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Partition coefficients of drugs in bilayer lipid membranes

J. J. Ramsden

Department of Biophysical Chemistry, Biozentrum, CH-4056 Basel (Switzerland)
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Abstract. The oil/water partition coefficient of drugs is widely accepted as a key parameter in drug design. The coefficients are usually determined using a bulk octanol phase to represent the lipid. The physiologically and pharmacologically relevant structure is, of course, the bilayer lipid membrane, but until now there has been no convenient means of measuring the partition coefficients of small molecules into a single bilayer. This paper demonstrates that the partition coefficient may be calculated from the change in membrane refractive index which occurs when a drug molecule partitions into the membrane. The refractive index is determined by an integrated-optics technique ideally suited to an ultra-thin structure such as a lipid bilayer.
Key words. Aspirin; caffeine; bilayer lipid membrane; drug partitioning; integrated optics.

It is widely accepted that the oil/water partition coefficient is a key parameter in drug design. This stems from the fact that the absorption, distribution, and metabolism of a drug involve its passage across phospholipid membranes of the cell. The effectiveness of a drug depends on its ability to cross these membranes, quite apart from its subsequent physiological action.

For the experimental determination of partition coefficients, the oleaginous phase corresponding to the lipid is represented by octanol. Since a bulk octanol phase is only a very rudimentary approximation to a bilayer lipid membrane, the ability to determine the partition coefficients of drugs into an actual membrane would be highly desirable.

This paper demonstrates that lipid membrane partition coefficients are accessible via the change in the refractive index of the membrane which occurs when a drug partitions into it. The refractive index of a thin film such as a lipid bilayer may very easily be measured by depositing it onto a planar optical waveguide, and measuring the phase velocities of guided waves in the waveguide. These velocities are influenced by the environment of the waveguide proper because the evanescent part of the guided waves penetrates beyond the confines of the waveguide into the surrounding medium, and die away exponentially[1].

Materials and methods

Figure 1 shows a planar optical waveguide upon which a bilayer lipid membrane has been deposited using the Langmuir–Blodgett technique (see below). The waveguide forms one wall of a small flow-through cuvette sealed to the membrane using an 'o'-ring. Guided waves propagate in the waveguiding layer F by total internal reflexion at the interfaces between F and the substrate S, and between F and B (buffer solution), where the overlayer M (membrane) is interposed. The evanescent part of the wave extends beyond the confines of F, however, and dies away exponentially in the media S and B. The interaction of the evanescent wave with these external media influences the phase velocities of the guided waves[1]. The effective refractive index N (defined as the ratio of the phase velocities of light in vacuum and in the waveguide) of the waveguide is therefore a function of the optical properties (refractive indices n and geometrical thicknesses d) of both the waveguiding film F and the layers S, M and B. If these properties change (e.g. the refractive index of the membrane M by the partitioning of a drug molecule into it), then the phase velocities of the guided modes and hence the effective refractive indices of the waveguide will also change.

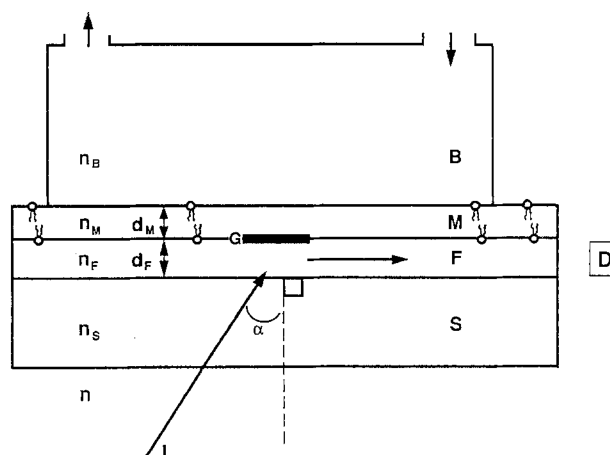


Figure 1. Schematic drawing of the optical waveguide. The various layers are, S, glass substrate; F, waveguiding film; M, bilayer lipid membrane; B, aqueous buffer. n and d are the refractive indices and thicknesses respectively of the various layers identified by the subscript; n is the refractive index of air. L is the incoming light, impinging with angle α on the grating G. D represents a photodiode detector. The cuvette is 1 cm in diameter and 0.89 mm high (volume = 70 μl).

