

# Experimental hydrophobicity parameters of perfluorinated alkylated substances from reversed-phase high-performance liquid chromatography

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**Environmental context.** Perfluorinated compounds are synthetic chemicals shown to be present in the blood of humans. To study how these contaminants get into our blood requires a good understanding of their physicochemical properties. We describe an alternative way to obtain values for how perfluorinated compounds distribute between water and fatty phases (mimicking e.g. gut content and gut wall), which is essential information for modelling and understanding the environmental fate of these chemicals.

**Abstract.** Capacity factors of perfluorinated alkylated substances were obtained from isocratic reversed-phase high-performance liquid chromatography–mass spectrometry experiments at different organic modifier strengths of the mobile phase. The resulting capacity factor *v.* modifier strengths plots were extrapolated to obtain capacity factors at 100 % water ( $k_0$ ) that can serve as indicators of the hydrophobicity of the perfluorinated acids. Values of  $\log k_0$  were shown to increase linearly with the number of  $\text{CF}_2$  units in the fluorinated alkyl chain.

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## Introduction

The hydrophobicity of organic molecules is a pivotal molecular property that governs their behaviour in environmental and biological systems. It has been widely accepted as providing a good indicator of the distribution of analytes between environmental compartments such as water and sediments,<sup>[1,2]</sup> or partitioning of chemicals into biological membranes.<sup>[3–5]</sup> The hydrophobicity of neutral organic chemicals is often expressed by their octan-1-ol/water partition coefficient ( $K_{ow}$ ) which can be obtained from shake-flask or slow-stirring experiments in which the chemical's concentration at equilibrium in the octanol and the aqueous phases is determined by chemical analysis. For surfactants the  $K_{ow}$  is ill defined because their experimental determination is hampered by, for example, formation of emulsions and micelles. The use of chromatographic methods such as thin-layer chromatography<sup>[6]</sup> and reverse-phase high-performance liquid chromatography (HPLC)<sup>[4,7]</sup> for obtaining an estimation of  $K_{ow}$  or hydrophobicity has been the subject of research since the 1950s in drug design and since the late 1970s in environmental chemistry.

Perfluorinated alkylated substances (PFASs) show persistence and long range transport in the environment and high concentrations of these compounds have been found in many environmental samples.<sup>[8]</sup> PFASs can bioaccumulate and have toxic effects.<sup>[9]</sup> However, their biotic and abiotic degradation

pathways are thus far only marginally known.<sup>[10,11]</sup> In Canada and Europe, some PFASs, notably perfluorooctane sulfonate (PFOS) are under restrictions for meeting criteria of persistent organic pollutants (POPs) that are controlled under the Stockholm convention (see <http://chm.pops.int/Programmes/New%20POPs/The%209%20new%20POPs/tabid/672/language/en-US/Default.aspx>, accessed November 2012).

Many PFASs are surfactants<sup>[12]</sup> (showing surface active dipolar behaviour). This characteristic renders traditional experimental methods such as shake flask or slow stirring unsuitable to determine their  $K_{ow}$ . The only experimentally determined partition coefficients reported for perfluorosulfonates and perfluoro carboxylates have been obtained by a voltammetric method.<sup>[5]</sup> To predict the partitioning behaviour of PFASs, mathematical models have been presented in the literature.<sup>[13]</sup> These relate distribution coefficients of these pollutants, such as e.g. air-to-water and octanol-to-water partitioning, to physicochemical or molecular properties of PFASs. The partition coefficients, in turn, are typically required for general environmental fate and transport models.<sup>[2]</sup> However, experimental partition coefficients for PFASs are largely missing for the reasons outlined above and therefore mathematical models like EPI Suite, ClogP, SPARC and COSMOtherm<sup>[13,14]</sup> applied to estimate or calculate these coefficients may suffer from poor calibration and lack of validation. The possibly resulting

inaccurate partitioning data could lead to misinterpretations, erroneous risk assessments and incorrect policies to regulate this new generation of pollutants.

Ideally, for a reversed-phase liquid chromatography system the capacity ratio ( $\log k'$ ) of a compound obtained with a mobile phase consisting of pure water would best represent its hydrophobicity.<sup>[15]</sup> In addition, the extent of end-capping of the stationary phase is an important factor.<sup>[16,17]</sup> Because  $\log k'$  values in pure water can be very large and, consequently, determination of exact peak maxima difficult,  $\log k'$  is usually measured in methanol–water or acetonitrile–water mixtures. For a given stationary phase in reversed-phase HPLC, the relationship between the capacity factor of a solute and the volume fraction ( $\varphi$ ) of the organic modifier in the mobile phase is given by<sup>[18]</sup>:

