A low-cost method for measuring the permeability of a pharmaceutical drug in a diffusion cell

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Abstract: A low-cost, *in vitro* laboratory experiment has been developed that mimics the absorption of a pharmaceutical drug in the body. It allows undergraduate chemistry students to gain experience in the measurement of drug permeability coefficients, a key indicator used by the pharmaceutical industry to identify the ease of absorption of any new drug candidate. The experiment requires a diffusion cell, coated membrane, peristaltic pump and UV-Vis spectrophotometer. The method has been validated by determining the permeability coefficients for a selection of acidic, basic and non-ionizable drugs. Using this assay, it is possible to classify drugs as exhibiting high or low permeability in a fast, facile and reliable manner.

Keywords

Pharmaceutical Chemistry; Pharmaceutical Drugs; Pharmaceutical Analysis; Permeability; Upper-Division Undergraduate; UV-Vis Spectroscopy

Introduction

The discovery and development of a modern pharmaceutical drug, from original design through clinical trials to marketing, is both lengthy and costly. Many, initially promising, drug candidates fail because of poor absorption in the body. To be absorbed easily, a drug needs to dissolve under physiological conditions, as well as be able to permeate through a non-polar membrane barrier upon passing from the intestine into the blood stream. Due to the importance of this factor, the pharmaceutical industry nowadays routinely conducts a series of physicochemical assays (*e.g.* determination of pK_a, lipophilicity, solubility and permeability) during the initial stages of the drug development process so that any potential problems with absorption may be identified early [1].

One of the most promising *in vitro* permeation assays reported to date is the parallel artificial membrane permeability assay (PAMPA) proposed by Kansy *et al*. in 1998. It probes the ability of a drug to permeate through a lipid-coated artificial membrane (around 0.2 mm thick) from a donor to an acceptor compartment [2]. PAMPA is an alternative to the permeability screens based on Caco-2 colon cancer cell lines, which have the disadvantage of being both costly and time-consuming [3,4]. Being designed for high-throughput screening, PAMPA has the potential to examine several drugs simultaneously and at varying pH in a single experiment through the use of a 96-well microtitre plate. Putting these advantages aside, the original PAMPA procedure suffers from a significant limitation due to the presence of an unstirred water layer surrounding the membrane; this can lower a drug's permeability by several orders of magnitude and is responsible for long assay times. However, it has recently been reported that efficient magnetic stirring during the permeability measurement can substantially reduce the unstirred water layer, from 2 – 4 mm (unstirred) to less than 0.1 mm when stirred at high speed [5,6,7]. Stirring was also found to widen the gap between

high-permeability drugs, which can have a permeability coefficient of up to 10^{-2} cm s^{-1}, and low-permeability drugs (where the permeability coefficient is $\leq 10^{-6}$ cm s^{-1}) as well as dramatically reduce the time needed to perform a permeability experiment, from normally 18 hours to just 1 – 2 hours for highly permeable drugs. PAMPA has further gained in importance since FDA and its European counterparts have started to consider a biowaiver and fast-track the approval of certain drugs that have been found in *in vitro* tests to be clearly both highly soluble and highly permeable (Class I drugs according to the Biopharmaceutics Classification System) [8].

Despite the success of PAMPA in the pharmaceutical industry, permeability measurements are rarely performed outside big companies and specialist research labs. There can be little doubt that students specialising in pharmaceutical or medicinal chemistry need to be provided with more hands-on experience in determining important physicochemical properties. Recent examples in educational journals included experiments for determining ionization constants [9], octanol–water partition coefficients [10] and performing dissolution tests [11,12] on common pharmaceutical drugs. Permeability assays, however, have received little attention in undergraduate teaching labs. In this paper, we describe a simple procedure for measuring permeability coefficients of pharmaceutical drugs with the help of a diffusion cell, a peristaltic pump and a UV-Vis spectrophotometer. Such equipment is available in most teaching labs or is affordable on a limited budget. The experiment mimics the way drugs are absorbed in the body, which allows students to learn and understand what happens following oral administration of a medicine. Finally, the method has been validated by determining the permeability coefficients for a selection of acidic, basic and neutral drugs and comparing the results afforded with those published in the literature.

