

Estimation of the oil water partition coefficient, experimental and theoretical approaches related to volatile behaviour in milk

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Abstract

Oil/water partition coefficients (K_{ow}) of 20 volatile compounds in model or milk-based emulsions were determined experimentally using atmospheric pressure chemical ionization-mass spectrometry (APCI-MS). The K_{ow} values were estimated by comparing the headspace concentration of volatiles above solutions with different lipid content (2, 7, 12, 27, 62 and 102 g/L). This involved curve fitting of a series of the six experimental values to an equation describing the expected behaviour of volatiles in lipid containing systems. Alternatively, a simpler experimental method based on the comparison of only two samples was used. Overall, the K_{ow} values determined in the milk-based emulsion were the same as those found in simple model emulsions, suggesting that the partitioning behaviour of volatiles between air and the two emulsion systems was the same. The Log P values of the 20 volatile compounds were calculated using four software programmes, the Log P values from EPI Suite™ were found to correlate best with experimental Log K_{ow} values.

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Keywords: Headspace analysis; Octanol–water partition coefficient; Oil content; APCI-MS

1. Introduction

Aroma release from foods under equilibrium conditions depends on the distribution (partition) of aroma molecules between air and food phases (Taylor, 1998). The partition of volatile compounds between different phases can be expressed in simple theoretical systems as the partition coefficient, e.g. air/water, air/oil or oil/water. The application of these partition coefficients to food systems (and measurement) can be complicated by the fact that foods generally contain several phases. The importance of these phases on volatile partitioning is well known (Reynolds & Land, 1982). Many volatile compounds are more soluble in oil than water, in oil/water systems like emulsions. The proportion of volatile compounds in the gas phase, depends on their affinity for the fat phase and the amount

of oil present (Landy, Courthaudon, Dubois, & Voilley, 1996; Malone, Appelqvist, Goff, Homan, & Wilkins, 1999). The affinity for the oil phase can be measured through the compound's oil/water partition coefficient (K_{ow}) (Sangster, 1997). Typically, this is measured in the absence of additional factors such as emulsifiers, which may confound experimental observations (McNulty & Karel, 1973).

A relationship between partition coefficients was proposed using a simple mathematical equation which theoretically allows the calculation of one partition coefficient if the others are known (Buttery, Guadagni, & Ling, 1973). The purpose of the work presented in this paper was to examine whether the equation of Buttery et al. (1973) describes volatile behaviour in complex systems such as milk-based emulsions. This involved using two separate methods to measure K_{ow} , which could be compared between milk-based and simple model emulsions.

Lipophilicity can also be estimated from models developed from studies of the partitioning of compounds

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between octanol and water, $\text{Log } P$ (Rekker & Mannhold, 1992). $\text{Log } P$ values were estimated using different software packages; these were compared with experimental K_{ow} values to assess the quality of the estimates.

2. Materials and methods

2.1. Chemicals

The 20 volatile compounds (98–99% purity) including carvone, benzaldehyde, 3-octanone, ethyl octanoate, furfural, linalool, octanal, methyl acetate, 2-heptanone, ethyl butyrate, ethyl hexanoate, hexanal, methyl butyrate, butanol, menthone, anethole, 3-hexenol, octanol, safranal and hexanal were obtained from Sigma–Aldrich (Gillingham, UK). Sorbitan monooleate (Span80) and polyoxyethylene sorbitan monostearate (Tween60) as surfactants were supplied by Fisher Scientific (Loughborough, UK). Sunflower oil and skimmed milk powder were purchased from a local shop (Greater Nottingham Coop., Nottingham, UK).

2.2. Emulsion preparation

Tween60 (3.5 g/L) was dispersed in distilled water or skimmed milk using a high-shear blender (Silverson Machines Ltd., Chesham, UK) for 10 min at 300 rpm. Thereafter, lipid (2, 7, 12, 27, 62 and 102 g/L of sunflower oil) containing Span80 (6.5 g/L, a lipophilic surfactant, Fisher Scientific, Loughborough, UK) was added to the aqueous phase dropwise with stirring for 15 min. The emulsion was instantly transferred into a homogenizer (Emulsiflex C50, Avestin, Heidelberg, Germany) through which it was passed four times at two different pressure ranges (5000–10,000 psi and 10,000–15,000 psi) at room temperature. The emulsions were stored in a refrigerator prior to use. Particle size measurements were obtained using a Malvern Mastersizer (Malvern Instruments, UK) in a stirred cell system using distilled water as the medium. Particle size distributions were measured to obtain droplet sizes of the emulsions. The samples had a $d_{3,2}$ (sum of volume/sum of surface) between 0.33 and 0.45. The droplet sizes of the emulsions had a narrow range, which is consistent with the presence of sufficient emulsifier to make stable emulsions.