$$\log k' = \log k_0 + a(\varphi)^2 - b\varphi \quad (1)$$

in which  $a$  and  $b$  are constants depending on the nature of the solute and the modifier, and the  $\log k_0$  intercept represents the  $\log k'$  value extrapolated to  $\varphi = 0$  (or a mobile phase consisting of pure water). Several authors have demonstrated<sup>[19–21]</sup> that for neutral compounds the quadratic term can be neglected if methanol is used as the organic modifier. Hence, the following equation results:

$$\log k' = \log k_0 - b\varphi \quad (2)$$

The slope  $-b$  represents the decrease in retention of the solute as a function of increasing organic modifier content. Extrapolation of the plot of  $\log k'$  v.  $\varphi$  yields the intercept at  $\varphi = 0$ , i.e.  $\log k_0$ , and the slope  $b$ . This slope depends on the chromatographic conditions (type of column, chemical composition, temperature, back pressure, etc.). It has been suggested<sup>[17]</sup> that, as an indicator of  $K_{ow}$ ,  $\log k'$  values obtained in a C18 HPLC column with methanol–water as mobile phase could be used at a single value of  $\varphi$  (above 0.25 but below 0.70 methanol) without extrapolation, because most of the curvature for methanol in Eqn 1 occurs at volume fractions of methanol below 25 % and above 70 %. It was argued that extrapolated  $k_0$  values determined from  $k'$  values outside the  $\varphi = 0.25$ – $0.70$  range will therefore deviate from the true  $k_0$  value and give poor correlations to  $K_{ow}$ . However, because the slope  $b$  is different for different solutes and hence the elution order of solutes can change with different values of  $\varphi$ <sup>[22]</sup> (even within the range of  $\varphi$  indicated above<sup>[20]</sup>), recording of the hydrophobicity at a single value of  $\varphi$  is not recommended.

The objectives of the work presented in this study were to employ reversed-phase HPLC to study the partitioning behaviour of PFASs into stationary phases commonly used in chromatography, and to obtain in this way quantitative information on their hydrophobicity. As in recent years stationary phases have become available that allow reversed-phase HPLC runs with a mobile phase consisting of pure water, we investigated such a stationary phase to evaluate its applicability for PFASs.

## Experimental

### Chemicals

Perfluorobutyl sulfonate (PFBS, 98%), perfluorohexyl sulfonate (97%), perfluorooctane sulfonamide (98%), perfluorobutanoic acid (PFBA, 99%), perfluorohexanoic acid (98%), perfluorodecanoic acid (98%), and perfluorododecanoic acid (96%)

were purchased from ABCR, Karlsruhe, Germany. Perfluoroheptanoic acid (96%), perfluorooctanoic acid (PFOA, 96%), perfluoroundecanoic acid (97%) and perfluorotetradecanoic acid (99%) were obtained from Acros, Geel, Belgium. Perfluorooctyl sulfonate (98%) was purchased from Fluka, Buchs, Switzerland, 6 : 2 fluorotelomer sulfonate (6 : 2 FTS, 98%) from Interchim, Montlucon, France, perfluorononanoic acid (97%) from Aldrich, Milwood, WI, and uracil (2-oxy-4-oxypyrimidine, 99%) from Sigma–Aldrich, Schnellendorf, Germany. CAS numbers, abbreviations used and compound characteristics are provided in Table S1 of the Supplementary material.

The solvents used were methanol of LC-MS grade (Biosolve, Valkenswaard, the Netherlands) and double distilled water (in-house production). The aqueous mobile phase used for the Lichrospher (Lich) column was buffered with phosphoric acid to pH values of 2.2, 2.4, 6.0 and 7.8. For the Aqua column, the pH values were set at 2.2 and 5.2. Investigating different pHs was considered necessary in order to study a possible effect of degree of dissociation, in view of the debate on actual  $pK_a$  values of perfluorinated acids.<sup>[23]</sup> We expect, however, that at the different pHs investigated all acids will be in their ionic form, and hence that no appreciable effect of the pH on  $k'$  will be apparent. The ionisation of the compounds studied was enhanced by adding salts of sodium acetate or ammonium acetate (both from Acros, Geel, Belgium) to the aqueous mobile phase. To avoid any contamination from glassware or from the chromatographic system, any contact with fluoropolymers was prevented at all times and polytetrafluoroethylene material was avoided during sample treatment and analysis.