Experimental Section

Apparatus for Measuring Drug Permeability. All permeability experiments were carried out in a vertical diffusion cell. The 100 mL diffusion cell was made in-house from glass and standard flat flanges with a 35 mm bore. A typical set-up is shown in Figure 1. The donor compartment of the diffusion cell was filled with a 0.15 – 0.5 mM drug stock solution in a suitable buffer. The acceptor compartment contained a pH 7.4 phosphate buffer adjusted with KCl to an ionic strength of 0.15 M (this solution mimicked blood in both pH and ionic strength). The two compartments were separated by a mixed cellulose ester membrane (Millipore MF, 0.025 μm pore size, 70% porosity, 105 μm thickness, 47 mm diameter) which had been coated with a 2% solution of phosphatidyl choline in hexadecane [5]; the lipid-coated membrane served as a model for the lining of the stomach or intestine. The side arm of the diffusion cell allowed a drug stock solution to be added, the pH to be adjusted or samples to be taken from the donor compartment.

Permeability Measurement Procedure. The donor compartment of the diffusion cell was filled with drug stock solution (63 mL) through the side arm. The diffusion cell was immersed in a water bath thermostated at 37 ± 1 °C. The pH of the drug stock solution was adjusted by dispensing 0.5 M aqueous NaOH or 0.5 M aqueous HCl. The experiment started upon addition of pH 7.4 buffer solution (20 mL) to the acceptor compartment of the diffusion cell. To minimize the size of the unstirred water layer, the donor solution was magnetically stirred at 620 rpm (which is the maximum setting for this equipment) throughout the duration of the experiment. A peristaltic pump circulated the acceptor solution, at a flow rate of 75 mL min^{-1}, to a UV flow cell and back. Typically 10 UV spectra (210 – 400 nm) of the acceptor solution were recorded every 3 minutes over a period of 30 minutes. Both the donor and acceptor solutions were replaced before the start of a new experiment.

Analysis of Permeability Data. The change in absorbance (or concentration) in the acceptor compartment, $C_A(t)$, with time t was fitted to a generic exponential curve

$$C_A(t) = C_{A,max} \times \left(1 - e^{-kt}\right) + C_A(0) \quad (1)$$

using a non-linear least squares procedure [13] and three fitting parameters, $C_{A,max}$, $C_A(0)$ and k. $C_{A,max}$ represents the extrapolated maximum concentration (absorbance) of the drug in the acceptor compartment, $C_A(0)$ the initial drug concentration (absorbance) at the start of the experiment, and k is formally a permeation rate constant. At time t = 0, the slope of the generic exponential curve is readily obtained by differentiation of Equation 1 and equals $k \times C_{A,max}$. The apparent permeability coefficient, P_{app}, of the drug is directly proportional to this "initial slope" and calculated according to Equation 2

$$P_{app} = \frac{kC_{A,max}V_A}{AC_D(0)} \quad (2)$$

where A is the effective area of the coated membrane (6.7 cm^2) and V_A the volume of the acceptor compartment (20 mL). The initial drug concentration (absorbance) in the donor solution, $C_D(0)$, was determined by recording a reference UV spectrum of the drug stock solution.

In practice, the order of magnitude of the permeability coefficient is generally more important and, therefore, log P_{app} was considered instead (Equation 2a).

$$\log P_{app} = \log\left(\frac{kC_{A,max}V_A}{AC_D(0)}\right) \quad (2a)$$

Log permeability *vs.* pH plots were fitted by a non-linear least squares procedure to Equation 3 [5,14]

$$\log P_{app} = \log P_{max} - \log\left[10^{\pm\left(pK_a^{flux} - pH\right)} + 1\right] \quad (3)$$

where pK_a^{flux} is the aqueous ionization constant under permeability or flux conditions and log P_{max} is the logarithm of the maximum permeability coefficient where the curve levels

off. A positive sign in the exponent is required for bases and a negative sign for acids. Both pK_a^{flux} and log P_{max} served as fitting parameters.

