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Modelling alterations in the partition coefficient in in vitro biological systems using headspace gas chromatography

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Abstract

Headspace gas chromatography was used to determine the physiological media–air partition coefficient (K) of four volatile organic solvents of industrial importance. The experimental conditions were those likely to be used in in vitro metabolic and toxicological studies on volatile compounds. The addition of solvent to the liquid phase from a stock solution in ethanol, or the presence of organic material at concentrations normally seen in in vitro studies, did not significantly alter the K value. Binary solvent addition resulted in a dose-dependent decrease in K for each solvent that was also influenced by the solvent solubility and the constituents of the liquid matrix. The aromatic solvents exerted the greatest effect and showed the greatest change in K value. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Partition coefficient

1. Introduction

In vitro systems are routinely used to determine the cytostatic and cytotoxic effects of a wide range of chemical compounds. The scarcity of exposure–effect data generated in vitro for volatile compounds such as organic solvents is noticeable. This is probably a reflection of the logistical problems inevitably encountered when studying such compounds [1]. Accurate determination of the concentration of the volatile compound to which the in vitro system is exposed can be problematic. However, it is essential to the production of accurate and reproducible exposure–effect profiles. Construction of defined aqueous solvent solutions has usually been achieved by the shake-flask method. This is subject to significant error especially if the generation of a biological response requires a high concentration of solvent. A prerequisite for any accurate determination of the concentration is an enclosed in vitro test system. Once the volume of each physical phase within the system is known it is possible to determine the concentration of added solvent in any phase by prior determination of K.

In this paper, we report liquid–air K values for four volatile organic solvents of industrial importance – dichloromethane (DCM), dimethyl disulphide (DMDS), toluene and xylene. Three liquid matrices were investigated – distilled deionised water, physiological buffer and McCoys 5A synthetic tissue culture medium [2]. The latter two matrices are representative of solutions routinely used in in vitro studies. For three solvents, the maximum initial volume of solvent used to determine the K value was close to the estimated limit

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of solubility [3]. This confirms the utility of K in in vitro experiments even at these high solvent exposures. The biological material within the liquid matrix can establish a third, primarily lipophillic phase to an otherwise biphasic system and, given the intrinsic lipophilicity of organic solvents, potential alterations in the K value cannot be ruled out. Similarly, the use of a diluent to obtain a homogenous aqueous solution of what are fundamentally hydrophobic compounds may alter the K value. Consequently, K was also established under conditions where cellular material was present and where the test solvent was added to the liquid phase from a stock solution in ethanol. Changes in the K value due to the presence of a second test solvent were also examined. While solvent–solvent interactions in the gas phase are theoretically improbable, potential interactions in the liquid phase cannot be overlooked especially where the presence of salts and dissolved compounds can aggravate the effects of an added second solvent. These effects are potentially significant in the determination of toxic interactions between solvents in vitro.

2. Experimental

2.1. Materials

DCM, DMDS, toluene and xylene were obtained from BDH chemicals (Poole, Dorset, UK). The solvents were of the highest purity (>99%). Glass headspace vials (21.74 ml; RSD=1.50%; N=20) and glass chromatography vials (nominal volume=2.00 ml; 1.88±0.01 ml; RSD=0.74%; N=20) were obtained from Chromacol Ltd. (Herts., UK). Aluminium crimp-caps and teflon-faced butyl rubber septa were obtained from Supelco (Poole, Dorset, UK).

2.2. Solvent addition limits to the headspace vial at 37°C

The maximum amount of solvent that will completely vaporise under these specific experimental conditions was estimated. The vapour pressure of each solvent at 37°C was calculated using the Antoine equation, $\mathrm{Log}_{10} p = A - [B/(T + C)]$, where T was 37°C and A, B and C were compound-specific constants. The moles of solvent that would give this vapour pressure were calculated from the ideal gas equation $PV = nRT$, where V=0.02174 l. Since commercial xylene is a mixture of three isomers, we estimated a maximum addition value from the percentage of each isomer present in solution. The percentages were 20.5% (*o*-xylene), 77.5% (*m*-xylene) and 2.0% (*p*-xylene). The maximum solvent addition to achieve complete vaporisation within a headspace vial at 37°C was calculated to be 61.50 mg (DCM), 5.45 mg (DMDS), 5.37 mg (toluene), 1.76 mg (xylene) and 5.87 mg (ethanol). Using the formula [4]:concentration (ppm) = (concentration (mg/m^3) × 25.464)/molecular weight, the corresponding air concentrations were 848,171 ppm, 67,766 ppm, 68,257 ppm, 19,415 ppm and 149,306 ppm, respectively. (The value 25.464 was calculated from the ideal gas law and is the volume occupied by 1 mole of a gas at 37°C.)