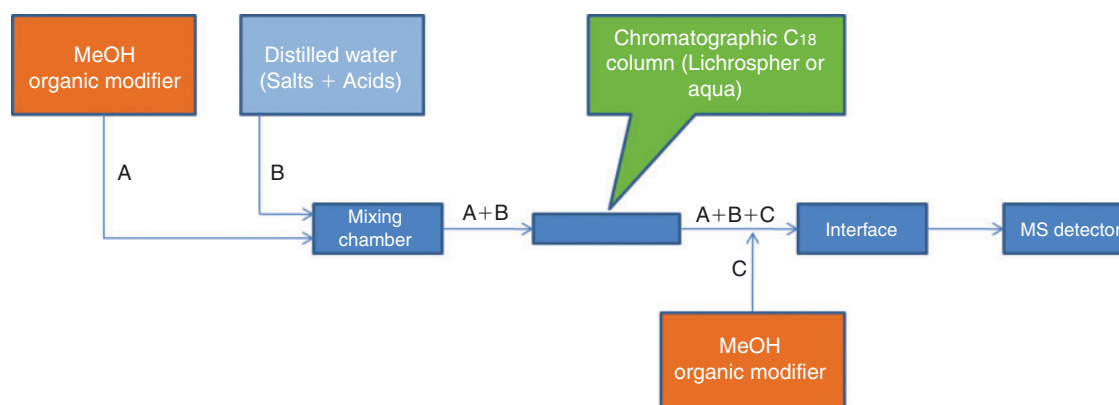
For the analyses, a standard mixture in methanol was used and this mixture was stored at 4 °C. Uracil was used for the determination of the dead time of the chromatographic system and methanol was the organic modifier in the mobile phase.

A solution of a mixture of 14 PFAS standards, containing 12 perfluorinated alkyl acids (PFAAs), a telomer acid and a neutral PFAS, was mixed with uracil for each isocratic condition. A blank consisting of only methanol was run before each injection of the standard mixture. The capacity factors of the PFASs studied were calculated under isocratic conditions for 11 methanol fractions ( $\varphi_{MeOH}$ ) of 0 to 1 increasing by 0.1 for each sub-step. Finally, the average capacity factor was calculated from three injected samples.

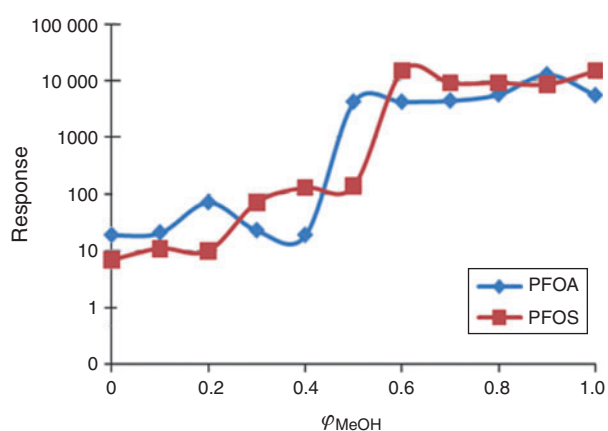
### Liquid chromatography–mass spectrometry (LC-MS)

Experiments were performed with a liquid chromatographic system from Thermo Separation Products (Thermo Scientific, Waltham, MA) consisting of a vacuum degasser, a P4000 gradient pump, a AS3000 auto-sampler and a SN4000 system controller. The analyses were performed with two different columns at a flow of 2 mL min<sup>-1</sup> and at a constant temperature of 25 °C.

The chromatographic columns used were: Aqua C<sub>18</sub> (150 × 2.0-mm internal diameter; Phenomenex, Utrecht, the Netherlands) with a particle diameter of 3 μm (p/no F-4311-B0). This column has a surface that can be 100% ‘wetted’ with water because of the polar embedded group that allows this stationary phase to be run under 100% aqueous conditions without loss of retention. The other column was a Lichrospher RP18 (125 × 2.0-mm internal diameter; Merck, Darmstadt, Germany) with a particle diameter of 3 μm (p/no 00E-3043-B0). A Phenomenex pre-column (C<sub>18</sub>; 2.0-mm internal diameter) was used to avoid any contamination from entering the analytical columns.



**Fig. 1.** Schematic set up of the chromatographic system used including a post-column pump to provide optimal mobile phase composition to the electrospray ionisation interface.



**Fig. 2.** Mass detector signal intensity for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) as a function of the fraction of methanol in the mobile phase.

Mass spectrometric detection was performed using a Thermo Quest Finnigan AQA-MS detector (ThermoQuest, Breda, the Netherlands) in selected ion monitoring mode. MS data were collected and integrated using *Xcalibur* software. The ions monitored for each analyte are provided in Table S1. Example chromatograms are shown in Fig. S1. The *GraphPad Prism* program (version 3.02, <http://www.graphpad.com>) was applied for all statistical analyses and linear regression.

