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Use of solid-phase microextraction for measuring oil–water partition coefficients and correlation with high-performance liquid chromatographic methods for lipophilicity

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Abstract

For flavour compounds, lipophilicity is often estimated by the partition coefficient between oil and water (log $K_{oil-water}$), which is highly relevant to food. A modification of the shake-flask method is reported here where compounds are quantified in the two phases using solid-phase microextraction (SPME). SPME's highly sensitivity to non-polar compounds facilitates quantification in the water phase. Twelve flavour compounds representing a broad range of lipophilicities and functional groups were analysed by two methods. Their log $K_{oil-water}$ was determined using SPME quantitation and their log k_w using a reversed-phase HPLC methodology. The isocratic capacity factor at 60% methanol and predicted log *P* value also showed high correlation factors with other methods. The octadecyl silylated surface of the HPLC column provides a matrix that interacts with lipophilic compounds where the retention time is the indication of lipophilicity. Both methods gave reproducible results (median 3% and 4% RSD) and similar but not identical values for lipophilicity. The relationship between the two methods is log $k_w = 0.85 \log K_{oil-water} + 0.48$ with a correlation coefficient of 0.94. The new SPME detection method, with the ability to quantify limonene and 2-pentylfuran at 1 ppm in the water phase, is preferred for flavour compound analysis due to the applicability of oil–water partitioning in food.

Keywords: Partition coefficients; Oil–water partition coefficients; Lipophilicity; Extraction methods; Aroma compounds; Volatile organic compounds

1. Introduction

Flavour compounds are small molecules (M_r 300) which have diverse chemical formulas and structures. Given the possibility for different functional groups within a molecule such as thiols, alkane chains, ketones, aldehydes, pyrazines, pyridines, acetates, alcohols, sulphides, etc., the degree of lipophilicity has a large range. One of the most polar flavour volatiles is diacetyl (2,3-butanedione) which partitions more into water than into oil. Most flavouring compounds, however, partition more into oil than water. A measure of the lipophilicity of flavour compounds is important for understanding how the compounds behave in food systems. For instance, most flavour compounds will prefer to reside in the oil phase of a food and this has clear consequences for the flavour of reduced fat foods.

The traditional value of compound lipophilicity is

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log *P*, which is the partition coefficient between octanol and water. This has been used for decades in establishing biological quantitative structure–activity relationships. For food systems, however, it is the partition coefficient between food oils and water that accurately represents the compound behaviour [1]. While shake-flask methods are normally used, determinations of the values for highly lipophilic compounds are difficult because of the minute quantities found in the water phase. Improvements in the shake-flask method could be envisioned by a method that exhibits high sensitivity to lipophilic compounds. Solid-phase microextraction (SPME) is an ideal method for this [2,3]. In headspace or immersion mode, the SPME fibre performs an extraction of the sample without the use of solvents. Lipophilic compounds in water can be easily detected at ppb levels [4,5].

An alternative to the shake-flask methods is a method based on high-performance liquid chromatography (HPLC), which has often been chosen for pharmaceutical analysis [6]. An overview of the method is found in Ref. [7]. Advantages of this method include applicability for compounds with higher lipophilicities and reduced loss of volatile compounds. The alkyl-bonded phase (C_{18}) slows the movement of compounds through the column. The more lipophilic the compound, the later its retention time. The capacity factor, *k*, is determined based on the retention time of the compound.

$$k = (t_R - t_0)/t_0 \quad (1)$$

where t_R is the retention time of the analyte and t_0 is the retention time of a non-retained compound.

Extrapolation of the capacity factor to 100% water is often done in order to minimise the selective effect of the stationary and mobile phase on solute retention. This is done by analysis at several % organic solvent concentrations and linear extrapolation of the retention times to 100% water. The log form is used: log k_w. Recently this method was used for analysis of flavour compounds [8,9].