2.3. Sample preparation

The 20 volatile compounds tested were chosen according to their octanol/water partition coefficients ($\text{Log } P$) to ensure a wide range of compound lipophilicities for method evaluation. Each volatile compound was dissolved individually in either distilled water or 1,2-propanediol (propylene glycol) appropriate to their solubility to produce stock solutions, which were diluted in the appropriate medium to prepare the final emulsions. All stock solutions were stored at 4 °C. Volatile compounds were added to emulsions (80 mL) in sealed glass bottles at various concentrations ranging from approximately 0.001 to 5.8 g/L. Samples were placed

on a roller bed (SRT2; Stuart Scientific, Redhill, UK) for 2 h to mix them and then equilibrated for 1 h.

2.4. Headspace analysis

Headspace concentrations above a series of emulsion containing different oil fractions were measured by atmospheric pressure chemical ionization–mass spectrometry (APCI–MS) (Micromass, Altrincham, UK). The headspace above each solution was sampled into the APCI–MS for approximately 50 s at a flow rate of 6.5 mL/min. The volatile compounds were ionized by a 4 kV positive ion corona discharge (cone voltage 16–26 V depending on the analyte). Data were expressed as relative headspace intensity obtained from experimental observations (RHI_O) calculated as

$$\text{RHI}_O = \frac{\text{HI}_E}{\text{HI}_C} \times 100 \quad (1)$$

HI_E and HI_C represent volatile headspace intensity above the emulsion sample and the control. Skimmed milk (2 g/L oil content) and water were used as controls for the milk and basic emulsion system, respectively.

2.5. Octanol–water partition coefficient ($\text{Log } P$) calculation

Four chemical modelling programs were used to obtain $\text{Log } P$ values; MOE (Chemical Computing Group Inc., 1997–2003), CAChe (Ghose et al., 1988), Hyperchem (Hypercube, 2003) and EPI Suite™ (US Environmental Protection Agency Office of Pollution Prevention Toxics & Syracuse Research Corporation (SRC), 2003).

3. Results and discussion

The K_{ow} of volatile compounds is an important physico-chemical descriptor to give an insight into aroma partitioning in many food systems. For lipophilic compounds, the air/product partition coefficient decreases substantially on the addition of lipid, whereas hydrophilic molecules typically have low air/water partition coefficients (K_{AW}) which change little on the addition of emulsion. Great differences in partitioning occur as K_{ow} increases from 1 to 100 particularly at low oil fractions (0–10 g/L), thereafter the curves are more similar (Fig. 1).

3.1. Six-point curve fitting for $\text{Log } K_{ow}$ estimation

Static equilibrium headspace from a series of six milk samples varying in oil content was measured to understand volatile behaviour in a complex system and estimate K_{ow} . The data was normalised to 100%, the volatile concentration of the low fat control sample (skimmed milk, 2 g/L oil content). To estimate K_{ow} , theoretical relative headspace intensities (RHI_T) were calculated using Eq. (2) (modified from the equation for the air/emulsion partition coefficient of Buttery et al., 1973) and fitted to the trend observed in the experimental RHI_O values from Eq. (1)

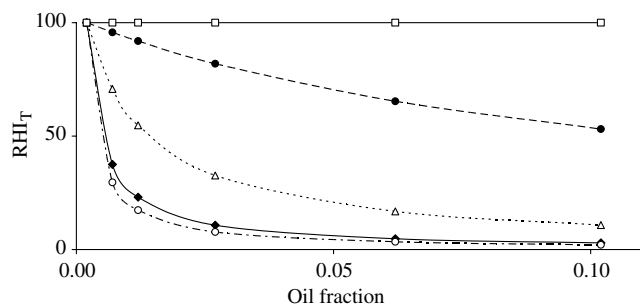


Fig. 1. The theoretical relative headspace intensity (RHI_T) above emulsion systems with K_{ow} of 1 (\square), 10 (\bullet), 100 (Δ), 1000 (\blacklozenge) and 10,000 (\circ) as oil fraction increases.