#### Quality of electrospray

In order to obtain capacity factors at high water fractions of the mobile phase, we studied the quality of the electrospray. For this experiment, solutions of PFOA and PFOS were injected directly to the electrospray ionisation (ESI)-MS system without a chromatographic column at all isocratic conditions ( $\varphi_{\text{MeOH}} = 1$  to 0 in steps of 0.1). Batch injection analysis for optimising the MS were carried out using a KD Scientific (Antec, Zoeterwoude, The Netherlands) syringe pump equipped with a 1-mL Hamilton syringe (Hamilton, Bonaduz, Switzerland). The quality of the electrospray was maintained by installing an extra pump into the chromatographic system, see Fig. 1. This pump provided a constant flow of MeOH directly post-column in order to optimise the composition of the eluent entering the ESI interface. The flow of the additional pump was adjusted

( $<1 \text{ mL min}^{-1}$ ) to ensure that no excessive contribution of methanol would reach the inlet of the ESI.

#### Results and discussion

##### *Influence of mobile phase composition on signal intensity of the MS detector*

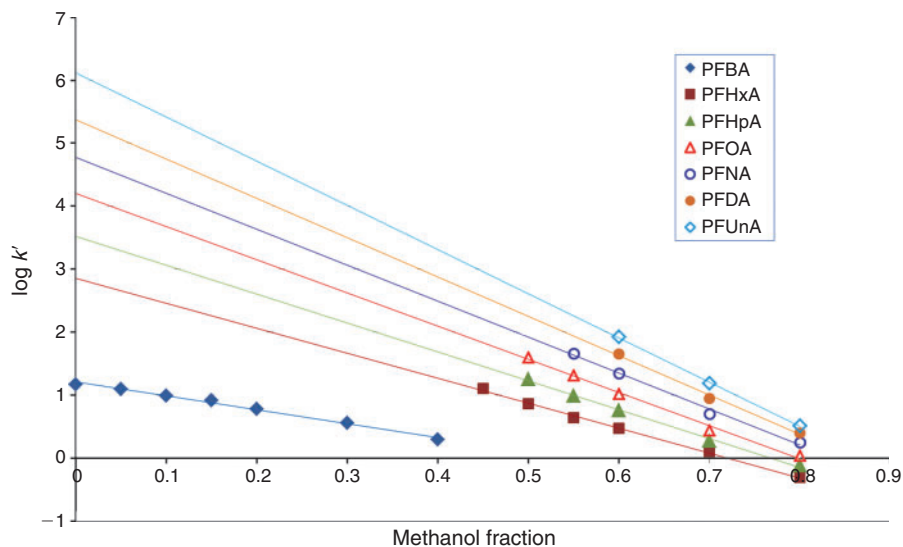
The linearity of the  $\log k'$  v.  $\varphi_{\text{MeOH}}$  relationship was tested over the whole range of  $\varphi_{\text{MeOH}} = 0$  to 1. As expected, the electrospray is influenced by the composition of the mobile phase as it enters the LC-MS interface. At low values of  $\varphi$ , the electrophoretic mobility of the ions is low leading to inefficient charge separation and difficulties in producing a stable spray.<sup>[24]</sup> Fig. 2 shows the influence of the mobile phase composition on the signals of PFOA and PFOS detected by the mass selective detector. At low values of  $\varphi$  no appreciable signal was detected. No difference in signal intensity was observed when sodium acetate was used instead of ammonium acetate. The optimal composition for the operation of the electrospray appeared to be at values of  $\varphi_{\text{MeOH}}$  between 0.6 and 1.

As a consequence, when working at low values of  $\varphi_{\text{MeOH}}$ , post-column addition of methanol is required and this can be provided by the setup illustrated in Fig. 1.

##### *Elution behaviour at low methanol fractions*

The elution behaviour of perfluorinated acids appears to be linear over a large range of  $\varphi_{\text{MeOH}}$  values. Fig. 3 shows the general picture of the  $\log k'$  v.  $\varphi_{\text{MeOH}}$  plots of the PFAAs as an example. First we looked at the elution behaviour at low methanol fractions in order to understand the behaviour over the whole range of solvent compositions. Capacity factors at low methanol fractions were therefore determined experimentally with the Aqua column and the  $\log k_0$  value for PFBA was directly determined without requiring extrapolation. Due to a decreased quality of the electrospray (see previous section), the capacity factor of PFBA could only be determined by installing an additional pump in the system that increased the fraction of methanol in the eluent entering the LC-MS interface.