In the development of the log k_w method, numerous studies correlated the values with log *P* [10]. Correlation of chromatographic retention to liquid–liquid distribution has been shown to be thermodynamically valid [11]. Even for 60 compounds with very distinct solute classes, a close correlation was established where the slope was 0.988 ($\pm$0.51). High correlations have been often demonstrated within a homogeneous series. Log k_w can be used a priori as a hydrophobic parameter and does not need an additional reference system. Log k_w is the equivalent to log *P* in its ability to describe the hydrophobic nature of bioactive compounds [10]. While the shake flask method gives reliable values for log *P* between -2 and 4, the HPLC method functions between 0 and 7. The slight differences in the two methods are partially due to the interactions of molecules with the incompletely derivatized silanol groups of the HPLC stationary phase and to more steric information included in the log k_w determination [10].

The aim of the present work is to develop a method based on SPME for the determination of oil–water partition coefficients and to compare the values with those obtained with the HPLC methodology.

2. Experimental

2.1. Oil–water partition coefficient determination

2.1.1. Presaturation of phases

Distilled water and sunflower oil (locally bought) were used. Sunflower oil (1 l) was presaturated in 10 ml water by agitation with a stirring bar for 2 h. After the mixture was centrifuged for 2 h at 5000 rpm, the water phase was removed. A clear oil phase was obtained after filtering through filter paper. This same procedure was repeated for 1 l of distilled water presaturated in 10 ml of sunflower oil. These presaturated oil and water solutions were used for the extractions.

2.1.2. Extraction

The previous and following procedure were performed at room temperature (22°C). 2-Methylpyrazine, 2,3-diethyl-5-methylpyrazine and 2-isobutyl-3-methoxypyrazine were from Pyrazine Specialities (Atlanta, GA, USA). All other aroma compounds were from Aldrich (Steinheim, Germany). Aroma compounds were dissolved in oil phase at concentrations ranging from 6.8 to 10.7 mg/l for replicate A and from 2 to 22 mg/l for replicate B. Concentrations were chosen so that the

amounts extracted in the water and oil phases could be detected. Each compound had a different concentration in replicates A and B as recommended by OECD guidelines [12]. As also recommended, two different volumes were used for the extractions. The two replicates were: (1) 20 ml of replicate A + 40 ml of water and (2) 40 ml of replicate B + 20 ml water. The extraction was made using a Mixor (Alltech, Deerfield, IL, USA) device for 10 min which gives a very efficient extraction. The mixture was transferred to a closed vial and centrifuged for 2 h at 5000 rpm. The phases were completely separated and clear. Samples of the oil phase (0.8 ml) and water phase (1.0 ml) were placed in 2-ml vials for SPME analysis and allowed to equilibrate.

2.1.3. Quantitation of compound amounts (SPME–GC–MS)

The oil phases were analysed by headspace-mode and the water phases by immersion-mode using a Varian 8200 autosampler. A polydimethylsiloxane SPME fibre was inserted into the headspace or water sample and allowed to equilibrate for 1 h. The fibre was placed into the injection port of the GC system for 5 min at 250°C containing a 0.75 mm I.D. liner (Supelco). During the first 3 min of desorption, the purge was off and the last 2 min with purge on further cleaned the fibre. GC separation and MS detection in the selected ion monitoring (SIM) mode was used for quantification of the aroma compounds (Hewlett-Packard 6890 GC system and 5973 mass-selective detector, DBWAX, J&W, 30 m × 0.25 mm I.D., 0.25 μm film, 1.0 ml/min helium at constant flow). SIM conditions are shown in Table 1. The oven was held at 20°C for 1 min and heated at 4°C/min up to 220°C where it was held for 10 min. Quantification was done using an external standard curve prepared from the presaturated phases. The sum of the amounts found in both phases were verified to be close to the original amount added.

2.2. Log P predictions

Predictions for log P and pK_a were made using Pallus 3.0 (Compudrug, San Francisco, CA, USA).

2.3. HPLC lipophilicity measurement

Compound lipophilicity was determined based on its retention time on a reversed-phase HPLC column [6]. The HPLC system used was a Hewlett-Packard series 1100 with diode array and HP1097A refractive index detection (Avondale, PA, USA). The column used (250 mm × 4 mm) was packed with Nucleosil 50-5 C_{18}, particle size 5 μm (Macherey–Nagel, Oensinger, Switzerland) and used at room tempera-

Table 1
Mass spectrometric conditions for SIM mode analysis (Electron Multiplier was 1529 with a dwell time of 30 ms)