$$RHI_T = \frac{SF}{\phi_o(K_{ow} - 1) + 1} \quad (2)$$

where ϕ_o , K_{ow} and SF were the oil fraction, the oil/water partition coefficient, and a scaling factor, respectively. K_{ow} effectively controlled the shape of the curve as oil fraction changed. SF was used to adjust the Y-axis dimension of the theoretical curves, to match them to the experimental RHI_O values. Curves were fitted by iterative fitting using solver in Excel (Microsoft Corporation, Seattle,

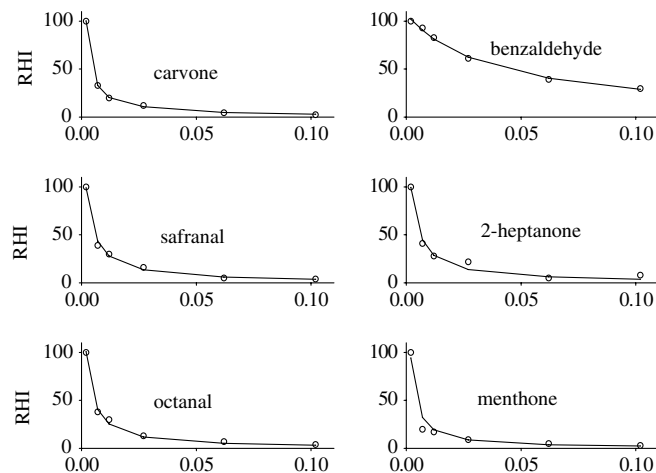


Fig. 2. Typical curves of the relative volatile headspace concentration above milk-based emulsions (experimental RHI_O values, \circ) and lines of best fit generated using solver in Excel and Eq. (2).

WA, USA). The fitting process minimizes the sum of the squares of the vertical distances of the data points from the curve (between experimental and theoretical values). Typical examples of the theoretical and observed relative

Table 1

Log K_{ow} for 20 volatiles determined in milk-based emulsion (containing sunflower oil) by curve fitting to data from six samples with varying oil content using a theoretical model

	Compound						
	Carvone	Benzaldehyde	3-Octanone	e.Octanoate	Furfural	Linalool	Octanal
Log K_{ow}	1.5 ^a	1.5	3.1	3.8	0.6	1.8 ^a	2.8
	3.0	1.4	2.4	3.7	0.7 ^a	2.4	2.8
	3.0	1.4	2.4	3.8	0.6	2.4	2.8
	2.8	1.4	2.7	3.8	0.2 ^a	2.4	1.9 ^a
	3.0	1.0 ^a	2.7	3.8	-0.1 ^a	2.4	2.0 ^a
	3.0	1.4	2.7	3.8	0.1 ^a	2.4	2.9
	3.0	1.3	2.5	3.8	0.7 ^a	2.4	2.9
	2.2	1.0 ^a	2.6	3.8			
Mean	2.8	1.4	2.6	3.8	0.6	2.4	2.8
SD	0.29	0.09	0.23	0.02	0.00	0.00	0.04
CV (%)	10.1	6.4	8.8	0.5	0.0	0.0	1.5
	Macetate	2-Heptanone	e.Butyrate	e.Hexanoate	Hexanal	Safranal	m.Butyrate
	Log K_{ow}	0.6 ^a	1.1 ^a	1.7	2.8	1.9	2.7
	0.0 ^a	1.7	1.6	2.8	1.5	2.7	1.5
	0.5 ^a	2.7	1.9	2.8	1.5	3.0	1.5
	0.3	2.2	1.9	2.8	1.5	3.0	
	1.2 ^a	2.1	2.1		1.4	3.0	
	0.4	2.7	1.9				
Mean	0.3	2.3	1.9	2.8	1.6	2.9	1.5
SD	0.10	0.41	0.18	0.01	0.19	0.15	0.05
CV (%)	29.0	17.9	9.4	0.4	12.2	5.3	3.3
	Butanol	Menthone	Anethole	3-Hexenol	Octanol	Hexanol	
	Log K_{ow}	0.6	3.2	3.7	1.7	3.1	1.2
	0.5	3.2	3.7	1.7	3.0	1.2	
Mean	0.5	3.2	3.7	1.7	3.0	1.2	
SD	0.06	0.00	0.00	0.00	0.02	0.00	
CV (%)	10.4	0.0	0.0	0.0	0.6	0.0	

Abbreviations: the letters 'm' and 'e' denote methyl and ethyl.