2.3. Matrices investigated in this study

Three matrices were used in these experiments: distilled deionised water, physiological buffer and McCoy's 5A synthetic tissue culture medium [2]. The physiological buffer was based on the recipe for the tissue culture medium and consisted of $CaCl_2 \cdot 2H_2O$ (1.0 mM), KCl (5.4 mM), $MgSO_4 \cdot 7H_2O$ (1.1 mM), NaCl (106.5 mM), $NaH_2PO_4 \cdot 2H_2O$ (6.0 mM), 4-(2-hydroxyethyl)-1-piperazineethane sulphonic acid (HEPES) (25.0 mM), D-glucose (10.0 mM) and KOH (6.0 mM). The final pH of the buffer was 7.4 and the ionic strength was 0.15 M. The matrices were incubated on an orbital shaker (200 rpm) at 37°C for 24 h. A_{600} measurements and the colour of the tissue culture medium confirmed the absence of microbial growth.

2.4. Standard and sample preparation

A series of headspace standards was prepared for each separate solvent by the total vaporisation technique [5]. Standard air solvent concentrations ranged from approximately 1000 ppm for each solvent to approximately 100,000 ppm (DCM), 65,000 ppm (DMDS), 64,000 ppm (toluene) and 19,000 ppm (xylene). For the lower solvent standards, solvent was added from a stock solution in ethanol. These

stocks were constructed gravimetrically using glass chromatography vials. Aliquots of 5 μl of stock were added to an empty headspace vial. Stock solutions were stored at −20°C in complete darkness to avoid the possibility of solvent loss due to evaporation or photodegradation.

Aliquots of 10 ml of the liquid phase matrix were added to each headspace sample vial. In vials containing organic material, an equivalent volume of liquid was removed prior to the addition of the cell aliquot. Solvent was added from a stock solution in ethanol. A sample of 50 μl of the appropriate solvent stock was added to the headspace vial using a gas-tight syringe by the open vial technique [5]. The ratio of the volumes of stock added and liquid phase (1:200) was maintained for all solvent additions. The maximum liquid concentration of solvent reached was approximately 0.14% (v/v). The vial was rapidly sealed. All vials were incubated at 37°C for 12 h on an orbital shaker (100 rpm) in complete darkness and in an inverted position. Preliminary experiments proved that this time is sufficient for equilibration. Where two solvents were included in the same sample, two separate stocks were used. The first stock aliquot was delivered as previously described. The second stock aliquot was delivered through the sealed septum. These vials were incubated in an inverted position and the caps were wrapped in parafilm (American National Can, Greenwich, CT, USA). No disintegration of the parafilm was observed and therefore, it was concluded that no loss of solvent was incurred. The amount of solvent transferred to the gas phase during equilibration does not cause a significant change in the volume of the original liquid phase (<0.04%). The magnitude of the solvent addition is reported in terms of C_0, the initial liquid phase solvent concentration.

2.5. Headspace gas chromatographic analysis

The HP19395A autosampler (Hewlett Packard, Palo Alto, CA, USA) had a sample loop volume of 1 ml. The headspace in the vial was pressurised for 20 s before sample extraction. The time for venting the headspace aliquot to the sample loop was optimised to 6 s. The time allowed the transfer of the headspace aliquot from the sample loop to the gas chromatograph column in 15 s. After each sampling event the loop was flushed with carrier gas to avoid carry-over. Samples of each liquid matrix were analysed for background solvent levels. Headspace aliquots were analysed on a gas chromatograph equipped with a flame ionisation detector. The gas chromatograph was operated in split mode with a split ratio of 64:1. A HP-1 capillary column (30 m×0.53 mm I.D., 0.88 μm film thickness, (Hewlett Packard) was used with nitrogen carrier gas (>99% purity). The injector and detector temperatures were 200 and 250°C, respectively. The multiramp oven temperature program was 40°C for 1 min followed by a ramp to 150°C at 10°C per min followed by an 8-min isothermal period at 150°C. Total run time was 20 min. The retention time for DCM, DMDS, toluene, *o*-, *m*-, and *p*-xylene was 1.75, 3.85, 4.30, 6.15, 6.32 and 6.72 min, respectively.