The compound PFBA has the lowest  $k_0$  value of all the PFAAs tested, i.e. the shortest retention time. Retention times of PFAAs with longer fluorocarbon chains pose a problem in the method applied because the *Xcalibur* software is not able to create retention time windows longer than 200 min. At very low methanol fractions ( $\varphi_{\text{MeOH}} \leq 0.25$ ), the retention times of the larger homologues were much longer than the retention time



**Fig. 3.** Plots of  $\varphi_{\text{MeOH}}$  v.  $\log k'$  for perfluoroalkyl carboxylic acids obtained with the Aqua column at pH 2.2. See Table S1 in the Supplementary material for definitions.

window supplied by the software and  $\log k_0$  values for these compounds could therefore not be determined directly experimentally, contrary to what was done for PFBA. Eqn 3 presents the results obtained for PFBA on the Aqua column at pH 2.2, where the intercept, 1.21, represents the value of  $\log k_0$ ,  $r$  represents the correlation coefficient and  $n$  is the number of data points used in the regression. Values in parentheses represent standard errors. Note that in the case of PFBA the  $\log k'$  values could only be determined below methanol fractions of 0.4. At higher methanol fractions, PFBA elicited a very short retention time that could no longer be discerned from the uracil, and hence no results were obtained for PFBA under these conditions.

$$\log k' = 1.21 (\pm 0.01) - 2.18 (\pm 0.04) \varphi \quad (3)$$

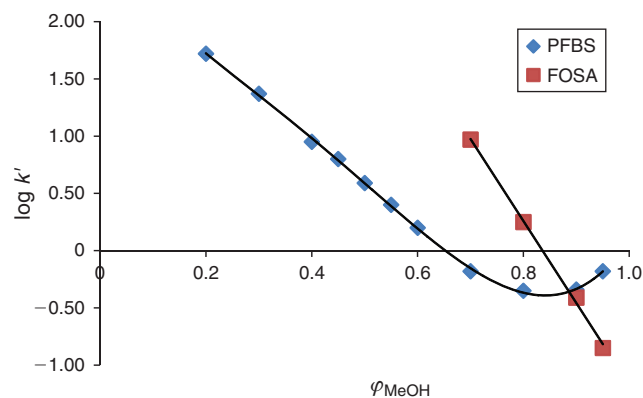
where  $n = 12$ ,  $r = 0.996$  and  $\varphi$  ranged from 0.0 to 0.4.

As can be seen from Eqn 3 and Fig. 3, for PFBA a linear relationship was observed between  $\log k'$  and  $\varphi_{\text{MeOH}}$  at very low methanol fractions.

#### Elution behaviour at high methanol fractions

The experiments in this study have shown that at certain isocratic conditions in both analytical columns used, such as at high methanol fractions:  $0.8 \leq \varphi_{\text{MeOH}} \leq 1$ , the relationship between the capacity factors of PFAAs and  $\varphi_{\text{MeOH}}$  becomes non-linear. This non-linear behaviour of PFAAs is illustrated in Fig. 4 for the example of PFBS. This type of behaviour was observed for all perfluorinated carboxylic and sulfonic acids (data not shown).

When the fraction of water in the mobile phase is increased, starting at  $\varphi_{\text{MeOH}} \leq 0.8$ , the relationship becomes linear, see Fig. 4 for PFBS. Linear regression has therefore only been applied in this study for methanol fractions of  $0 \leq \varphi_{\text{MeOH}} \leq 0.8$  for all experiments. Because the elution behaviour of PFBA (see previous section) appeared to be linear (see Fig. 3), we assumed that the relationship between capacity factors and methanol fractions is linear from  $\varphi_{\text{MeOH}} 0.4$  to 0. The plot for PFBS (Fig. 4) indeed shows that between  $\varphi_{\text{MeOH}} = 0.5$  and 0.2 linearity is observed. It is therefore assumed that all other PFASs will elicit a similar elution behaviour and any non-linear effects



**Fig. 4.** Elution behaviour of perfluorobutyl sulfonate (PFBS) and perfluorooctane sulfonamide (FOSA) at different methanol fractions in the mobile phase at pH 2.2 (Aqua column).

are not expected in the range  $0 \leq \varphi_{\text{MeOH}} \leq 0.8$ . This has allowed us to calculate the  $\log k_0$  values of the PFASs studied by applying Eqn 2.

It is not well understood what type of interactions the PFASs can have with the stationary phase at the very high methanol fractions. We assume that at high methanol fractions, surfactants with ionic groups (carboxylate and sulfonate) probably prefer to interact with the silanol groups of the stationary phase rather than be dissolved in the mobile phase. The acidity of surfactants apparently plays an important role. The compound FOSA<sup>[25]</sup> is a neutral compound and for this compound linear behaviour can be observed at high methanol fractions (i.e.  $\varphi_{\text{MeOH}} > 0.8$ ), see Fig. 4. The elution behaviour of this neutral compound is therefore linear over the whole range of methanol fractions that were possible to study.