^a Values rejected due to poor fit between experimental values and theoretical curves.

headspace concentrations above milk-based emulsion are shown in Fig. 2. Partitioning behaviour showed that the most lipophilic compounds studied were ethyl octanoate and anethole (Table 1), whilst methyl acetate and furfural were the most hydrophilic ones. The majority of data presented in Table 1 is consistent with values in the literature (Pollien & Roberts, 1999).

The quality of fit between the two sets of values depended on the whether the model accurately described the partitioning of compounds in the presence of milk as well as the overall accuracy of the data. There were some instances in the estimation of $\text{Log } K_{ow}$, where there was a poor fit between experimental and theoretical curves. These were discarded when they appeared to be due to experimental error (e.g. the first estimate of $\text{Log } K_{ow}$ for carvone was a clear outlier). Generally, this was a rare occurrence for the majority of compounds with high lipophilicity, for instance octanal showed a 1% coefficient of variation ($\text{CV} = \text{SD}/\text{mean}$) for five independent measurements. In contrast, the least lipophilic compounds such as methyl acetate showed much greater error ($\text{CV}\% = 29$ in 6 runs), with many more cases where the fit between observed and theoretical values were poor. Such compounds show little effect of lipid on their partitioning behaviour, greater error may be a direct result of attempting to fit curves to such minor trends. The fact that the RHI_O values fitted the theoretical curves (generated from the model that described the behaviour of volatiles in simple emulsions) strongly implies that the milk-based emulsions behaved as a simple emulsion. Overall, the 6 samples method appeared reliable, robust and worked for a wide range of compounds with different K_{ow} values.

3.2. Two-sample analysis for $\text{Log } K_{ow}$ estimation

The previous section described a six sample approach to estimate $\text{Log } K_{ow}$ but it involved the preparation of many samples. Given that the equation of the curve is known and appeared to accurately describe the changes in volatile behaviour (Fig. 2), it should be possible to estimate $\text{Log } K_{ow}$ based on only two samples. This should simplify the method to measure $\text{Log } K_{ow}$ of volatile compounds and lessen the number of samples. The $\text{Log } K_{ow}$ values were estimated using Eq. (3), again in milk-based emulsions with the volatile dissolved in water as the control sample

$$K_{ow} = \frac{\frac{100}{\text{RHI}_O} - 1}{\phi_o} + 1 \quad (3)$$

Headspace measurements showed a broad range of changes in volatile concentration for the emulsion compared to the water control, from virtually no effect to a 99% change in RHI_O . The data obtained from the 2 sample method (Table 2) for $\text{Log } K_{ow}$ estimation were compared with the values from the six sample measurements (Table 1). Average differences between the two sets of data were obtained for three ranges of RHI_O values. When the headspace concentration (RHI_O) changed between 45% and 75%, the $\text{Log } K_{ow}$ values obtained showed the least differences (0.25; from 39 measurements in total). Above and below this range the average differences in $\text{Log } K_{ow}$ between the 2 and 6 sample methods were 0.49 and 0.57, respectively (each based on 18 measurements), showing that the estimates were not as accurate.

The results from the samples with the 45–75% change in headspace concentration correlated well with the values

Table 2
 $\text{Log } K_{ow}$ data were calculated in milk-based emulsions (containing sunflower oil) based on 2 samples measurements with different lipid content

	Compound						
	Safranal	Menthone	e.Octanoate	3-Hexenol	2-Heptanone	Hexanal	Octanol
$\text{Log } K_{ow}$	3.1	3.0	4.0	1.6	1.9	2.0	2.4
	3.0	2.9	4.1	1.6	2.1	2.3	3.1
	2.8						
Mean	2.9	3.0	4.0	1.6	2.0	2.2	2.7
SD	0.14	0.01	0.04	0.00	0.11	0.21	0.50
CV (%)	4.8	0.2	1.1	0.0	5.5	9.6	18.3
	Carvone	Octanal	e.Hexanoate	Linalool	3-Octanone	Anethole	Furfural
$\text{Log } K_{ow}$	2.3	2.9	2.9	2.4	2.5	3.8	0.6
	2.1	3.0	2.9	2.4	2.7	4	0.3
Mean	2.2	3.0	2.9	2.4	2.6	3.9	0.5
SD	0.10	0.03	0.00	0.00	0.07	0.18	0.21
CV (%)	4.5	0.9	0.0	0.0	2.8	4.5	47.1
	Hexanol	e.Butyrate	m.Butyrate	m.Acetate	Benzaldehyde		
$\text{Log } K_{ow}$	1.8	2.0	1.3	0.5		1.3	
	1.8	1.9	1.7	0.5		1.9	
Mean	1.8	2.0	1.5	0.5		1.6	
SD	0.00	0.12	0.28	0.00		0.42	
CV (%)	0.0	5.9	18.9	0.0		26.5	

Abbreviations: the letters 'm' and 'e' denote methyl and ethyl.