Table 1
Water–air, buffer–air and medium–air partition coefficient values for dichloromethane, dimethyl disulphide, toluene, *o*-xylene, *m*-xylene and *p*-xylene at 37°C

Solvent	Matrix	K^a
DCM	water	8.77±0.03
	buffer	7.50±0.01
	medium	7.10±0.13
DMDS	water	9.23±0.06
	buffer	9.07±0.11
	medium	9.95±0.19
Toluene	water	2.20±0.05
	buffer	2.14±0.02
	medium	2.52±0.06
o-Xylene	water	2.48±0.03
	buffer	2.28±0.01
	medium	2.61±0.06
m-Xylene	water	1.70±0.04
	buffer	1.52±0.02
	medium	1.97±0.06
p-Xylene	water	1.74±0.03
	buffer	1.73±0.03
	medium	2.18±0.05

[a] Values are $K \pm SD$, $N=8$, $r^2 \geq 0.98$.

2.6. Partition coefficient calculation

Defining K as C_L/C_G, where C_L and C_G are the liquid and gas solvent concentrations, respectively, we can deduce a working formula for its calculation. We also define W as the total amount of solvent added to the system, V_L and V_G are the volumes of the liquid and gas phases, A_S and A_G are the equilibrium gas chromatograph peak areas of the standard and sample, respectively, and f is the gas chromatograph response factor relating the peak area to the gas phase solvent concentration.

According to the mass balance equation,

$$W = C_L V_L + C_G V_G$$

From this

$$C_L = (W - C_G V_G)/V_L$$

But $A_G = fC_G$. Therefore,

$$C_L = (W - (A_G V_G/f))/V_L$$

Therefore,

$$K = ((W - (A_G V_G/f))/V_L)/(A_G/f) = (fW - A_G V_G)/A_G V_L (= C_L/C_G)$$

This is effectively the 'vapour phase calibration' method for K determination [6].

We can rearrange this to a form more amenable to graphical representation:

$$(fW - A_G V_G)/fV_L = K \times (A_G/f)$$

2.7. Data analysis

All data distributions were tested for deviations from Gaussian distribution using the Kolmogorov–Smirnov test. The P value comes from Dallal and Wilkinson's approximation to Lilliefors' method [7]. Regression analysis was used to analyse the relationship between $((fW - A_G V_G)/f\ V_L)$ and (A_G/f). The significance of the regression line was determined at $P \leq 0.05$ and where two slopes (Ks) were compared, the formula for the difference between two regression lines was used at $P \leq 0.05$. The SD of the K estimate is calculated from 0.5 times the 95%

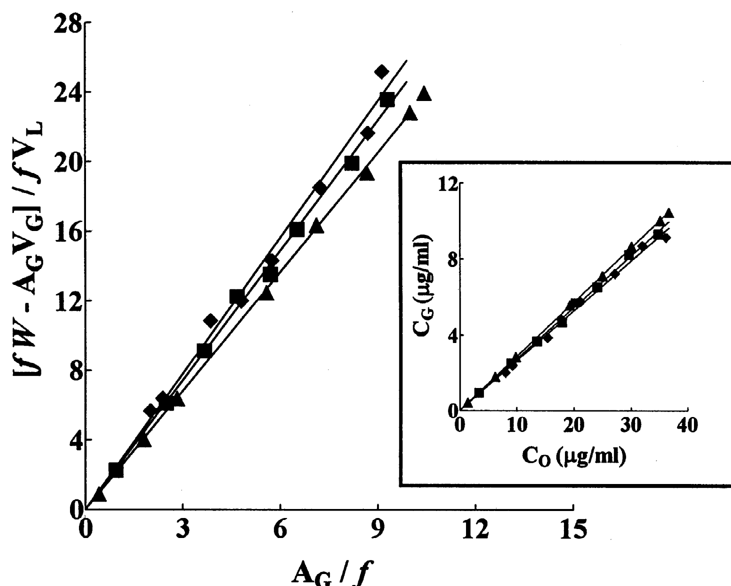

Fig. 1. Determination of the water–air (■), buffer–air (▲) and medium–air (♦) K. This specific example is for o-xylene. Similar traces were obtained for DCM, DMDS, toluene, m-xylene and p-xylene. W, V_L, V_G and A_G are measured directly. Each data point represents a single determination. A minimum of eight data points was used for each regression analysis. The temperature of the system was controlled at 37°C (±0.1°C). r^2 was invariably >0.98. Inset: Headspace linearity for o-xylene at 37°C.

confidence interval of the slope of the regression line.

