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RESEARCH ARTICLE

# A Novel Method for the Investigation of Liquid/Liquid Distribution Coefficients and Interface Permeabilities Applied to the Water-Octanol-Drug System

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## ABSTRACT

***Purpose*** In this work a new, accurate and convenient technique for the measurement of distribution coefficients and membrane permeabilities based on nuclear magnetic resonance (NMR) is described.

***Methods*** This method is a novel implementation of localized NMR spectroscopy and enables the simultaneous analysis of the drug content in the octanol and in the water phase without separation. For validation of the method, the distribution coefficients at pH = 7.4 of four active pharmaceutical ingredients (APIs), namely ibuprofen, ketoprofen, nadolol, and paracetamol (acetaminophen), were determined using a classical approach. These results were compared to the NMR experiments which are described in this work.

***Results*** For all substances, the respective distribution coefficients found with the two techniques coincided very well. Furthermore, the NMR experiments make it possible to follow the distribution of the drug between the phases as a function of position and time.

***Conclusion*** Our results show that the technique, which is available on any modern NMR spectrometer, is well suited to the measurement of distribution coefficients. The experiments present also new insight into the dynamics of the water-octanol interface itself and permit measurement of the interface permeability.

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## INTRODUCTION

The distribution properties of substances between immiscible liquid phases have considerable impact on processes both in nature and in industry, spanning from global phenomena related to the enforcement of nutrients and accumulation/washing out of environmental toxins to numerous manufacturing technologies based on distribution phenomena such as supercritical gas extraction and two-phase partitioning bioreactors, just to name prominent examples. Any chromatographic method for purification or quantification is based on similar distribution phenomena.

In particular, the lipophilicity of a compound is commonly quantified by its distribution coefficient ($D_{ow}$), the ratio of the concentrations in an organic ($c_o$) and a water ($c_w$) phase ($D_{ow}=(c_o/c_w)$) (1). Such distribution processes are of special significance for drug-like molecules in the body: while hydrophilic molecules—with a negative value of $\log(D_{ow})$—preferentially distribute to hydrophilic compartments (*e.g.* blood serum and urine), hydrophobic molecules preferentially accumulate in fat tissues and cell membranes. When distribution coefficients of drug molecules were for the first time correlated with their pharmacological effects more than 100 years ago (2), biomembranes were not yet described. Until today, partition coefficients of drugs and drug-like molecules have been heavily used in medicinal chemistry and drug design to estimate possible oral uptake of drug molecules, permeability through biological membranes, and receptor binding. In order to standardize distribution experiments with respect to oral uptake, octanol is the most commonly used organic phase, and the drug molecules are

regarded in their respective unionized state (as many drugs are weak acids or weak bases). In this case, the logarithm of the partition is reported ($\log(P_{ow}) = \log(c_o/c_w)$) (1). Many have attempted to correlate this value to the biopharmaceutical properties of drug candidates (*e.g.* Lipinski rule of five for possibility of oral uptake (3)).

However, despite the significance of distribution coefficients and the long period they have been intensively discussed, they are notoriously difficult to measure in a reliable manner; for example, reported values of the partition coefficient of ibuprofen at room temperature range from 1.07 to 4.50 at the same pH value (4). Apart from the direct methods of distribution between phases, numerous techniques to estimate distribution and partition coefficients in a more efficient way have been developed to match the high throughput of synthesis of drug candidates. These methods avoid the time-consuming equilibration between the phases by correlating retention properties of solutes, *e.g.* in reverse-phase chromatography. These methods may in general provide a reasonable estimate by a correlation to classical logP; however, for structurally diverse compounds, significant outliers may frequently be observed (for a review see ref (5)). Considerable effort has also been devoted to pure modelling and calculation procedures in order to estimate distribution coefficients *in silico* (6).

The classical experimental method is still considered the most reliable; it consists of three stages.