Table 1. Parameters used in the solution of the mode equation

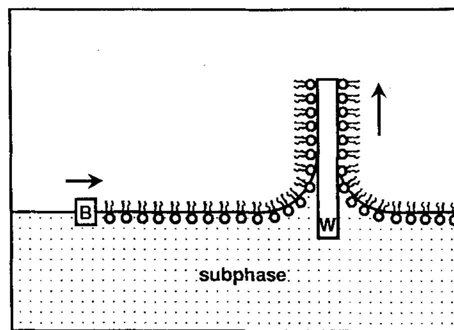
Parameter	Value
n_S	1.525781[e]
n_B	1.331603[b]
n_F	1.811358[a] 1.811241[c]
d_F/nm	177.18[a] 177.34[c]
d_M/nm	5.14[d]
measurement temperature	24.0 ± 0.4 °C

[a]For the aspirin measurements; [b]measured using a Rayleigh interferometer (type LI3, Carl Zeiss, Jena, Germany); [c]for the caffeine measurements; [d]from reference 3; [e]provided by the glass manufacturer.

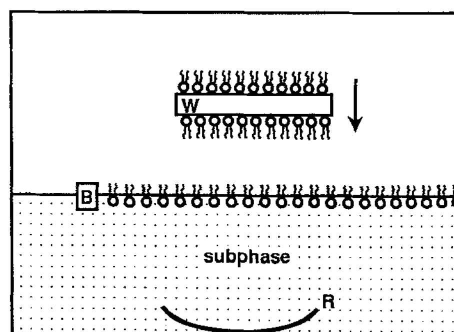
The effective index can be conveniently measured if a diffraction grating (fig. 1, G) is incorporated into the surface of the waveguide. When incident light falls onto the grating with a certain angle α, the light enters the waveguide and travels along to its end, where its intensity is measured by a detector (fig. 1, D). If this intensity is recorded while the angle α is varied, a very sharp maximum is observed, from which the effective refractive index, N, can be determined using a simple formula[2]. By solving the mode equations for the guided waves, the membrane refractive indices can be determined, provided the other unknowns (refractive indices of the substrate, buffer and F, and the thickness of the membrane) can be measured independently.

Planar optical waveguides made of $Si_xTi_{1-x}O_2$, $x \approx 0.3$, and incorporating a grating (2400 lines/mm) in the upper surface, were obtained from ASI AG, Zürich, Switzerland (type 2400), and were pretreated in buffer-solution for 24 h. They were then mounted in an integrated optics scanner (type IOS-1, ASI AG, Zürich, Switzerland) in order to measure N for the zeroth order transverse electric (TE_0) and transverse magnetic (TM_0) modes in the absence of the membrane. From the data d_F and n_F, the thickness and refractive index of the layer F could be calculated by solving the 3-layer mode equations[2] (see table 1).

The waveguide was then placed in a laboratory-built Langmuir trough filled with the same buffer. A solution of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) in hexane:ethanol 9:1 was spread on the surface. After the solvent had evaporated, the lipid was compressed with a pressure of 32 mN/m by slowly moving the barrier (fig. 2). The waveguide was then withdrawn at a speed of 0.04 mm/s up through the compressed monolayer, which is thereby transferred to the waveguide surface[4]. A feedback circuit connecting the transducer measuring the surface pressure and the motor driving the moving barrier ensured that the surface pressure was kept constant. Surface pressure and barrier position were plotted on an x, y pen recorder during monolayer transfer, enabling the amount of lipid taken up by the waveguide to be read off directly. The amount always corresponded to twice the area of one face of the waveguide. For the second monolayer, the waveguide was rotated at right angles and brought rapidly down onto and through the floating compressed monolayer[5]. The waveguide was then remounted in the integrated optics scanner and N remeasured. After filling the cuvette with a drug solution, equilibrium was rapidly established (in less than 30 s). Typically, about a dozen readings of N for the TE_0 mode were made and averaged. Measurements were carried out with acetylsalicylic acid (aspirin) and 1,3,7-trimethylxanthine (caffeine). Both these molecules interacted reversibly with the membrane, i.e. when the cuvette was refilled with buffer solution alone, the signal rapidly returned to the baseline.