The acidity of PFASs enables electronic and hydrogen bond interactions with the silanol groups in the stationary phase to occur<sup>[11,17,19,21]</sup> (instead of, or complementary to, van der Waals interactions). This non-linear behaviour may be enhanced at high methanol fractions in the mobile phase. The non-linear contribution to the elution behaviour could probably be



**Table 1. Capacity factors extrapolated to 100 % water ( $\varphi = 0$ ) for perfluorinated substances obtained with different chromatographic columns ( $C_{18}$  (Aqua) and  $C_{18}$  (Lich)) at different pH values**

The intercept ( $\log k_0$ ) calculated by linear regression is shown. n.d., no data. The values in parentheses represent the standard deviation of the means based on  $n = 4$  (column 2) and  $n = 2$  (column 3) values

Perfluorinated substance	Partition coefficient ( $\log k_0$ )		
	$C_{18}$ (Aqua) and $C_{18}$ (Lich) pH 2.2 and 2.4	$C_{18}$ (Aqua) and $C_{18}$ (Lich) pH 5.2 and 6.0	$C_{18}$ (Lich) pH 7.6
Perfluorobutyl sulfonate	2.35 (0.24)	2.38 (0.46)	2.12
Perfluorohexyl sulfonate	3.70 (0.49)	3.41 (0.50)	3.45
Perfluorooctyl sulfonate	5.10 (0.70)	4.78 (0.18)	5.11
Perfluorooctane sulfonamide	5.57 (0.24)	5.87 (0.31)	5.88
6 : 2 Fluorotelomer sulfonate	3.91 (0.49)	3.91 (0.37)	4.20
Perfluorobutanoic acid	1.56 (0.68)	2.04	2.01
Perfluorohexanoic acid	2.97 (0.42)	2.70	2.87
Perfluoroheptanoic acid	3.27 (0.54)	3.29 (0.45)	3.34
Perfluorooctanoic acid	3.92 (0.51)	4.00 (0.48)	4.23
Perfluorononanoic acid	4.61 (0.36)	4.74 (0.24)	5.11
Perfluorodecanoic acid	5.41 (0.45)	5.58 (0.11)	5.65
Perfluoroundecanoic acid	5.90 (0.83)	6.01 (0.45)	6.37
Perfluorododecanoic acid	6.27 (0.56)	n.d	7.47
Perfluorotetradecanoic acid <sup>A</sup>	6.6 (–) <sup>A</sup>	n.d	n.d

<sup>A</sup>Indicative value for perfluorotetradecanoic acid; only one value from measurements at Lichrosphere  $C_{18}$  (at pH 2.2) available from acetonitrile– $H_2O$ , because in methanol– $H_2O$  no clear peak maxima could be obtained due to poor peak shape.

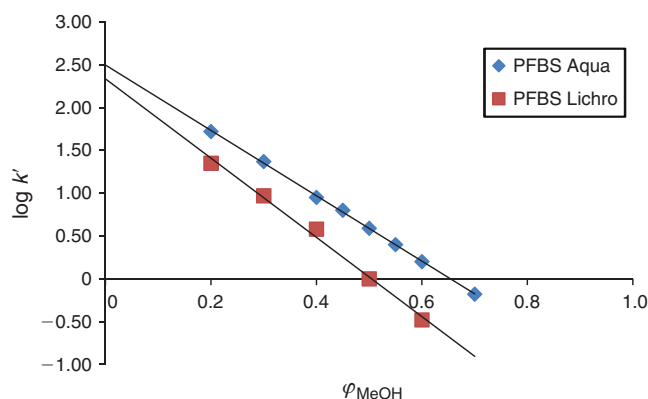
minimised by changing the pH of the mobile phase to lower values than the acid dissociation constants of the PFASs studied to correct for ionisation of PFASs. The columns applied in this study (and also most other commercially available columns) unfortunately have a pH limitation because the stationary phase can dissolve at pH values lower than or close to 2, whereas the reported  $pK_a$  values of, e.g. perfluorocarboxylic acids, vary between  $-0.2$  and  $3.5$ .<sup>[26,27]</sup>

It should be noted that in the guidelines of the Organisation for Economic Co-operation and Development (OECD), the principles and limitations to determine  $\log K_{ow}$  by HPLC have been stated.<sup>[21]</sup> According to the OECD guidelines, the method is not applicable to strong acids and bases, metal complexes, substances which react with the eluent or surfactants. The  $\log K_{ow}$  of acids can however be obtained by adjustment of the pH value to values below the  $pK_a$  of the compounds studied.