1. The first stage is to obtain the equilibrium of distribution between the octanol and water phases. Most approaches are based on the shake-flask method, which is regarded as "the golden standard" (7). There is no common understanding for the time needed to reach equilibrium, not even for the same surface area and volume relation; the literature for experiments on 96-well plates reports any time between 10 min and 18 h (8,9).
2. The second stage concerns sampling for quantification. The samples should ideally be taken from each of the phases; however, analysis of one of the phases and calculating the other as the difference of the total amount of drug used is also very common.
3. The third stage consists of measuring concentrations in the samples, where detection by UV–vis absorption is most commonly used, as well as *e.g.* MS, pH titration, and recently $^1$H NMR (10)

The second step, sampling, is most critical, as it may disturb the water-octanol interface and hence hamper accurate concentration measurements. In this work, we present a novel approach using localized $^1$H NMR spectroscopy for measuring the concentration of the API in both the water and octanol phase simultaneously *in situ*, without disturbing the interface. We present data on four commonly known pharmaceutical compounds as examples: ibuprofen, ketoprofen, paracetamol (acetaminophen) and nadolol. Furthermore, the spatial and temporal resolution of this method is discussed with respect to the kinetic parameters of transport processes through the interface, which may directly relate to permeability.

It should be mentioned that the presented experiments may be performed on any modern liquids NMR spectrometer, which is equipped with pulsed field gradients in at least one direction.

## MATERIALS AND METHODS

### Materials

Ibuprofen (Caesar&Lorenz GmbH, Hilden, Germany), ketoprofen (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), paracetamol and nadolol (Sigma-Aldrich) were used, and their molecular structures are reported in Fig. 1. For the preparation of the buffer solutions, sodium di-hydrogen phosphate ($NaH_2PO_4$) (Riedel de Haen AG, Seelze, Germany), di-sodium hydrogen phosphate ($Na_2HPO_4$) (Sigma-Aldrich) and sodium chloride (NaCl) (Sigma-Aldrich) were dissolved in micro filtrated water. For the distribution coefficient determination, 2-(±)-octanol (Sigma-Aldrich) was used. Results were compared to 1-octanol (Fluka Chemie AG, Buchs, Switzerland); no significant differences were observed (data not shown).

### Determination of the Partition Coefficient at pH 7.4 (logD(7.4)) Using Classical Approach

Solutions for each of the respective compounds were prepared dissolving approximatively 100 mg of the substance in 50 ml of isotonic and isohydric (pH = 7.4) phosphate buffer (73.4 mM). The solutions were filtered through a 0.2 μm filter, and the initial concentration of the drug was quantified by UV spectroscopy using a Genesis 10 UV Scanning (Thermo Electron Corporation, Cambridge, UK) using a calibration curve. Twenty ml of each solution were placed in a 100 ml separation flask, and similar 20 ml of phosphate buffer was used as blank. Twenty ml of octanol were added to each sample. Two phases solutions were agitated for 72 h at room temperature (23–24°C) at 130 rpm using a shaking bath model SW 23 (Julabo Labortechnik GmbH, Seelbach, Germany). For each compound, four samples (4 ml each fraction) of the water phase were analyzed using UV spectroscopy. In a similar way, the concentrations of the drugs in each sample of the octanol phases (4 ml each)