Table 3
Log K_{ow} values obtained from 2 samples headspace measurements for a simple emulsion

	Compound						
	Butanol	Octanol	e.Hexanoate	Carvone	3-Hexenol	Anethole	m.Acetate
Log K_{ow}	0.4	2.6	3.0	2.5	1.7	4.2	0.5
	0.3	2.6	2.9	2.4	1.9	4.1	0.7
	0.3	2.7	2.9	2.3	1.7	3.9	0.6
Mean	0.3	2.6	2.9	2.4	1.8	4.1	0.6
SD	0.06	0.10	0.05	0.12	0.11	0.13	0.10
CV (%)	17.3	3.7	1.9	5.0	6.2	3.1	16.7
	Hexanal	Octanal	2-Heptanone	e.Butyrate	Benzaldehyde	Furfural	m.Butyrate
Log K_{ow}	2.0	3.0	2.2	2.0	2.0	0.6	1.4
	2.1	2.9	2.2	1.9	1.9	0.8	1.3
	2.3						
Mean	2.2	3.0	2.2	2.0	2.0	0.7	1.3
SD	0.15	0.09	0.00	0.07	0.06	0.14	0.04
CV (%)	6.8	2.9	0.0	3.3	3.0	20.0	3.3
	Hexanol	Linalool	3-Octanone	Menthone	e.Octanoate	Safranal	
Log K_{ow}	1.9	2.6	2.6	3.0	4.2	3.0	
	1.9	2.6	2.8	3.1	4.1	3.0	
Mean	1.9	2.6	2.7	3.1	4.1	3.0	
SD	0.00	0.00	0.15	0.07	0.05	0.00	
CV (%)	0.0	0.0	5.4	2.3	1.3	0.0	

Abbreviations: the letters 'm' and 'e' denote methyl and ethyl.

from the six-point curve fitting procedure ($R^2 = 0.87$ and slope 0.97) as shown in Fig. 3. If only two samples were used to determine Log K_{ow} values, the changes in headspace concentration between the two samples should be in the region of 45–75% for greater accuracy. This is difficult to achieve without any prior knowledge. However, it may be possible to achieve this using Log P values to give an initial estimation of the lipophilicity, and hence the amount of lipid to be added in order to obtain these differences in partitioning behaviour.

3.3. Comparison of a simple emulsion and a milk-based emulsion

Milk-based emulsions are complex biological entities, whereas model emulsions are composed of fat droplets

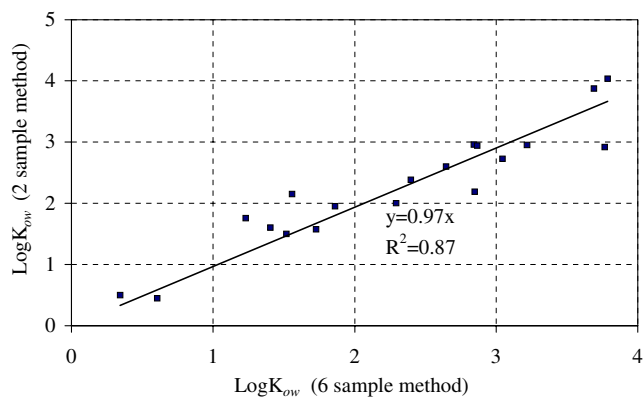


Fig. 3. Comparison of Log K_{ow} estimates from 6 sample vs. 2 sample method in milk-based emulsion (containing sunflower oil).

with regular shapes and sizes depending on the composition of the emulsion (emulsifier and oil) as well as the processing conditions. According to Eq. (2), partition should depend solely on oil fraction and K_{ow} , which disregards any emulsifier effect. This hypothesis was tested by comparing the data from the milk-based emulsions (Tables 1 and 2) with a series of model emulsions with different oil levels. These were analyzed using the 2 sample method and Log K_{ow} values obtained for a simple emulsion (Table 3). These were compared with the milk-based emulsion K_{ow} data (Tables 1 and 2) using a paired sample t -test. There was no statistically significant difference ($P < 0.05$) in partitioning between the two emulsion systems. This can be seen in Fig. 4, where a plot of Log K_{ow} values of simple emulsion and milk-based emulsion showed a good correlation ($R^2 = 0.86$ and slope = 0.97). Hence, the results