#### General results of PFASs

The order of the PFASs eluting from the column remained the same over the whole range of the solvent compositions tested ( $0 \leq \varphi_{MeOH} \leq 0.8$ ) despite clear differences in the slope of the  $\varphi_{MeOH}$  v.  $\log k'$  relationships. Indeed, it has been shown that for individual homologues a linear  $\log k'$  v.  $\varphi_{MeOH}$  dependence would lead to a fan-like shape of the  $\log k'$  v.  $\varphi$  plots with a common convergence point<sup>[21]</sup> (see Fig. 3). Hence, the capacity factor becomes higher when the chain length of the PFAS increases (see below and Fig. 3).

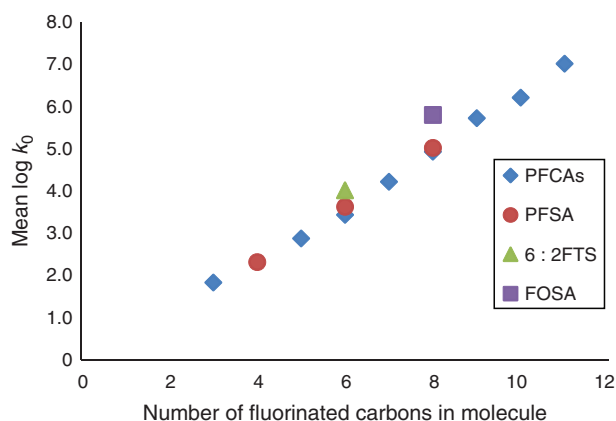
As stated above, the long retention times of long chain PFASs form a limitation to obtain an accurate value for the capacity factor at lower values of  $\varphi_{MeOH}$ . In Fig. 3 it is shown that not all capacity factors were measured for every PFAS due to long retention times and the inability of the software to handle these long retention times as has been mentioned above. Moreover, at long retention times peaks flatten out and it becomes difficult to accurately assess the apex of a peak. As a consequence, fewer capacity factors could be obtained experimentally for long chain



**Fig. 5.** Plots of  $\varphi_{MeOH}$  v.  $\log k'$  for perfluorobutane sulfonate (PFBS) obtained with two different columns at pH 2.2 demonstrating that extrapolation to 100 % water ( $\varphi = 0$ ) leads to similar results for  $\log k_0$ .

PFASs compared with short chain PFASs. This is the reason why there is no data for perfluorododecanoic acid (PFDA) and perfluorotetradecanoic acid (PFTeA) at pH 5 and no data is present for PFTeA at pH 7, see Table 1.

The results obtained with both columns were grouped according to the pH range used in the experiments (pH  $\sim 2.3$ , pH  $\sim 5-6$  and pH 7.6). Table 1 provides the capacity factors extrapolated to  $\varphi_{MeOH} = 0$ . The second column in Table 1 lists data of the average of four different experiments performed at pH values of 2.2 and 2.4 using both analytical columns (see Table S2 for all separate results). The third table column is an average of two experiments performed at pH values of 5.2 and 6.0 using both analytical columns. Finally, the data in the fourth table column is from one single experiment performed at pH 7.6. The  $\log k_0$  values determined for each analytical column are similar to each other (see Fig. 5, Table S2) and increase with increasing chain length of the PFASs.



**Fig. 6.** Plot of the relationship between  $C_F$ , the number of fluorinated carbon atoms in the perfluorinated alkylated substances (PFASs), and the overall mean  $\log k_0$  value obtained from all high-performance liquid chromatography experiments. See Table S1 in the Supplementary material for definitions.

#### Retention and length of the fluorocarbon chain

As can be seen from Fig. 5, the hydrophobicity of perfluorinated sulfonates, 6:2 FTS and perfluorinated carboxylates, expressed by their  $\log k_0$  value, is linearly related to the number of fluorinated carbon atoms in the PFAS molecule. This is in agreement with earlier findings reported for water–methanol eluents.<sup>[28]</sup> For example for 6:2 FTS a  $\log k_0$  value corresponding to six perfluorinated carbon atoms is found, rather than to eight carbons. This compound is not fully fluorinated and has four hydrogen atoms instead of fluorine atoms; its  $\log k_0$  value is 1.1 log units lower than its C8 homologue PFOS. Obviously, the number of fluorine-attached carbon atoms of these compounds controls for a large part the partitioning behaviour in the chromatographic system and thereby the  $\log k_0$  value which is shown in Fig. 6.