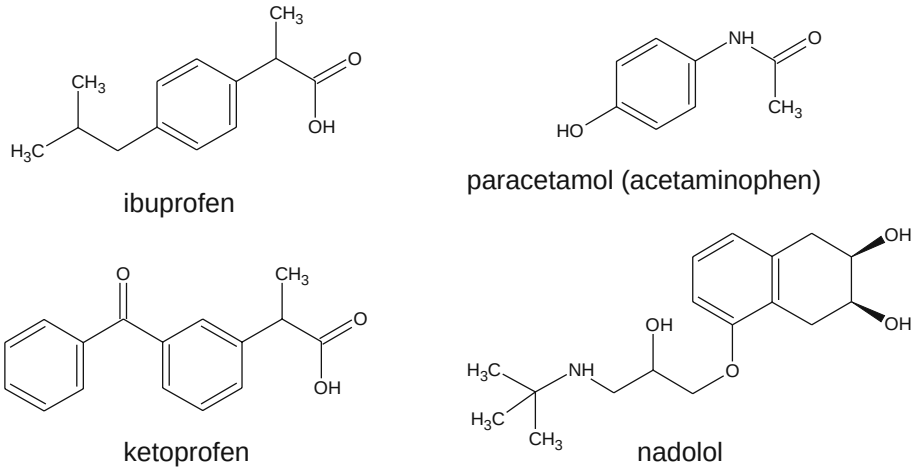


**Fig. I** Molecular structures of the investigated compounds.

were analyzed. For the calculation of the partition coefficient at pH = 7.4 the following equation was used:

$$\log(D_{ow}(pH)) = \log\left(\frac{c_o^i}{c_w^i}\right)$$

where $c_w^i$ is the sum of the ionized and unionized forms of compound i in the buffered water phase and $c_o^i$ its concentration in the octanol phase. We compare two approaches in Table II. For the first approach we measured *both* concentrations with UV–vis absorption. These values are given as $UV_m$ in the table. An estimation of $\log(D_{ow})$ (given as $UV_e$ in Table II) was obtained by assuming that all amounts not measured in the water phase after 72 h had migrated to the octanol phase.

## NMR

The NMR experiments were carried out on a Varian INOVA 500 spectrometer operating at a proton frequency of 499.845 MHz and equipped with Performa II gradient amplifiers. Temperature was stabilized at 25°C during all experiments. The water signal was suppressed using presaturation. The experiments were performed without deuterium lock, as no $^2H$ was present in the utilized solvents. A standard sample containing phosphate buffer and 10% $D_2O$ was used for shimming and referencing purposes. No further shimming and referencing was performed, the samples being too heterogeneous.

Slice selection (11) was achieved by combining a field gradient of 14.96 G/cm (149.6 mT/m) with a frequency selective $\pi/2$ pulse (iburp1) of 4.0175 ms, the offset of the transmitter frequency determining the distance of the centre of the slice with respect to the centre of the magnetic field. The resulting slice thickness was 176 µm.

## RESULTS

### Classical Approach

For each substance, a calibration curve for the UV–vis analysis was prepared in phosphate buffer. For all drugs, linearity in the concentration range from 1 µg/ml to 300 µg/ml was observed; the extinction coefficients are reported in Table I together with the respective correlation coefficients $R^2$. For measuring the concentration in the octanol phase another calibration curve was determined in octanol for each compound. After 72 h the concentration of the respective drug in the water phase as well as in the octanol phase was measured and logD(7.4) calculated. Results are reported in Table II. As expected, and considering the concentrations of the respective drug detected after reaching the equilibrium in both phases, ibuprofen and paracetamol were the substances of highest lipophilicity ($\log D_{ow}(7.4)$ of 1 and 0.75, respectively) followed by ketoprofen (0.19) and nadolol (−0.6). All $\log D_{ow}(7.4)$ values are in close accordance with literature (9). For each drug the distribution coefficient was approximately the same if it was estimated just from the aqueous phase by comparing concentrations in the buffer initially and after 72 h ($\log D_e(7.4)$). Ibuprofen and ketoprofen showed the highest deviation between the two approaches (0.13 and 0.20 respectively), probably due to accumulation of small amounts of the respective drug at the interface between the two phases. It is also possible to see that the buffer capacity of the phosphate buffer was not sufficient for the stock solutions depending on the acidity/basicity of the drugs. However, after migration of a considerable amount of drug into the octanol phase, the pH re-adjusted to 7.4. In the case of ketoprofen, the pH in the aqueous phase was found to be 7.25 at equilibrium due to higher solubility of this substance.