Results and Discussion

Permeability experiments were carried out in a diffusion cell consisting of a donor compartment (containing the drug stock solution) and an acceptor compartment (containing pH 7.4 buffer at the same ionic strength as blood) separated by a lipid-coated commercial membrane (Figure 1). Drug concentrations selected for use in the donor compartment ensured that a single permeability test was completed in about 30 minutes. The pH 7.4 buffer solution in the acceptor compartment was circulated through a UV flow cell and back. The UV-Vis spectrophotometer was programmed to record a UV spectrum (210 nm – 400 nm) every 3 minutes and save the spectra for later data analysis. Alternatively, the change in drug concentration with time was followed by recording the change in absorbance at a single wavelength.

We chose the drug naproxen for our first model permeability study since this drug has been studied extensively in a variety of permeability assays and is well known to be highly permeating. Naproxen is a weak acid, with a pK_a of 4.18, and has a strong absorption maximum at 229 nm and a weaker absorption at 264 nm. At low pH, naproxen is in its neutral form which makes it easy for the molecules to permeate a non-polar membrane, be it a cell membrane or a lipid-coated artificial membrane. However, with increasing pH, naproxen becomes more and more ionized, which improves its aqueous solubility but reduces its permeability. When tested in our diffusion cell set-up, naproxen was found to permeate quickly from the donor compartment through the coated membrane into the acceptor

compartment; this was readily apparent by observation of an increase in the corresponding absorbance peak at 229 nm with time in the UV spectra recorded of the acceptor solution.

Like a standard diffusion process [15], with which students should be familiar from physical chemistry lectures, the permeation of a drug through a membrane under steady-state conditions can be described by Fick's first law (Equation 4)

$$\frac{dC_A}{dt} = \frac{P_{app}A}{V_A} \times (C_D - C_A) \quad (4)$$

The solution for this differential equation is an exponential function. Figure 3 shows how the absorbance at 229 nm changed over time at three representative pH values. At pH 3.0, the plot had a characteristic exponential shape and absorbance readings started to approach a maximum value within half an hour. The data could be fitted to a generic exponential curve (Equation 1). However, at pH 4.3, the permeability of naproxen was not only lower but the curve's shape became almost a straight line with a reduced slope. When the pH of the donor solution was raised to 5.3 or higher, the gradient decreased even further. Although the curve's exponential shape was no longer evident at increased pH, the apparent permeability could still be calculated from the initial gradient observed during a 30 minute experiment.

For ionizable drugs, the permeability coefficient depends on the pH of the donor solution and it is therefore important that this parameter is evaluated over an appropriate pH range. In the case of a weakly acidic drug, such as naproxen, the pH of the donor solutions selected for evaluation typically covered the range from 3 to 6. Figure 4 shows the "log permeability versus pH profile" obtained for naproxen and it is characteristic of a weak acid. At low pH, as the drug was in its neutral form, log P_{app} was at its highest value (log P_{max}). However, upon steadily increasing the pH of the donor solution, a point was eventually reached where the value deduced for log P_{app} began to decrease; this happened above naproxen's pK_a^{flux}, the aqueous ionization constant under flux conditions where 50% of the drug was in its ionized

form [3]. The pK_a^{flux} differs from the aqueous pK_a due to the partitioning of the ionizable drug between the aqueous solution and the lipophilic coating of the membrane [3,16].

Whereas weakly acidic drugs were found to permeate most easily at low pH, the maximum permeability coefficient (log P_{max}) for a weak base such as propranolol was reached instead at high pH (Figure 5a). Non-ionizable drugs (*e.g.* nifedipine) showed, as expected, virtually no variation in their log P_{app} values as a function of pH, over the wide range explored (Figure 5b).

The maximum permeability coefficients that could be determined using our diffusion cell procedure were, for the majority of cases, similar to or slightly smaller than those reported in the literature, which had been obtained using a PAMPA assay where the donor compartment was stirred. More examples are included in the Supporting Material. Minor deviations were attributed to the lower stirring efficiency in our method. From one operator to another, log P_{app} values could be reproduced within ±0.3 (see Supporting Material). It has been estimated that the lower limit for log P_{app} that can be successfully determined using our approach is about –4 to –5, provided that the drug has a sufficiently strong UV absorbance.