3. Results and discussion

The liquid–air K value was determined experimentally for DCM, DMDS, toluene and xylene (Table 1). Defining the equilibrium partitioning of a solvent in terms of the quantities $((fW - A_G V_G)/f V_L)$ and (A_G/f) permits the application of linear regression analysis to the actual experimental measurements taken (Fig. 1). A deviation in the linearity of the relationship, which would indicate a decrement in the headspace linearity, can be readily visualised. However, no deviations from linearity were observed. The r^2 values confirmed the goodness of fit of the linear regression model (invariably >0.98). Henry's Law is obeyed over this range and the maximum headspace solvent concentration possible under these experimental conditions was not exceeded. The addition of solvent directly to the liquid phase not only reduces sample loss but allows us to achieve C_0 as well. This is the pre-equilibrium liquid phase solvent concentration obtained at the moment of addition and is significant in in vitro studies where it may be the actual solvent exposure that elicits a physiological response.

K is numerically equivalent to the slope of the regression line fitted to the plotted data values. The SD of the slope can be taken as the SD of the K estimate. The magnitude of the SDs reported here is very much lower than that associated with previous determinations and probably reflects the variability intrinsic to the manual techniques employed in other studies. Automation of the analytical component of headspace analysis clearly enhances the reproducibility of the K estimation.

Comparison of K values with those previously reported in the literature is limited to the water–air values since distribution coefficients have not been deduced between air and the physiological buffer and medium investigated here. The water–air value for DCM compares favourably with a previous estimate of 8.71±0.50 (mean±SD) [8]. The water–air toluene K value is within the range of 2.5±0.5 and 2.23±0.16 (mean±SD) reported by Lindqvist [9] and Sato and Nakajima [10], respectively. The values for *o*-, *m*- and *p*-xylene are within the ranges of 2.63±0.32, 1.66±0.24 and 1.57±0.36 (mean±SD) reported by Sato and Nakajima [11]. Certain buffer–air values compare favourably with documented K values reported for liquids of similar ionic strength and pH. The buffer–air DCM K value (7.50) is within the mean±SD range of 5.96±2.75 reported by Gargas et al. [12] and 7.2±0.6 reported by Sato and Nakajima [13] for 0.9% saline–air. The buffer–air values of *m*- and *p*-xylene are within the ranges of 1.92±0.46 and 1.77±0.27 (mean±SD) reported by Gargas et al. [12]. However the toluene buffer–air K value is outside the 0.9% saline–air value of 1.75±0.13 (mean±SD) documented in the same report. No K values have been documented for DMDS.

As a general rule, salt concentrations up to 5% (w/v) do not produce a significant salting-out effect

Table 2
Buffer–air and medium–air partition coefficient values for dichloromethane, dimethyl disulphide, toluene, *o*-xylene, *m*-xylene and *p*-xylene at 37°C[a]

Solvent	Partition coefficient			
	Pure[b]	EtOH[c]	Pure/cells[d]	EtOH/cells[e]
Buffer				
DCM	7.74±0.11	7.58±0.06	7.64±0.10	7.70±0.09
DMDS	8.95±0.15	9.21±0.08	9.14±0.12	9.01±0.14
Toluene	2.17±0.07	2.11±0.07	2.10±0.02	2.10±0.02
o-Xylene	2.27±0.03	2.28±0.02	2.25±0.02	2.25±0.03
m-Xylene	1.53±0.03	1.54±0.02	1.51±0.02	1.51±0.03
p-Xylene	1.74±0.02	1.75±0.03	1.70±0.03	1.72±0.03
Medium				
DCM	6.67±0.26	7.10±0.23	6.49±0.23	6.84±0.09
DMDS	9.83±0.17	9.95±0.15	9.63±0.27	9.62±0.19
Toluene	2.67±0.06	2.52±0.06	2.53±0.06	2.51±0.07
o-Xylene	2.81±0.05	2.61±0.06	2.82±0.04	2.64±0.06
m-Xylene	2.01±0.08	1.97±0.06	2.13±0.12	2.09±0.07
p-Xylene	2.23±0.08	2.18±0.05	2.33±0.05	2.09±0.07

[a] Values are the K±SD, $n \geq 8$, $r^2 \geq 0.98$.

[b] Pure solvent added to the liquid matrix.

[c] Solvent added from a stock solution in ethanol to the liquid matrix.

[d] Pure solvent added to the liquid matrix containing an organic phase.