The reproducibility of the concentration measurements was very good. The standard deviation in the log $D_{ow}$

**Table I** Common Names, IUPAC Names, Molecular Weight, and UV–vis Spectroscopic Parameters in Water of the Investigated Compounds

| Compound | Systematic (IUPAC) name | Molecular weight [g/mol] | $\lambda_{MAX}$ [nm] | Extinction coefficient [ml/(mg*cm)] | $R^2$ |
|---|---|---|---|---|---|
| Ibuprofen | (RS)-2-(4-(2-methylpropyl)phenyl)-propanoic acid | 206.29 | 265 | 1.74 | 0.9998 |
| Ketoprofen | (RS)-2-(3-benzoylphenyl)-propanoic acid | 254.30 | 260 | 64.83 | 1.0000 |
| Paracetamol (Acetaminophen) | N-(4-hydroxyphenyl)-acetamide | 151.17 | 244 | 65.56 | 0.9999 |
| Nadolol | (2R,3S)-5-{[(2R)-3-(tert-butylamino)-2-hydroxypropyl]oxy}-1,2,3,4-tetrahydronaphthalene-2,3-diol | 309.40 | 271 | 3.62 | 1.0000 |

ranged from 0.002 to 0.02. The measurements are thus very precise but not necessarily very accurate, as witnessed by the wide spread in literature values (4).

## NMR

Measurements were done on the same samples as described above, and distribution coefficients were calculated by comparing the integral of the aromatic protons of the compound of interest in the water and the octanol phases (Table II).

We followed the paracetamol (acetaminophen) signal intensity as a function of time and position in the sample (see Fig. 2); time resolution was approximately 2 min per spectrum, while the slice thickness was 176 μm. The results show that equilibrium is only obtained after several days and that there is a position situated ca. 1 mm below the lowest point of the water/octanol meniscus where the paracetamol signal intensity appears to be zero. The values of the distribution coefficient did not change significantly over the next six days, although the appearance of the water resonance did. After several days, two distinct OH resonances can be observed, whereas the OH line in the octanol phase is too broad to be detected during the first few days. Subsequently, we have measured the paracetamol and octanol signal intensities more precisely as a function of the position in the sample (see Fig. 3). The spectra were acquired with 64 scans per spectrum (ca. 8 min.) acquired using interleaved acquisition with 4 scans per block.

Spectra of the aromatic region of paracetamol were recorded in a separate series of experiments (Fig. 4), which is why the position of the interface with respect to the center of the magnetic field is different from the previous experiments. The spectra were acquired with 64 scans per spectrum (ca. 8 min.) acquired using interleaved acquisition with four scans per block.

# DISCUSSION

## Validation of the NMR Method

The distribution coefficients as measured by NMR and the classical method agree very well. One of the advantages of the NMR method is that the distribution coefficient is obtained directly from the ratio of the signal intensities *in situ* without disturbing the sample. It is not necessary to know the concentrations, but concentrations can be estimated assuming that the excess volume of mixing is negligible (12), and using the values given in reference (13), the concentration of octanol in octanol ($c_o^o$) was obtained *via*

$$c_o^o = 1000^* \left( \frac{1 - x_o^w}{x_o^w \left(\frac{M^w}{\rho^w}\right) + (1 - x_o^w)\left(\frac{M^o}{\rho^o}\right)} \right)$$

**Table II** Distribution Coefficients ($D_{ow}$), Concentrations in Octanol ($c_o$) and the pH in the Water Phase for the Investigated Compounds

| Compound | pH water phase | | log($D_{ow}$) | | | $c_o$ [mmol/l] | |
|---|---|---|---|---|---|---|---|
| | Initial | Final | $UV_m$ | $UV_e$ | NMR | $UV_m$ | NMR |
| Ibuprofen | 7.00 | 7.40 | 1.00 | 1.13 | 1.07 ± 0.10 | 6.4 | 9.75 |
| Ketoprofen | 7.13 | 7.25 | 0.19 | −0.01 | −0.10 ± 0.10 | 4.7 | 4.1 |
| Paracetamol | 7.36 | 7.40 | 0.75 | 0.64 | 0.27 ± 0.10 | 12.7 | 7.9 |
| Nadolol | 7.61 | 7.40 | −0.60 | −0.60 | −0.72 ± 0.10 | 1.2 | 1.1 |
| Octanol | | | | | 3.21 | | 5966[a] |
| $H_2O$ | | | | | −1.45 | | 1940[a] |