To date, this experiment has been performed by 11 advanced level students (2007 – 2012) who worked alone and determined entire log permeability *vs*. pH profiles for selected drugs as part of their dissertation projects. In addition, three 16 to 17-year old pupils from local schools, who had applied through the Nuffield Science Bursary scheme for a placement for a summer project, have also helped testing the method. For adaption to an undergraduate teaching experiment, students could be asked to work in small groups and, rather than determine full log permeability *vs*. pH profiles, measure permeability for one drug only at selected pH values.

Conclusions

This experiment provides students with an insight into how a drug is absorbed in the body and introduces the concept of permeability coefficients for pharmaceutical drugs. It does not rely on any specialized equipment and illustrates within 30 minutes whether a drug permeates rapidly at a certain pH or not. By curve fitting absorbance–time curves, students are able to determine the actual permeability coefficients for a range of drugs for themselves. The method works best for drugs with medium to high permeability (log $P_{app} \geq -4$). Like PAMPA, the diffusion cell method allows drugs to be categorized into low and high permeability compounds according to the Biopharmaceutics Classification System. Undoubtedly, the opportunity to obtain experience in this area will be of considerable benefit to chemistry students, particularly those who are interested in seeking future employment in the pharmaceutical sector.

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Supporting Materials. Detailed experimental and analytical procedures, results for other drugs studied, discussion of reproducibility and scope are given in the supporting materials. Typical analysis spreadsheets for determining permeability coefficients and fitting "log permeability vs. pH profiles" are also provided (http://dx.doi.org/10.1333/...).

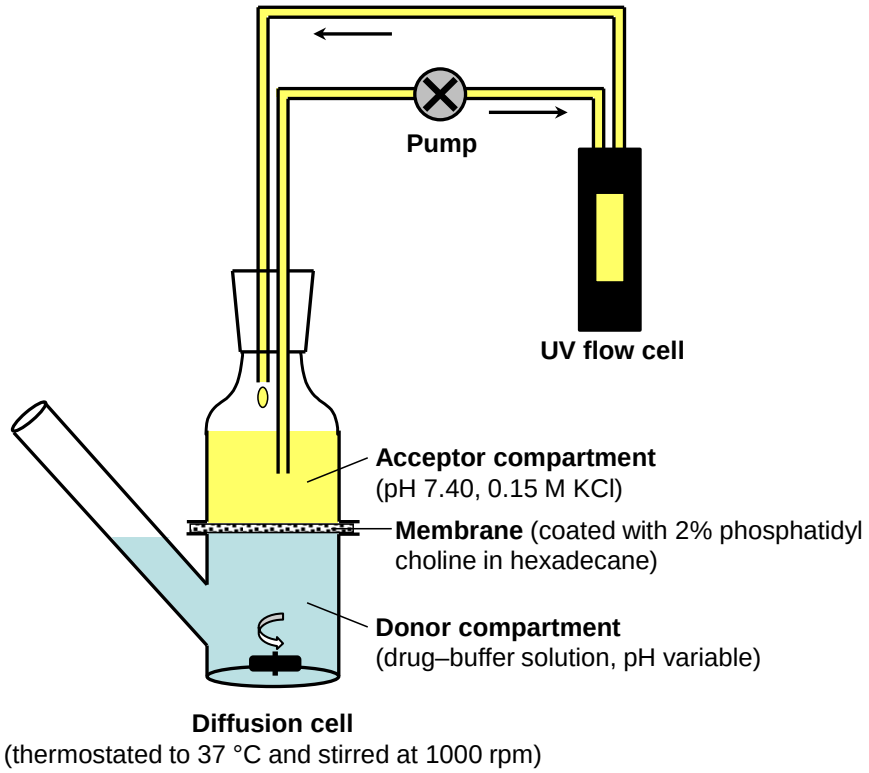

Figure 1. Schematic drawing of the set-up used for measuring drug permeability.