Group	Compounds	Time windows	m/z
1	Diacetyl	≥8 min	43/86
2	Pyridine	8–13.2 min	52/79
	Limonene,		93/121/136
	2-Pentylfuran		81/138
3	2-Methylpyrazine	13.2–17 min	67/94
	1-Octen-3-one		70/97
4	1-Octen-3-ol	17–26 min	57/85
	2,3-Diethyl-5-methylpyrazine		135/150
	2-Isobutyl-3-methoxypyrazine		124/151
5	β-Damascenone	26–34 min	69/121/190
	Guaiacol		109/124
6	4-Ethylguaiacol	34–44 min	137/152

ture. The mobile phase was made up volumetrically from various combinations (30–70%) of methanol, and a solution containing 3-morpholinopropanesulphonic acid buffer (0.01 *M*) and *n*-decylamine (0.2%, v/v). The pH of the aqueous solution was adjusted beforehand to 7.4 by addition of HCl. As seen by their predicted pK_a values, all compounds were in their neutral form at pH 7.4. Retention times (t_R) were measured at room temperature with a 1.0 ml/min flow-rate. The column dead time (t_0) was determined with uracil. The capacity factor was defined as $k=(t_R-t_0)/t_0$. Log *k* for 100% water (log k_w) was linearly extrapolated from results obtained for between three and five different mobile phase compositions.

3. Results and discussion

The aim of this study was to compare the newly developed variation of the shake flask method with SPME detection to the already developed HPLC methodology. Predicted log *P* values have also been compared it is a standard method used in many industries.

3.1. Determination of log $K_{oil-water}$ by Mixor extraction, centrifugation, and SPME–GC–MS quantitation

The OECD chemical testing program published guidelines for determination of the *n*-octanol–water partition coefficient by the log $K_{oil-water}$ method [12]. One of these is that the quantity of substance as well as the ratio of solvent volumes be varied. For this reason, replicates were done at different concentrations and different solvent volumes. Also, presaturation of the phases and analysis of the compound concentration in both phases was done as recommended. Especially for volatile compounds, it is important to analyse the amounts in both phases because depending on the handling procedure, losses due to volatilisation could have occurred. In order to determine the concentration of compounds that were used and the necessary range for the standard curve, we performed a preliminary test.

The partition coefficient results can be seen in Table 2. The 12 compounds tested were chosen because they span the range of flavour compound lipophilicities and are stable when in solution together. At the concentrations that they were present, we did not expect any interaction between the

Table 2
Values of log $K_{oil-water}$, log k_w, log k_{60} and predicted log *P* and pK_a values for different compounds showing standard deviation (SD) and number of measurements (*n*)[a]

Compound	Log $K_{oil-water}$			Log k_w[b]			Log k_{60}			Predictions	
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Log *P*	pK_a
Diacetyl	−0.43	0.06	2	−0.30	0.01	2	−0.88	0.17	2	−0.6	Neutral
2-Methylpyrazine	−0.43	0.02	2	0.26	–	1	−0.50	–	1	−0.19	0.86
Pyridine	−0.25	0.13	2	0.61	0.07	5	−0.35	0.04	5	0.73	5.25
Guaiacol	0.97	0.02	2	0.97	–	1	−0.53	–	1	1.48	9.92
2,3-Diethyl-5-methylpyrazine	1.48	0.11	2	1.73	0.12	2	0.25	0.10	2	1.33	1.41
1-Octen-3-ol	1.68	0.00	2	2.63	0.09	3	0.51	0.05	3	2.75	15.43
Ethyl guaiacol	1.66	0.05	2	1.92	0.07	2	0.14	0.15	2	2.43	10.25
1-Octen-3-one	2.27	0.02	2	2.18	0.17	3	0.34	0.14	3	2.94	Neutral
2-Isobutyl-3-methoxypyrazine	2.44	0.09	2	2.43	0.06	2	0.52	0.04	2	1.58	0.53
β-Damascenone	3.09	0.05	2	2.79	0.17	2	0.57	0.07	2	3.7	Neutral
2-Pentylfuran	3.57	0.11	2	3.52	0.10	2	1.02	0.07	2	3.66	Neutral
Limonene	3.68	0.05	2	3.91	–	1	1.33	–	1	4.03	Neutral

[a] Log $K_{oil-water}$ used a Mixor for efficient extraction followed by centrifugation and SPME quantitation in the two phases, in which the volume ratio varied. Log k_{60} was the capacity factor on a C_{18} HPLC column at 60% methanol. Log k_w was the capacity factor for 100% water, as extrapolated from the value at multiple methanol phase concentrations. Predictions were made using Pallus 3.0.