[e] Solvent added from a stock solution in ethanol to the liquid matrix containing an organic phase. The second mode of addition (solvent added from a stock solution in ethanol) is that used in the previous K determinations. No significant difference was recorded between the values obtained here and those presented in Table 1.

[5]. The salt or, more appropriately, the 'ingredient' concentration of the physiological buffer and culture medium used in these studies was 1.7 and 1.5%, respectively. However, in all cases, the buffer–air *K* value was lower than that of the corresponding water–air value suggesting a salting-out effect. The difference was statistically significant in the case of DCM, *o*-xylene and *m*-xylene. The difference between the medium–air and water–air *K* values was statistically significant and, with the exception of DCM, so too was the difference between the buffer–air and medium–air values. Only for this solvent was the medium–air value the lowest in magnitude, suggesting the possibility of a chemical reaction between solvent and one or more medium components for DMDS, toluene and the three xylene isomers. This has potential pharmacokinetic consequences since the effective solvent concentration to which an in vitro biological model is exposed may be altered by such interactions. Assuming that a *K* value calculated for a liquid with a given ionic composition will suffice for a liquid of similar ionic composition is therefore a flawed approach to take as the chemical nature of the components themselves also influences the final distribution of solvent. The hypothetical reaction observed here must be an equilibrium process since the magnitude of the medium–air *K* value is not a function of the total amount of solvent added to the headspace vial. No precipitates were observed in the medium after incubation with solvent.

The smaller the dimensions of the in vitro test system, the lower the amount of solvent required to give a specific initial concentration. Conventional equipment may not permit the accurate measurement and delivery of small volumes of pure solvent. In such circumstances a diluent, such as ethanol, is required for the addition of solvent. The stock solution in the diluent can be easily constructed and the delivery of a pre-determined volume of this solution can be accurately made. The effect of co-addition of diluent on the *K* value was investigated (Table 2). Ethanol did not significantly alter *K*. This solvent is completely miscible with aqueous solutions and probably does not contribute significantly to any 'salting-out' effect at this final concentration (0.5%, v/v). Methanol, which is also completely miscible in water, is used in the construction of solvent stock solutions and was previously shown to have no effect on Henry's Law constants (and

Table 3
The binary partition coefficient values, expressed as a percentage of the individual partition coefficient values, for the maximum inital liquid phase solvent concentrations[a]

Second solvent	Percentage of individual solvent *K* values[b]					
Water						
DCM	–	99.14	89.17	99.94	91.49	101.77
DMDS	97.92	–	91.90	100.19	95.02	102.41
Toluene	95.07	96.15	–	96.96	87.32	101.95
Xylene	94.20	97.61	88.58	–	–	–
Buffer						
DCM	–	93.89	79.12	96.33	76.55	100.15
DMDS	92.35	–	91.90	100.15	87.18	99.68
Toluene	85.55	91.71	–	93.38	64.20	95.84
Xylene	75.31	91.28	74.37	–	–	–
Medium						
DCM	–	98.29	90.14	95.12	88.20	102.18
DMDS	93.18	–	92.45	97.04	92.34	100.46
Toluene	93.11	91.18	–	95.42	76.69	103.82
Xylene	87.74	89.84	85.40	–	–	–

[a] The C_0 values were 8.26 m*M* DCM, 5.85 m*M* DMDS, 5.36 m*M* toluene and 1.64 m*M* xylene (0.33 m*M* *o*-xylene, 1.28 m*M* *m*-xylene and 0.03 m*M* *p*-xylene).
[b] The percentage *K* values were extrapolated from the 3D plots for these solvent additions.

therefore on K) [14]. The buffer–air and medium–air K value for each solvent was determined with cellular material added to a final concentration of 250 μg cellular protein per ml. This corresponds to the concentration of biological material typically used in in vitro studies. The lipid content of this material provides a hydrophobic environment into which an apolar solvent can freely diffuse. An alteration in the K value is possible if the dimensions of the material are sufficient to contribute a third phase to the system. However, no effects on the K value were evident proving that, at these levels, the organic phase is an irrelevant factor in solvent distribution.