A



B

Figure 2. *A* Langmuir–Blodgett[4] and *B* Langmuir–Schaefer[5] techniques for depositing lipid monolayers onto planar substrates. W, waveguide; B, moving barrier; R, receptacle for coated waveguide.

The buffer used throughout was a 10 mM solution of 2-(N-morpholino)ethane-sulphonic acid-NaOH (MES) adjusted to pH 6.0, purchased from Calbiochem (Ultrol grade). Aspirin and caffeine were from Fluka (purum grade, >99% pure), and synthetic POPC was from Avanti Polar Lipids, and all were used as received. Water (conductivity 18 Ω cm) was supplied from a Barnstead Nanopure installation.

The mode equations can be derived from the Fresnel equations[2]. For light rays parallel to the optic axis of

the lipid film (normal to the waveguide surface), i.e. the TE mode (s-polarized), the membrane behaves as an isotropic medium of refractive index n_M. The index corresponding to the p-polarized rays varies with the angle of refraction[4]. Therefore we shall confine our attention to the index n_M. The relevant mode equation is:

$$n_M^2 = \frac{(g(S) + g(B) - kd_F\sqrt{n_F^2 - N^2})(n_F^2 - n_B^2)}{kd_M\sqrt{n_F^2 - N^2}} + n_B^2, \quad (1)$$

where

$$g(Y) = \arctan\left(\frac{\sqrt{N^2 - n_Y^2}}{\sqrt{n_F^2 - N^2}}\right). \quad (2)$$

The thickness of the POPC bilayer, d_M, was taken from literature values established by X-ray diffraction[3].

Results and discussion

A plot of n_M vs bulk aqueous concentration c is shown in figure 3 for the two substances investigated, caffeine and aspirin. The next stage is to calculate the mole fraction of incorporated drug from the membrane refractive index data. This may be done with the help of the Lorentz–Lorenz formula which connects the refractive index of a medium with its molecular parameters, i.e. partial molar volume $\bar{v}$ and molar refractivity R_M:

$$n^2 = \frac{1 + 2R_M/\bar{v}}{1 - R_M/\bar{v}}. \quad (3)$$

Since, for a mixture of substances[6],

$$R_M = \sum x_i R_{M,i}, \quad (4)$$

where $R_{M,i}$ is the molar refractivity of the ith component present with mole fraction x_i, and

$$\bar{v} = \sum x_i \bar{v}_i, \quad (5)$$

for a two-component system equation (3) becomes

$$n^2 = \frac{x(\bar{v}_2 - \bar{v}_1 + 2(R_{M,2} - R_{M,1})) + \bar{v}_1 + 2R_{M,1}}{x(\bar{v}_2 - \bar{v}_1 + R_{M,1} - R_{M,2}) + \bar{v}_1 - R_{M,1}}, \quad (6)$$

where x is the mole fraction of component 2, identified as the solute (drug), and component 1 is the lipid. The molar refractivity of a molecule is, to a very good approximation, simply the sum of its bond refractivities[7]. Therefore, provided the structural formula is known, R_M can be calculated. An analogous summation may be carried out for molecular volume (as the sum of atomic volumes)[8], albeit with reduced accuracy. The calculated values are given in table 2. Equation (6) evaluated with the parameters given in table 2 is plotted as n_M vs x in figure 4. Using this curve, the mole fraction x partitioned into the membrane can be obtained from the membrane refractive index data in figure 3. x is plotted as a function of the mole fraction

Table 2. Molecular parameters (R_M, molar refractivity, and $\bar{v}$, partial molar volume) of components of the system. Five water molecules per POPC molecule have been included to allow for lipid hydration.