[a] calculated from the known concentrations, see text. $UV_m$: Measured by UV–vis Absporbtion in both Phases, $UV_e$: Concentration in Octanol Estimated Form Known Starting Amount and Concentration in Water, NMR: Measured by Localized $^1$H NMR Spectroscopy

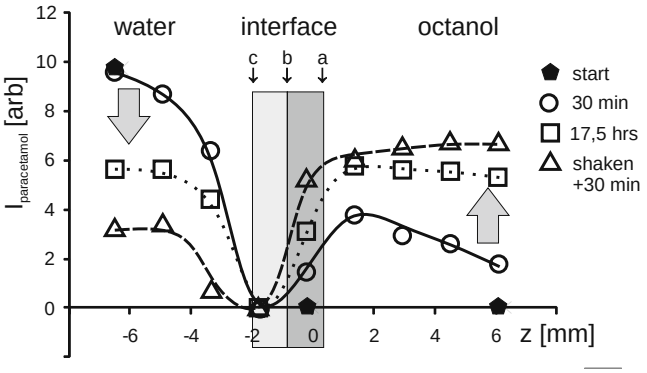


**Fig. 2** Paracetamol concentration as a function of time and the position in the sample. Data recorded at 1 h, 2 h, 4 h and 6 days are not shown for clarity (see Supplementary Material). Times indicate the moment where half of the experiment was finished (1 min 57 s per spectrum); *a* top of the meniscus, *b* bottom of the meniscus and *c* the position where the octanol concentrations reaches its bulk value in the water phase.

where $x_o^w(0.22)$ is the mole fraction of water in octanol, $M^w$ (18.02 g/mol) and $M^o$ (130.23 g/mol) the molar masses of water and octanol, and $\rho^w$ (0.9969 g/ml) and $\rho^o$ (0.8223 g/ml) their densities at 25°C. The other concentrations were subsequently found by comparing the integral of the signal of a known number of protons with the integral of the octanol $CH_3$ group. The resulting concentrations are reasonably close to those obtained by the classical method (Table II).

The resonances of the OH in water and octanol are only observable after several days. Assuming the mole fraction of octanol in water is small ($x_w^o \approx 10^{-4}$ (14), $c_w^o = c_o^o/1622$, our data), we can obtain the concentration of water in octanol from its concentration in water, the ratio of the integrals of the OH protons and the integral of the CH in 2-octanol (the integrals measured with $^1$H NMR without using presaturation). The result (1.92 mol/l) is in close agreement with the literature value (2.22 mol/l (13)).

## The Water-Octanol Interface

The octanol and paracetamol signal intensities decrease in the same manner over the water-octanol interface. It should be noted that the position, where the octanol signal intensity attains its equilibrium value (indicated with c in