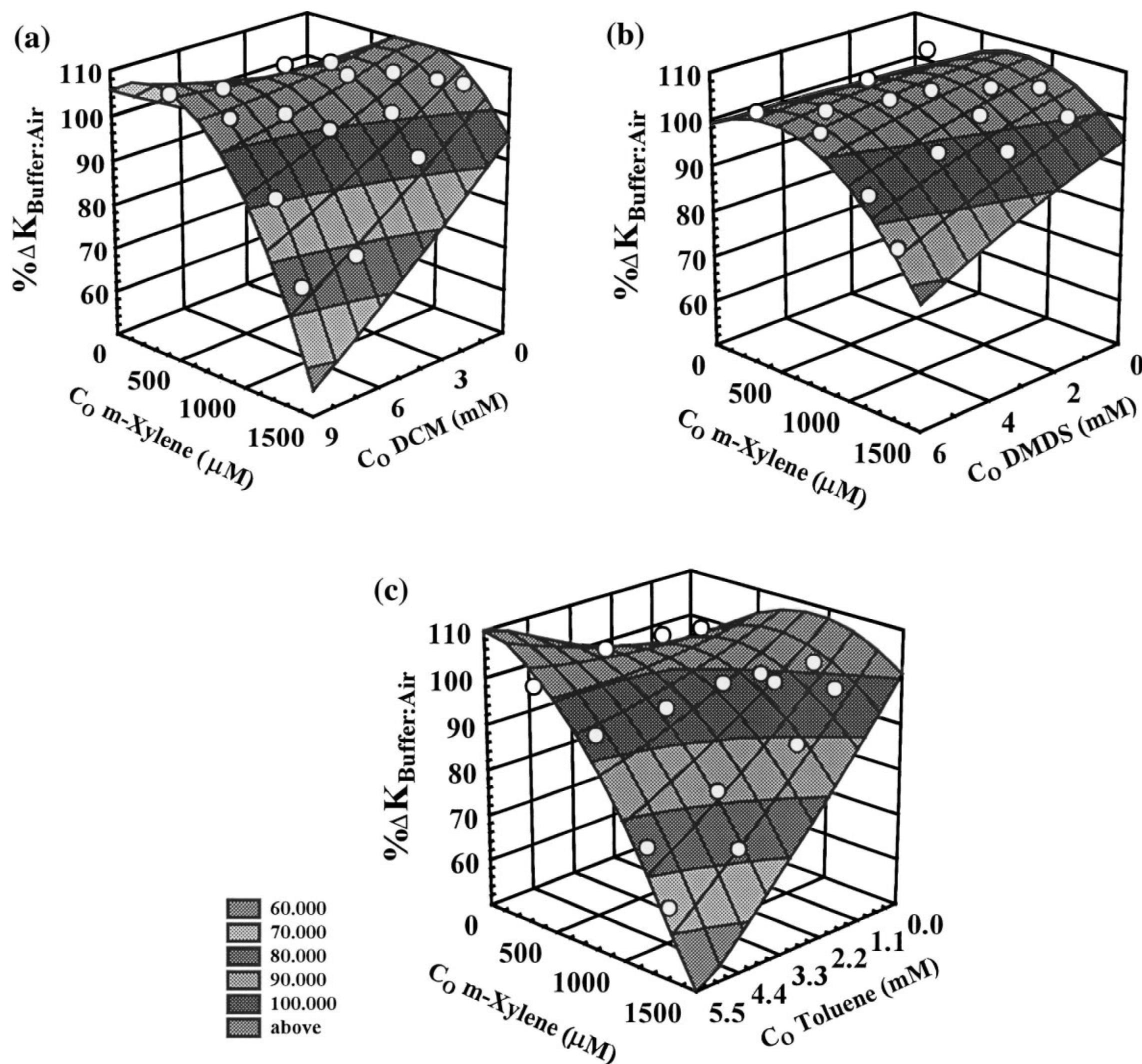

Fig. 2. Examples of the 3D surface plots obtained for each solvent combination showing the change in buffer–air K value observed for a given solvent (in this case m-xylene), in the presence of (a) DCM, (b) DMDS and (c) toluene. The individual data values are shown to which a quadratic surface plot was fitted to emphasise the trend in the data. Solvent exposures are given in terms of the initial liquid phase solvent concentration (C_0). A decrease in K was observed for each matrix investigated and for each solvent combination which became more marked at the higher additions of solvent. The aromatic solvents, toluene and xylene, had the greatest effect on the other solvents' K values as can be seen here in the specific case of m-xylene.

