizer temperature 375°C; capillary voltage 46 V; tube lens 75 V. D$_2$O and D$_3$-ACN were monitored in SIM mode at \(m/z = 21\) and 45, respectively (\([\text{M+H}]^+\)).
Figures

Figure S1: Experimental LC/MS chromatograms of deuterated water and ACN at different mobile phase composition. Numbers by each chromatogram indicate the volume fraction of ACN ($\theta^M_{ACN}$) in the mobile phase. Left: deuterated water. Right: deuterated ACN. For details about MS parameters see Experimental Section of Supporting Information.
Figure S2: Van’t Hoff plots, Eq. (7), for the four perfluoroalkyl acids PFPeA, PFHxA, PFHpA, PFOA at different ACN volume fraction ($\theta_{ACN}^M$). FA concentration in mobile phase: 0.1% v/v. Black squares: $\theta_{ACN}^M = 0.6$; empty circles: $\theta_{ACN}^M = 0.8$; black circles: $\theta_{ACN}^M = 0.9$. Dotted and dash-dotted lines: data linear regressions.
Figure S3: Dependence of natural logarithm of PFOA retention factors ($\ln k$) on ACN volume fraction ($\theta_{ACN}$) as a function of the percentage v/v of formic acid (FA) in the mobile phase.
Tables

<table>
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<tr>
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<th>$V^{*}_{R,\text{ACN}}$ (mL)</th>
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<th>$\theta^M_{\text{ACN}}$</th>
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Table S1: Retention volumes of deuterated water and acetonitrile ($V^{*}_{R,\text{wat}}$ and $V^{*}_{R,\text{ACN}}$) at different mobile phase composition ($\theta^M_{\text{ACN}}$ and $\theta^M_{\text{wat}}$) recorded by mass-spectrometric detector and corresponding excess volumes ($V^{\text{exc}}_{\text{wat}}$ and $V^{\text{exc}}_{\text{ACN}}$) calculated according to Eq. (1). The symbol “-“ means that the corresponding retention volume was not measured\(^1\).

\(^1\)Example of calculation. With data listed in the third row of Table S1 (corresponding to a mobile phase concentration 90/10 ACN/water) and by using Eq. (1), one gets for the excess volume of water, $V^{\text{exc}}_{\text{wat}} = (0.11 - 0.169) \times 0.9 \times 0.1 = -0.00531$ mL.
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<th>$k$</th>
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<th>$k$</th>
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Table S2: Retention factor $k$, Eq. (4), and chromatographic selectivity $\alpha$, Eq. (8), for PFPeA, PFHxA, PFHpA and PFOA at different mobile phase composition ($\theta_{ACN}^M$).
Table S3: Enthalpy ($\Delta H^\circ$) and entropy ($\Delta S^\circ$) change of the transfer of solute from the mobile to the stationary phase calculated by van’t Hoff analysis (Eq. (7)) for the perfluoroalkyl acids considered in this work. $\theta_{ACN}^M$: ACN volume fraction

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References


(S4) Ościk, J. Adsorption; Ellis Horwood Limited, Chichester, 1982.