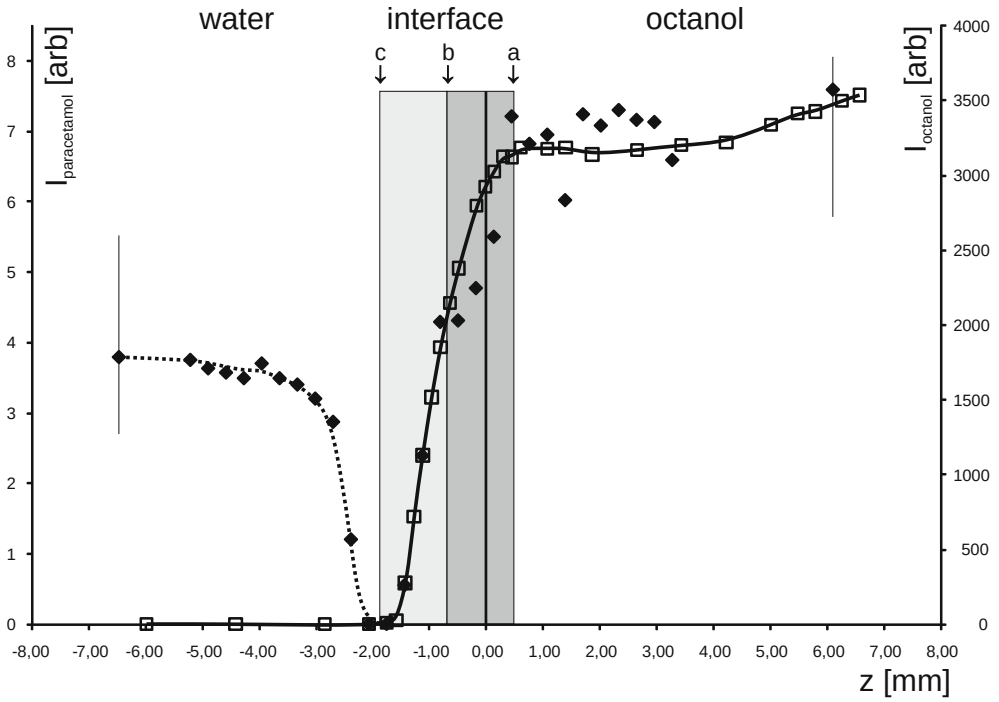


**Fig. 3** Paracetamol concentration (♦ *left hand scale*) and octanol concentration (□ *right hand scale*) as a function of the position in the sample after 6 days. $D_{ow}$ was obtained by dividing the paracetamol intensities at ca. −6.5 mm and +6.0 mm. *a* top of the meniscus, *b* bottom of the meniscus and *c* the position where the octanol concentrations reaches its bulk value in the water phase.

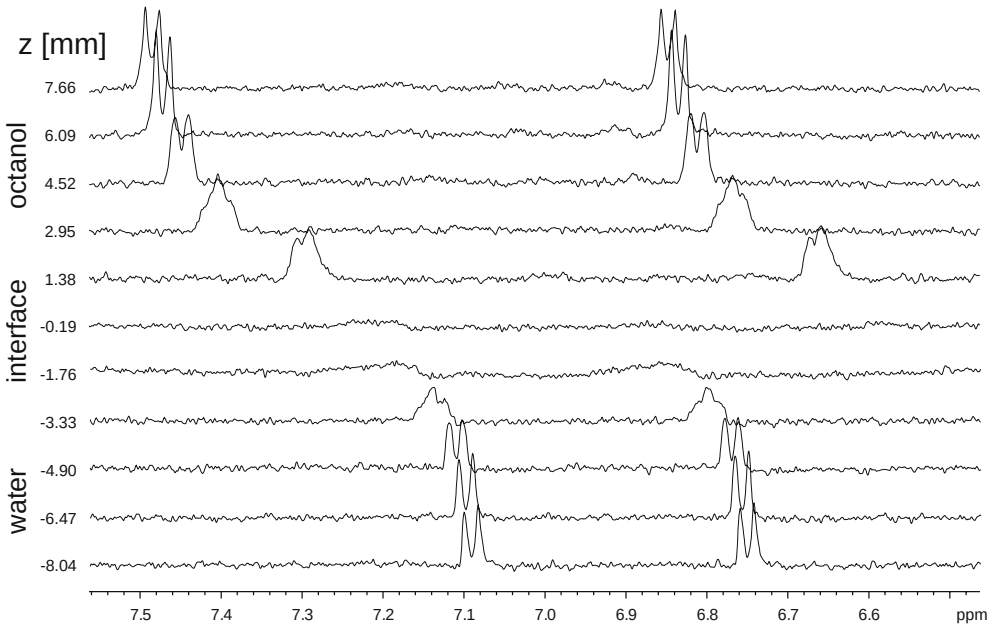


**Fig. 4** $^1$H NMR spectra of the aromatic protons of paracetamol as a function of the position in the sample. The bottom of water-octanol meniscus is situated at ca. 0 mm, its top at ca 1.5 mm; the position where the octanol concentrations reaches its bulk value in the water phase is at ca. −1.25 mm.