Dalton's Law of partial pressures holds for the air composition over a complex solution. The individual partial pressures are a consequence of the partitioning behaviour of each component at any temperature. Most significantly, the partial pressure of one component will not influence the partial pressure of another. The behaviour of the components in the liquid phase is more complicated. The four solvents exhibit varying degrees of solubility in water [3]. At 25°C DCM has a solubility of approximately 20 mg/ml, while that of toluene is 0.67 mg/ml. The solubility of the three isomers of xylene ranges from 0.146 to 0.175 mg/ml. No solubility data is available for DMDS. Since the dissolution of gases in water is an exothermic process, increasing the temperature to 37°C will reduce these solubility values. Therefore, in the case of DMDS, toluene and xylene, the higher solvent addition levels may approximate to the solubility limits under those conditions.

Because of the matrical nature of binary solvent studies we limited our investigation to four solvent concentrations. This gave sixteen combinations for

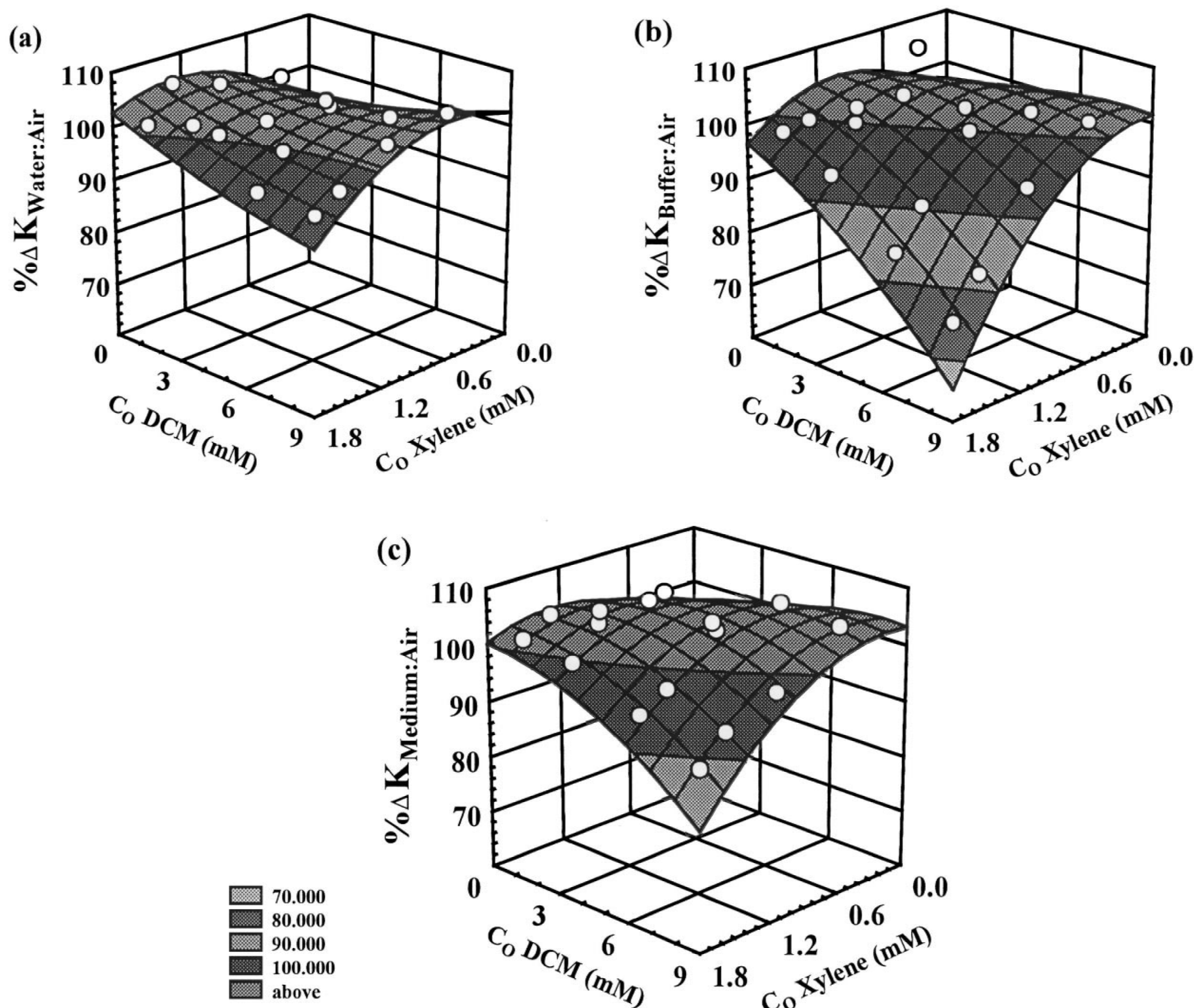

Fig. 3. Examples of the influence of the matrix on the magnitude of the alteration in K due to the addition of a second solvent. The specific example shown is the alteration in K for DCM in the presence of xylene (*o*-, *m*- and *p*-xylene combined). The greatest alteration was observed in buffer and the lowest in water. This was invariably the case for the other solvent combinations as well. The individual data values are shown to which a quadratic surface plot was fitted to emphasise the trend in the data. Solvent exposures are given in terms of the initial liquid phase solvent concentration (C_0).