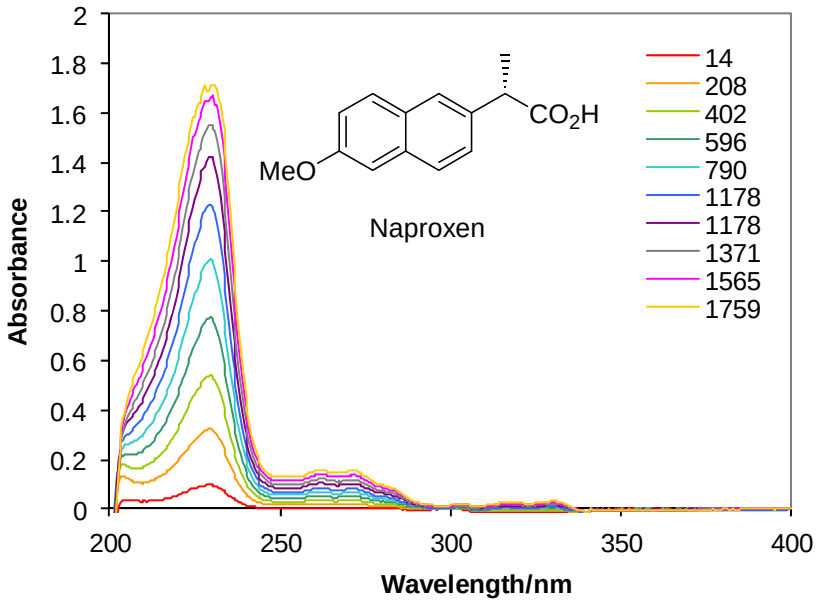

Figure 2. UV spectra of the acceptor solution recorded at set times (in seconds) during a typical permeability experiment using a naproxen donor solution at pH 3.0.

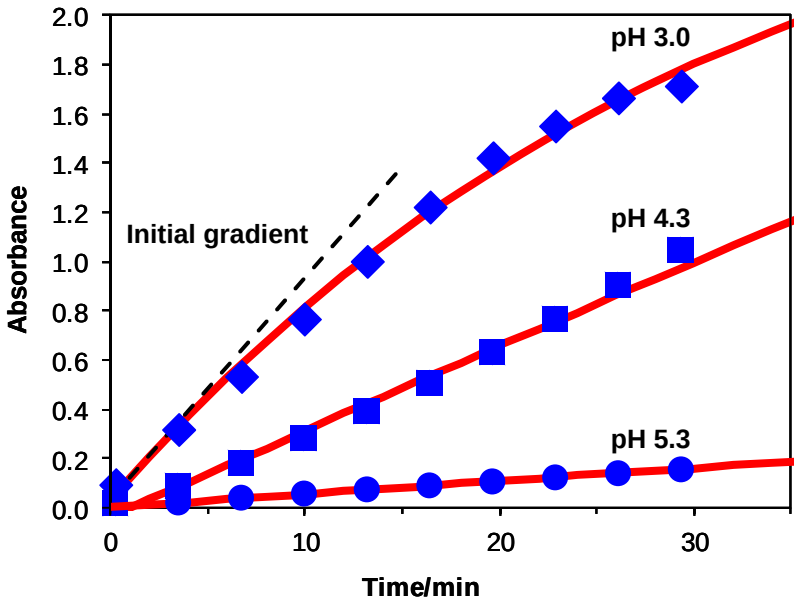

Figure 3. Plot of absorbance at 229 nm against time for naproxen at three different pH values (pH 3.0 – diamonds; 4.3 – squares; 5.3 – circles). The drawn curves represent the best fit of a generic exponential curve (Equation 1) through the data points. The initial gradient of the exponential curve at time $t = 0$ is shown for the curve fitted to the pH 3.0 data; from this, the logarithm of the permeability coefficient (log P_{app}) could be calculated using Equation 2a.