[b] Average R^2 value for k_w determination: 0.998.

compounds that would influence their oil–water partition coefficients. Evidence supporting this argument is that the two replicates gave very similar values even if the concentrations were different. The two replicates have a median difference of 0.07 log units, which is well within the recommended limits [12]. The median relative standard deviation (RSD) was 3%, which is very good for an analytical method. The advantage of using SPME as a detection method can be seen by the ability to quantify the amounts of highly lipophilic compounds, limonene and 2-pentylfuran, in the water phase. These compounds were present at 1 ppb in the water phase and this was in the quantifiable range.

Log $K_{oil-water}$ of diacetyl had been previously measured using a technique that only analysed the aqueous phase and a similar value was found (−0.5) [13].

3.2. Determination of log k_w by HPLC

In line with the recommended methods for correlation to log P [7,10], a C_{18} reversed-phase column was used with methanol–water elution and log k_w was determined by extrapolation of isocratic capacity factors to 100% water. The linear extrapolation of isocratic capacity factors had high correlation coefficients (average $R^2 = 0.998$), and used at least three points. The replicates were done throughout a year with columns of the same packing material but of a different lot number and age and hence had a slightly higher variation than the log $K_{oil-water}$ method. The median RSD among the compounds was 4%, which is good considering this variation in time of analysis. The literature values using the same method [9] are very close (difference of less than 0.08 log unit) for diacetyl, 2-methylpyrazine, pyridine, 2,3-diethyl-5-methylpyrazine and 2-isobutyl-3-methoxypyrazine. Only 1-octen-3-ol was somewhat different (ours/literature values of 2.63/2.31, respectively).

3.3. Inter-correlations of various lipophilicity parameters

Table 2 shows the values obtained for various methods of measuring lipophilicity and Table 3 shows the correlation coefficients between methods. These phases have different chemical interactions with the compounds and it is not expected that they will have correlation coefficients of 1.0.

3.3.1. Relationship between $K_{oil-water}$ and k_w

The current study used 12 compounds with many different chemical structures and functional groups, as is usually the case for flavour compounds. Fig. 1 shows the correlation between the two methods. The equation is:

$$\log k_w = 0.85 \log K_{oil-water} + 0.48 \quad (2)$$

If the intercept was equal to 0 and the slope was equal to 1, the methods would not have a systematic difference. Given the data in this experiment, the methods are very close but not identical. The difference between complete equality is seen in Fig. 1. The more lipophilic compounds have similar partitioning between the HPLC C_{18} phase and sunflower oil. However, the less lipophilic compounds have greater affinity for the HPLC C_{18} phase. This finding that polar solutes give higher than expected log k_w values has been previously noted and was explained by their inclusion in the solvation layer of the stationary phase [10]. This could be the case for 2-methylpyrazine and pyridine which are overestimated in log k_w as compared to log $K_{oil-water}$.

Likewise, compounds with hydrogen-bond donor

Table 3
Correlation matrix between different lipophilicity factors showing correlation factors (R)

	Log $K_{oil-water}$	Log k_w	Log k_{60}	Log P predicted
Log $K_{oil-water}$	1.00			
Log k_w	0.97	1.00		
Log k_{60}	0.94	0.98	1.00	
Log P predicted	0.93	0.94	0.89	1.00