each of six binary solvent additions in each of the three liquid matrices tested. When linear regression analysis was performed on all sixteen possible combinations, it was evident that the inclusion of two hydrophobic solvents does not significantly alter their respective K values, even at the higher additions of solvent (Table 3). However, two distinct influences on the magnitude of the K value can be discerned from the 3D surface plots constructed for each solvent combination (Figs. 2 and 3). The greatest change in the K value occurs at the higher additions of solvent (Table 3). This trend appears to be dependent on the solvent species. Toluene and xylene, which have relatively low solubility, elicit the greatest change in the K value of the other solvent when they are added to the system at high concentrations (Fig. 2). The liquid matrix also influences the perturbation observed in the magnitude of K, the effect being most significant on the buffer–air and medium–air K values (Fig. 3). The fugacity of the solvent is already enhanced in these matrices due to the 'salting-out' effect of the constituents. Conceivably, the presence of a second hydrophobic solvent acts in a manner similar to added salt, thereby enhancing the fugacity of the other solvent. The complete dissolution of a hydrophobic solvent requires a large water shell around each solvent molecule. The presence of a second solvent will undoubtedly sequester water molecules for dissolution and aggravate the effects of dissolved electrolyte on solvent solubility. In general, it was observed that the lower the solubility of the solvent, the greater the change in the K value on addition of a second solvent (Table 4). The partitioning of the solvent in the sample vials ensured that the vapour phase saturation was never reached for any solvent addition.

While the overall K value did not change significantly in all cases, a distinct trend in the K values was observed. This confirms that hydrophobic solvents can influence the partitioning of each other and the greater the addition of solvent the more pronounced this influence will be. The chemistry of the liquid phase is also an important determinant of this partitioning and potential interactions between solvent and liquid components cannot be overlooked. It is conceivable that a significant alteration in K could be registered for other solvent combinations, especially those involving sparingly soluble solvents in liquids with a high electrolyte content. Prudence requires the verification of any alteration in K as to assume otherwise could lead to erroneous conclusions concerning solvent exposure magnitudes with repercussions for in vitro dose-response curves. However, once these precautions are taken, the resulting K estimates allow a quantifiable biological

Table 4
Water–air, buffer–air and medium–air partition coefficient values for dichloromethane, dimethyl disulphide, toluene, *o*-xylene, *m*-xylene and *p*-xylene at 37°C in the presence of a second solvent

Second solvent	Binary solvent K^{a}					
Water						
DCM	–	9.31±0.05	2.13±0.03	2.51±0.02	1.69±0.02	1.78±0.02
DMDS	8.68±0.06	–	2.16±0.02	2.54±0.02	1.72±0.02	1.80±0.01
Toluene	8.70±0.06	9.30±0.07	–	2.50±0.02	1.65±0.03	1.83±0.01
Xylene	8.73±0.10	9.36±0.08	2.11±0.04	–	–	–
Buffer						
DCM	–	9.01±0.09	2.02±0.05	2.28±0.02	1.42±0.05	1.74±0.02
DMDS	7.37±0.09	–	2.15±0.03	2.33±0.02	1.48±0.03	1.77±0.01
Toluene	7.19±0.12	9.04±0.12	–	2.28±0.03	1.30±0.06	1.71±0.02
Xylene	6.70±0.21	8.85±0.13	1.94±0.07	–	–	–
Medium						
DCM	–	9.81±0.03	2.44±0.02	2.61±0.03	1.99±0.04	2.26±0.02
DMDS	7.02±0.07	–	2.45±0.03	2.64±0.02	1.99±0.03	2.24±0.02
Toluene	6.80±0.06	9.76±0.11	–	2.61±0.03	1.89±0.05	2.24±0.03
Xylene	6.89±0.11	9.62±0.13	2.40±0.04	–	–	–

[a] Values are K±SD, N=16.

effect to be directly related to an air solvent concentration, thereby allowing a more concise correlation analysis with in vivo data than that achievable with liquid solvent concentrations alone. This is important for the initial stages of the risk assessment of solvents and solvent mixtures.

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