The resulting overall relationship can be expressed by Eqn 4 ( $R^2 = 0.995$ ,  $n = 12$ ):

$$\log k_0 = 0.66C_F \quad (4)$$

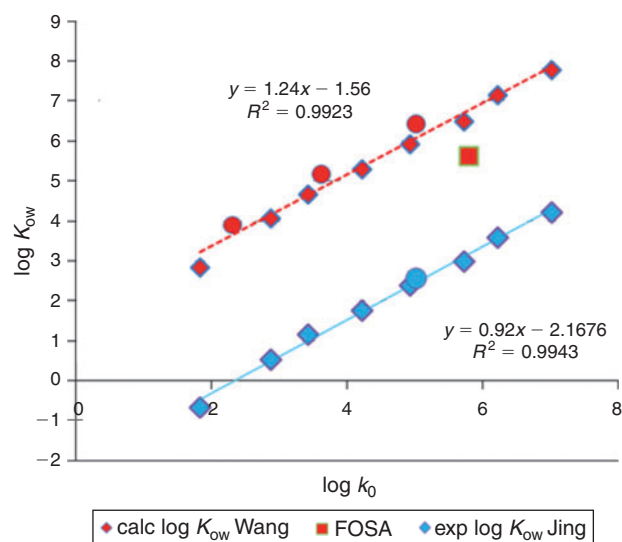
where  $C_F$  is the number of carbon atoms with fluorine attached.

The contribution of each  $CF_2$  group to the increase in hydrophobicity is therefore equal to 0.66, a value that is close to the value of 0.61 derived from voltammetric measurements,<sup>[5]</sup> between values of 0.50 and 0.80 estimated by earlier model calculations (COSMOtherm and SPARC models respectively)<sup>[13]</sup> and again close to the average value (0.62) calculated recently with COSMOtherm.<sup>[14]</sup> One should bear in mind though, that such models can probably only properly estimate the partitioning properties of neutral, monomeric species.<sup>[14]</sup>

The hydrophobicity of FOSA, for which  $C_F = 8$ , is slightly higher than the  $\log k_0$  value predicted by Eqn 3. This can be ascribed to the neutral character of PFOSA that results in a slightly lower affinity for water, and to its amide group that enables additional hydrogen bonding with the stationary phase and may also slightly neutralise the strong electron-withdrawing effect<sup>[5]</sup> of the perfluoroalkyl chain.

#### Influence of pH of the mobile phase

With increasing pH of the mobile phase, the  $\log k_0$  values show a small increase in the hydrophobicity overall (see Table 1). The



**Fig. 7.** Plot of the relationship between  $\log k_0$  values from the present study and available estimated  $\log K_{ow}$  values from Wang et al.<sup>[14]</sup> (red dashed line) and voltammetric  $\log P^{0'}$  values from Jing et al.<sup>[5]</sup> (solid blue line). The trendline and statistics correspond to all perfluorinated alkyl acids (PFAAs) ( $n = 11$ ) for the data of Wang et al.<sup>[14]</sup> ( $n = 11$ , red markers, dashed line), and Jing et al.<sup>[5]</sup> ( $n = 9$ , blue markers and blue line). Diamonds represent perfluoroalkyl carboxylic acids (PFCAs), circles are perfluoroalkyl sulfonic acids (PFSA).

$\log k_0$  values at pH 5.2 are approximately a half log unit higher and lower than their respective  $\log k_0$  values at pH 2.2 and pH 7.6. The  $\log k_0$  values are however not statistically different from each other (based on 95% confidence intervals). A similar trend is not observed for sulfonic acids, see Table 1. As a conclusion, the pH of the aqueous mobile phase only slightly affects the  $\log K_w$  of perfluorinated carboxylic acids irrespective of the chain length. The  $pK_a$  value of non-fluorinated carboxylic acids is close to 5. This value seems to be rather constant for different carbon chain lengths and it is estimated that  $pK_a$  values of perfluorinated carboxylic acids are also constant but much lower.<sup>[27]</sup>

#### Hydrophobicity

Most  $\log K_{ow}$  values reported for PFASs in the literature have been calculated using partitioning models. Apart from the experimental  $\log P^{0'}$  values determined using voltammetry by Jing et al.,<sup>[5]</sup> Kelly et al. provided an overview table with  $\log K_{ow}$  values using original data calculated by Arp et al.<sup>[13]</sup> and correcting these because of ‘apparent overestimation’ (see table S3 of the supporting information of Kelly et al.<sup>[29]</sup>). Wang et al.<sup>[14]</sup> presented new results obtained with the COSMO-therm software. These calculated and experimental values have been summarised in Table S3. As can be seen from Table S3, values are available for the  $C_4$ – $C_{12}$  carboxylic acids, PFOS and FOSA. A strong linear relationship is observed between the  $\log k_0$  values obtained in the present study and both the experimental and calculated  $\log K_{ow}$  values published (cf. Fig. 7). We conclude that the HPLC-derived hydrophobicity values obtained in the present study provide a robust set of indicators for the environmental partitioning behaviour of PFASs.

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