Substance	R_M/cm³ mol⁻¹	$\bar{v}$/cm³ mol⁻¹
POPC	236.5	1060
aspirin	43.3	136
caffeine	48.2	121

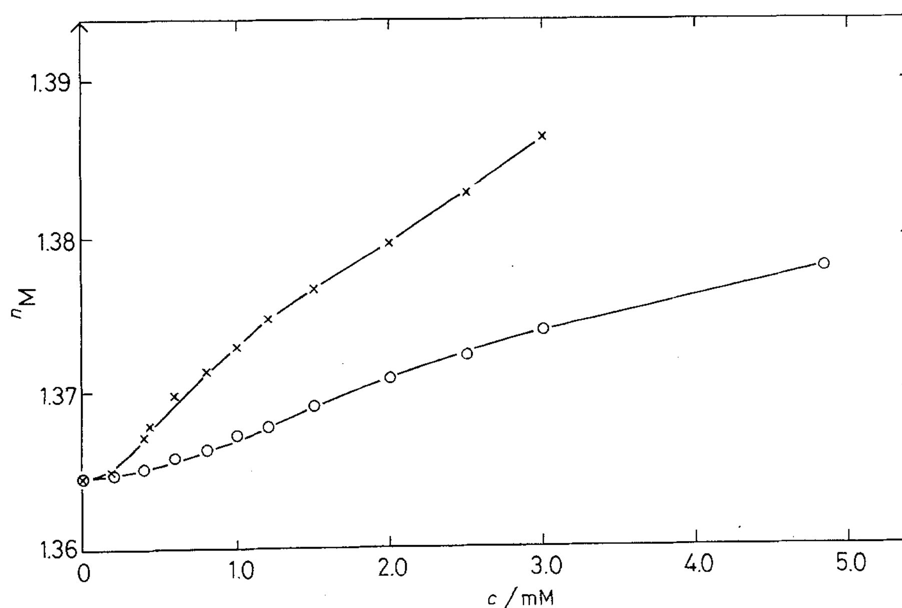


Figure 3. Plots of n_M vs c for aspirin (×) and caffeine (○). The measured N_{TE} data was converted to n_M using equation (1) and the parameters given in table 1.

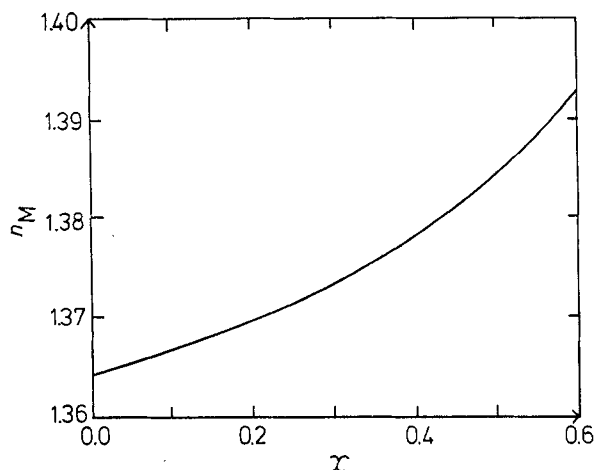


Figure 4. Plots of n_M vs x calculated according to equation (6) using values for $\bar{v}$ and R_M given in table 2. The curves for the two sets of parameters, corresponding to aspirin and caffeine, are indistinguishable at the resolution of the graph.