Figs. 2 and 3), is situated well below the lowest point of the meniscus (indicated with b) and coincides with the position where the paracetamol signal intensity disappears.

It is *a priori* not clear whether the NMR signal disappears because the paracetamol concentration is locally too low or because the NMR resonances are too broad to be detected. Spectra of the aromatic region of paracetamol are given in Fig. 4. The aromatic part of the paracetamol spectrum shows a characteristic AA'BB' pattern, which looks like two doublets. The lines broaden close to the interface. The same is observed for the OH resonance. The line width of the water in the water phase is about 3 Hz; it increases to 170 Hz just below the bottom of the meniscus and drops to about 10 Hz in octanol, where—only after several days—two OH resonances are observed.

The data are most easily explained by assuming that the paracetamol resonances become too broad to be detected due to dynamic exchange between different environments. Both fluctuations in the anisotropic magnetic susceptibility and in pH around octanol droplets are expected to have the strongest influence on the resonances of the aromatic protons. It is interesting to note that the layer where this occurs is situated *below* the bottom of the meniscus at the top of the water phase and that this interface layer has a macroscopic thickness in the mm range. In fact, this layer can easily be visualized using pH indicator paper. Although the paracetamol spectra show dynamic effects in a layer of ca. ±3 mm centered at the bottom of the meniscus, the most dramatic broadening coincides with the penetration of octanol into the water face, below the bottom of the meniscus.

## Time Dependence and Spatial Resolution

Knowing the concentrations as a function of time and position makes it possible to determine the diffusion coefficients in both the octanol and water phases and the permeability of the water-octanol interface from the diffusion equation:

$$\frac{\partial}{\partial t}c(z,t) = D\frac{\partial^2}{\partial z^2}c(z,t)$$

From Fig. 3, it is clear that the diffusion of paracetamol within each phase is fast with respect to the permeability of the interface. There is a small, but significant, dependence of the paracetamol concentration on the distance to the interface, but the quality of the data is not good enough to determine the diffusion constant of paracetamol in octanol. Indeed, we expect that inhomogeneities on mm length scales are averaged out on hour time scales for typical diffusion constants for small molecules ($10^{-6}$–$10^{-5}$ cm$^2$/s). Care should also be taken that other contributions, such as convection, to the transport processes can either be neglected or quantified. It is possible, though, to estimate the permeability of the interface.

Assuming that the permeability timescale is long with respect to the diffusion timescale, and assuming that the concentration is a delta function at $t=0$: $c(x,t=0)=C\,\delta(x)$, the diffusion equation can easily be solved, yielding

$$c(z=d,t) = A_1\frac{e^{-\frac{A_2}{t}}}{\sqrt{t}} + A_3$$

where $A_1$, $A_2 = d^2/D$ and $A_3$ are fitting parameters, $A_3$ corresponds to the equilibrium concentration, and D/d is the permeability of the interface and $d \approx 0.1$–$0.3$ cm its width. Fitting the expression to the average of four points in the octanol phase gives $D/d \approx 2$–$6\ 10^{-5}$ cm/s, which is in amazingly good agreement with the literature value for the intestinal wall permeability of paracetamol as measured in rats ($8.6 \pm 5\ 10^{-5}$ cm/s (15)).

### Perspectives

Localized NMR spectroscopy has considerable advantages with respect to other methods to determine distribution coefficients; it avoids the need to separate the phases and makes it possible to follow the concentrations in both space and time simultaneously. It is comparable to other methods in terms of measured values, but it may have a disadvantage in terms of equilibration times.