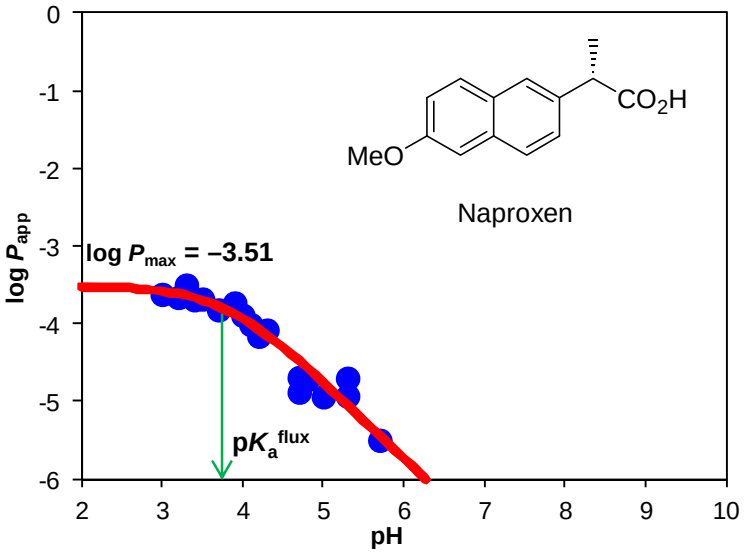

Figure 4. Plot of log P_{app} against pH for naproxen. The solid circles are experimental data points, whereas the drawn curve represents the best fit to Equation 3 with a log P_{max} of –3.51 and a pK_a^{flux} of 3.79.

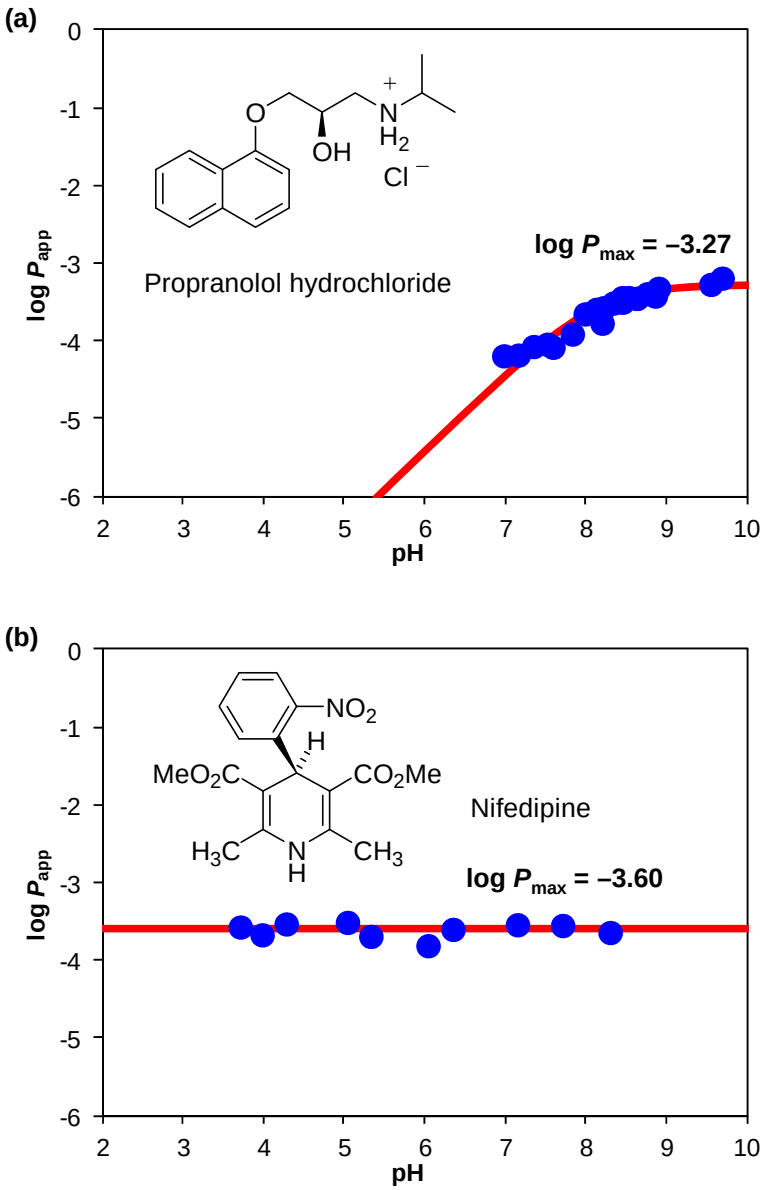

Figure 5. Plots of log P_{app} against pH for (a) a basic drug (propranolol) and (b) a non-ionizable drug (nifedipine).

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