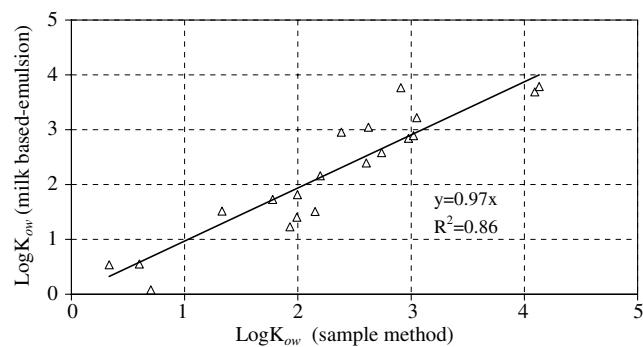


Fig. 4. The relationship between Log K_{ow} values for volatiles in a simple emulsion and milk-based emulsion.

Table 4
Correlation coefficient (R^2) of three sets of $\text{Log}K_{ow}$ data and $\text{Log}P$ values from four software programs

Program	6 sample (ME)	2 sample (ME)	2 sample (SE)	Data from the literature	Mean
EPI Suite™	0.80	0.85	0.86	0.91	0.86
Moe	0.66	0.80	0.78	0.79	0.76
CaChe	0.74	0.72	0.70	0.60	0.69
Hyperchem	0.28	0.14	0.03	0.30	0.19

The data from the literature (Pollien & Roberts, 1999) are included for comparison.

Abbreviations: 'ME' and 'SE' express milk-based emulsion and simple emulsion, respectively.

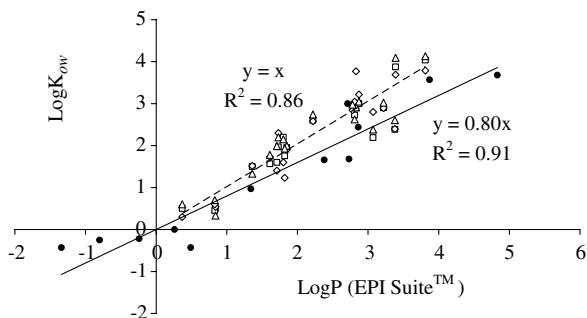


Fig. 5. Relationship between $\text{Log}P$ (calculated using EPI Suite™) and $\text{Log}K_{ow}$ values from the data in Tables 1–3 (Δ , \square and \diamond) and values (\bullet) from Pollien and Roberts (1999).

confirm that the milk-based emulsion was essentially behaving in a similar way to a simple emulsion.

3.4. Correlation of compound lipophilicity with octanol–water partition coefficient

For the purpose of seeking reliable and reproducible compound lipophilicity values, experimental $\text{Log}K_{ow}$ values (Tables 1–3) and data from the literature (Pollien & Roberts, 1999) were correlated with lipophilicity value estimates ($\text{Log}P$) from four computational programs including MOE, Cache, Hyperchem and EPI Suite™. The correlation coefficients (R^2) are shown in Table 4. The highest average R^2 value was 0.86 for the EPI Suite™ $\text{Log}P$ estimation, whereas the lowest R^2 was 0.19 for the Hyperchem program. The EPI Suite™, $\text{Log}P$ values were plotted vs. $\text{Log}K_{ow}$ from all four data sets; three sets of our data and some literature values. The R^2 values ranged from 0.80 to 0.91. The slopes from the two trend lines in Fig. 5 were 1.00 and 0.80 for our results and the literature ones, respectively. This deviation could be explained by the difficulty in obtaining good estimates of $\text{Log}K_{ow}$ values for the more hydrophilic compounds, either experimentally or when modelling in EPI Suite™ when values with $\text{Log}P$ values less than -0.5 acted as high leverage outliers.

The results above show that experimental methods could estimate molecular lipophilicity acceptably and with

a precision that is, at least in some cases, superior to computational approaches. However, the $\text{Log}P$ database, is a simple and effective substitute for experimental approaches which are time consuming and expensive. The choice of method for determining lipophilicity depends ultimately on the accuracy required.

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