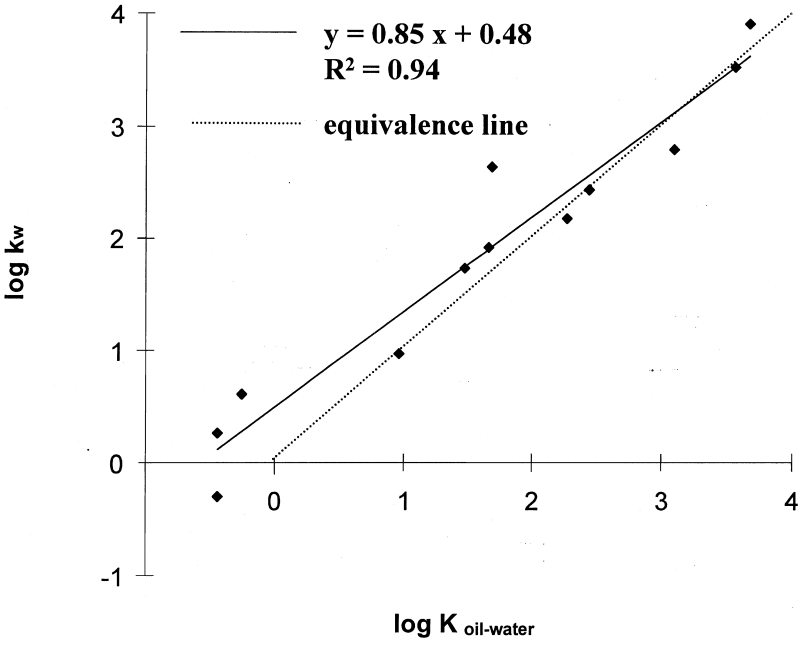

Fig. 1. Relationship between log $K_{oil-water}$ (shake flask-SPME) and log k_w (HPLC) using 12 compounds of diverse chemical structures. With 95% probability, the intercept is between 0.14 and 0.82 and the slope is between 0.70 and 1.01.

and/or acceptor capabilities, such as *ortho*-substituted aromatic compounds, are capable of forming intramolecular hydrogen bonds and have shown enhanced log k_w values due to their decreased ability to form hydrogen bonds with the mobile phase [10,14]. Indeed, triglycerides in the oil phase with their long hydrocarbon chains have little function as proton acceptors. This could be the case for 1-octen-3-ol, which has higher log k_w as compared to log $K_{oil-water}$. The literature value for 1-octen-3-ol is indeed lower than what was found here [9].

The finding that there was a close but not identical correlation between the two methods is what was expected as both are valid but slightly different measurements of lipophilicity.

3.3.2. Correlations between other lipophilicity methods

Isocratic capacity factors are also possible lipophilicity indexes [15]. In this study, the isocratic capacity factor at 60% had much larger standard deviations than log k_w. This is certainly because each log k_w extrapolation used between three and five independent measurements at different methanol contents; which in itself gives more accurate values than a single measurement. This study represents a realistic scenario where the analysis was performed over many months with different ages of columns. The possible advantage of using an isocratic capacity factor is that a value can be obtained with less chromatographic runs. In this study, the isocratic capacity factors at 60% methanol showed high correlations to log k_w but lower correlations to log $K_{oil-water}$. Caution to use isocratic factors has been noted because they may give a reduced range and sometimes even a different order of compound lipophilicity [15]. Thus, our conclusion is that log k_w is preferable to log k_{60} due to higher accuracy.

The predicted log P values showed the lowest correlations to the measured values of lipophilicity, although they can be useful to give a first indication. The relationship between predicted and measured log P values, using different models, has been extensively studied in the literature [16].

4. Conclusions

Partition coefficient methods between two phases should involve analysis of both phases. The SPME detection based shake-flask method for the determination of $K_{oil-water}$ was able to analyse both phases for an extended range of compound lipophilicities. The most lipophilic compounds, limonene and 2-pentylfuran, were quantified at 1 ppb in the water phase. As the compounds analysed here represent the range of flavour compound lipophilicities, this method can be broadly applied in flavour analysis. This new detection method had reproducible (RSD = 3%) and accurate calculations of the partition coefficient. Accuracy was assessed by comparison with standard methods of compound lipophilicity determination, mainly the C_{18} HPLC capacity factor method. This method, when extrapolated to 100% water, also gave reproducible values even over an extended period. However, the disadvantage is that the values are relative and must be compared with other studies run under the same conditions. In addition, the need to extrapolate to 100% water entails at least three analyses at different % methanol. In fact, all methods of lipophilicity investigated gave comparable but not identical results, which was expected (R^2 between 0.98 and 0.96). The SPME partition coefficient methodology for analysis of flavour compounds is preferred due to the determination of thermodynamic equilibrium parameters between two phases inherent in food systems and the ability to quantify highly lipophilic compounds.

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