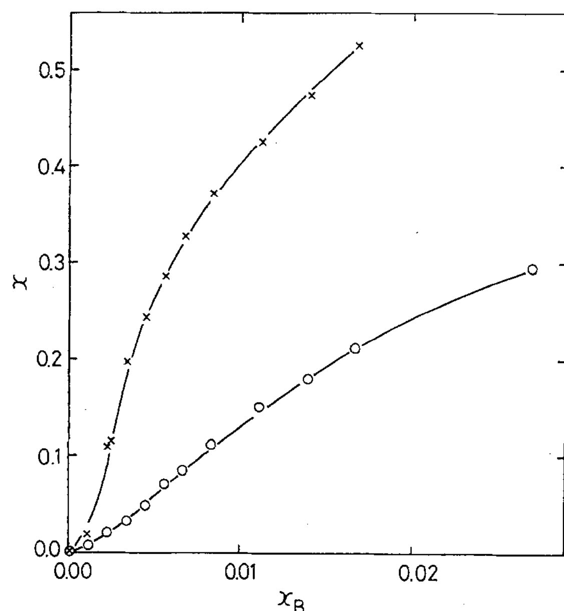


Figure 5. Plots of x vs x_B for aspirin (×) and caffeine (○). The data in figure 3 were converted using equation 6) (evaluated using fig. 4) and equation (7), and the parameters given in table 2.

of solute in the aqueous phase, x_B, in figure 5. The solutions are dilute enough for the equation

$$x_B = (c/mM)/18 \qquad 7)$$

to hold.

The final step is to evaluate the partition coefficients K for the two drugs. According to Nernst's distribution law, in the case of ideal partitioning, the following relation should hold:

$$K = x/x_B \qquad 8)$$

and K may be evaluated by measuring the slope of the x vs x_B plot. If a straight line is drawn through the points plotted in figure 5, values of 13 and 35 are obtained for caffeine and aspirin respectively. The latter value compares with a figure of 16 from the literature (n-octanol/aqueous at pH 7.4)[9]. It will be immediately noticed that the partition isotherm is not ideal, however, but sigmoidal. In the case of aspirin, with a pK_a of 3.5, the molecule will be ionized at neutral pH and hence negatively charged. One would therefore expect binding to be anticooperative since the membrane, initially uncharged, charges up as the solute partitions into it, repelling subsequent arrivals. Caffeine, on the other hand, is uncharged; the partition isotherm is reminiscent of that of the neutral oligopeptide alamethicin, the partitioning of which also demonstrates sigmoidal behaviour[10,11], which was interpreted as being due to aggregation of the solute in the membrane. In the case of alamethicine, independent evidence (ion channel formation) supports the idea of the formation of aggregates.

The application of equation (1) to determine n_M from the measured N values assumes that the membrane thickness is invariant. This is almost certainly the case for small x, but the presence of solute mole fractions exceeding 0.3 (the mass fraction is then about 7%) means that the possibility of some perturbation to the lipid structure, which could result in a change of the membrane thickness, should be considered. The application of equation (6) to determine x from n_M likewise implies that R_M and $\bar{v}$ remain constant. Since the molar refractivity is a function of chemical structure, it would only change in the event of marked changes in the bonding pattern, such as new covalent bond formation. This does not appear to be the case for the partitioning of these drugs into the lipid bilayer, as shown for example by the complete and rapid reversibility of partitioning. On the other hand some variation in partial molar volumes cannot be excluded; it is known that some molecules, e.g. hexane[12], have a partial molar volume of approximately zero when partitioned into phospholipid bilayers.

Conclusions

By depositing a bilayer lipid membrane on a planar optical waveguide and measuring its refractive index as it is brought into contact with aqueous drug solutions, the partition coefficients of the drugs into the lipid membrane can be determined. In contrast to the bulk octanol phase usually employed for such measurements, the lipid membrane is the physiologically and pharmacologically relevant medium. Comparison of the membrane partition coefficient with existing literature data on the octanol/water partition coefficient shows reasonable agreement. Whether the octanol/water partition coefficient is in general a good guide to the membrane partition coefficient will not be known until more extensive determinations of membrane partition coefficients have been made.

The combination of Langmuir–Blodgett technology for depositing the membrane on a planar support, and integrated optics for measuring the membrane refractive index (the support is a planar waveguide), allows the measurements to be made rapidly and accurately.
The technique can in principle be applied to any drug molecule, and by extension to other types of membrane, such as natural cell membranes, which can readily be deposited on the waveguide.

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