Due to the small surface of the water-octanol interface in an NMR tube, it takes a relatively long time to reach equilibrium. This turns into an advantage, though, as it permits following the transport processes with time. The difficulty of placing a sample in the NMR apparatus with a precision in the order of 100 μm in a reproducible manner is a challenge. The range of attainable partition coefficients ($-3 \lesssim \log(D_{ow}) \lesssim +3$) is comparable to other methods (5,8), but might be extended using other nuclei than $^1$H or modern solvent suppression techniques. The very big advantage of the NMR method lies in the possibility of following processes over time without disturbing the sample. Another advantage is that the method should be insensitive to eventual not dissolved material, sedimentation or accumulation at the interface as undissolved material yields NMR resonances too broad to be observed in solution NMR, and we determine concentrations at positions well within the bulk phases. We estimate that the NMR method can determine distribution coefficients with an accuracy of ca 5–10% if the sample has time to equilibrate.

Studying nuclei other then $^1$H, it should be possible to measure the diffusion coefficients of the API directly in both the water and the octanol phases, using standard NMR experiments with only slight modifications. Further work is necessary to separate eventual other contributions to the transport processes, such as convection, from the permeability and diffusion coefficients.

## CONCLUSION

We have determined octanol-water distribution coefficients of some active pharmaceutical ingredients using localized $^1$H NMR spectroscopy. The results compare well to literature values and to results obtained using a classical shake flask approach employing UV–vis spectroscopy. The timescale of the distribution process makes it possible to determine concentrations as a function of time and position, and to measure the permeability of the interface.

Our results indicate that the water-octanol interface has a macroscopic thickness in the mm range and is situated in the water phase below the octanol-water meniscus. The spectra of paracetamol indicate that this layer is characterized by dynamic exchange between water and octanol environments.

The investigation of liquid/liquid distribution coefficients and interface permeabilities is a novel application of localized NMR spectroscopy, and our results show the technique is well suited to these studies.

## REFERENCES


1. Leo A, Hansch C, Elkins D. Partition coefficients and their uses. Chem Rev. 1971;71:525–616.
2. Meyer H. Zur Theorie der Alkoholnarkose. Arch Exp Path Pharmak. 1899;42:109–18.
3. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 2001;46:3–26.
4. Sangster J. LOGKOW, a databank of evaluated octanol-water partition coefficients. http://logkow.cisti.nrc.ca/logkow/.
5. Poole SK, Poole CF. Separation methods for estimating octanol-water partition coefficients. J Chromatogr B. 2003;797:3–19.
6. Cheng T, Zhao Y, Li X, Lin F, Xu Y, Zhang X, *et al.* Computation of octanol-water partition coefficients by guiding an additive model with knowledge. J Chem Inf Model. 2007;47:2140–8.
7. Berthod A, Carda-Broch S. Determination of liquid-liquid partition coefficients by separation methods. J Chromatogr A. 2004;1037:3–14.
8. Hitzel L, Watt AP, Locker KL. An increased throughput method for the determination of partition coefficients. Pharm Res. 2000;17(11):1389–95.
9. Chiang PC, Hu Y. Simultaneous determination of LogD, LogP, and pK(a) of drugs by using a reverse phase HPLC coupled with a 96-well plate auto injector. Comb Chem High Throughput Screen. 2009;12:250–7.
10. Mo HP, Balko KM, Colby DA. A practical deuterium-free NMR method for the rapid determination of 1-octanol/water partition coefficients of pharmaceutical agents. Bioorg Med Chem Lett. 2010;20:6712–5.
11. Bernstein MA, King KE, Zhou XJ. Handbook of MRI pulse sequences. Elsevier Academic Press; 2004. p. 266–71.
12. Liltorp K, Westh P, Koga Y. Thermodynamic properties of water in the water poor region of binary water +alcohol mixtures. Can J Chem. 2005;83:420–9.
13. Sangster J. Octanol-water partition coefficients, fundamentals and physical chemistry. Wiley-Blackwell; 1997.
14. Marcus Y. Structural aspects of water in 1-octanol. J Solution Chem. 1990;19(5):507–17.
15. Stewart BH, Chan OH, Lu RH, Reyner EL, Schmid HL, Hamilton HW, *et al.* Comparison of intestinal permeabilities determined in multiple *in-vitro* and *in-situ* models—relationship to absorption in humans. Pharm Res. 1995;12(5):693–9.