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3.2

Passive diffusion of drug substances: the concepts of flux and permeability

Birger Brodin, Bente Steffansen and Carsten Uhd Nielsen

Experimental studies of the movement of molecules in solutions and molecular transport across artificial or biological barriers are used by the pharmaceutical scholar in a variety of contexts, ranging from simple diffusion and dissolution studies, to complex *in vivo* pharmacokinetic investigations. Movement of molecules in solutions and molecular transport across barriers may be described mathematically, and knowledge about these descriptions will aid in the design of experiments and interpretation of data. A number of textbooks explain the mathematical background necessary for transport studies (for example Schultz, 1980; Steen-Knudsen, 2002). In the present chapter, however, focus will be on presenting only the most commonly used equations and explaining the parameters involved, and the circumstances under which these equations can be applied. The aim of this section is thus to provide a basic framework of concepts describing transport of drug substances across biological barriers, hopefully enabling the reader to choose appropriate experimental models and data analysis for a given problem related to flux and permeability studies.

3.2.1 How do molecules move in solution? The concepts of flux, migration and diffusion

Mass transport of molecules in a solution or molecular transport across a barrier is normally measured by fluxes. The flux of a solute is simply defined as the mass or number of molecules moving through a given cross-sectional area during a given period of time (Equation 3.2.1):

$$J = \frac{m}{A\,t} \tag{3.2.1}$$

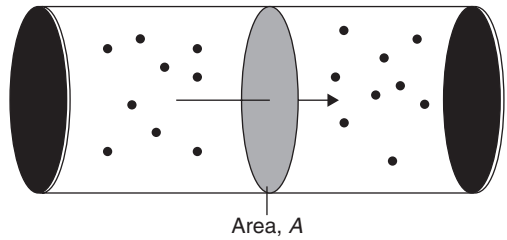

Figure 3.2.1 Flux, i.e. movement of molecules (•) through cross-sectional area (*A*) in a given time period (*t*).

where J is the flux of a mass of compound m, moving through a cross-sectional area A during time t as illustrated in Figure 3.2.1. The unit for a flux value could thus be mol $cm^{-2}\,min^{-1}$, or alternatively $\mu g\,cm^{-2}\,h^{-1}$.

Movement of molecules in solution or molecular transport across barriers can be caused by migration or diffusion. Migration is movement of molecules caused by an external force that is acting on each of the solute molecules. Such external forces can be gravity, electrical fields (in case of charged solutes) or hydrodynamic flow. Diffusion is the random thermal movement of molecules in a solution, and thus diffusion may only cause a net transport of molecules in the presence of a concentration gradient.

The velocity of diffusion is related to the diffusion coefficient of a solute, a constant related to the properties of a given molecule in a given solvent. The diffusion coefficient (D) is dependent on the size of the solute molecule and the viscosity of the solvent as described by the Stokes–Einstein equation (Equation 3.2.2):

$$D = \frac{RT}{6\pi\eta N_o r_A} \tag{3.2.2}$$

where R is the gas constant, T absolute temperature, r_A the radius of spherical solute, N_0 Avogadro's number, and η the viscosity of the solution. Thus the diffusion coefficient decreases with increasing molecule size and increasing viscosity of the solvent

Diffusional flux can be described by the relationship commonly known as Fick's law (or Fick's first law), normally accredited to the German physiologist Adolf Fick (Equation 3.2.3):

$$J(x,t) = -D\frac{\partial C(x,t)}{\partial(x)} \tag{3.2.3}$$

Fick's Law is a partial differential equation, describing a flux, J, down a concentration gradient, δC, in a plane over time, t, for a solute, x, with a diffusion coefficient D. This version of Fick's law is rarely used for interpreting simple transport studies. However, when assuming a time-independent linear concentration profile (Equation 3.2.4), Fick's law becomes more straightforward to use, as we shall see later.

In transport studies conducted in biopharmaceutical science or in preclinical development, most experimental designs aim at eliminating migrational flux components to solely study diffusional fluxes. Migrational fluxes will not be described further in this chapter, apart from being mentioned in relation to electrical fields in Sections 3.2.5 and 3.2.6.

3.2.2 Fluxes across barriers and the permeability coefficient

The most common biopharmaceutical use of flux studies is transport investigations of drug candidates or prodrug candidates, across a barrier tissue, such as small intestinal cell culture models or tissue models. A typical setup for the conduction of this type of experiment will include a donor compartment with a defined initial concentration of compound, a defined volume, a barrier structure with a defined cross-sectional area and thickness, and a receiver compartment with a defined initial concentration and a defined volume (see Figure 3.2.2).

Stirring should be complete in both donor and receptor compartments, in order to ensure that there is no concentration gradient within the two compartments, and thus the only gradient present is across the barrier structure separating the two compartments. Fluxes are then measured simply, by taking samples from the receiver compartment at given time points after addition of the test compound to the donor solution. In the simple situation, where the flux across the barrier only moves an insignificant amount of solute test compound from the donor chamber, the concentration gradient across the barrier is essentially constant, and the flux will thus be of zero order, i.e. be constant, since flux occurs as a function of the concentration gradient. In such cases, a simple version of Fick's law can be used to relate fluxes and concentration gradients (Equation 3.2.4):

$$J = P\,(C_{\text{donor}} - C_{\text{receiver}}) \tag{3.2.4}$$

where P is the permeability coefficient, C_{donor} and C_{receiver} are the concentrations of the drug substance in the donor and receiver compartments, respectively, and J is the flux from the donor to receiver compartment of the drug substance in question. Occasionally the notations P_{apparent} (P_{app})

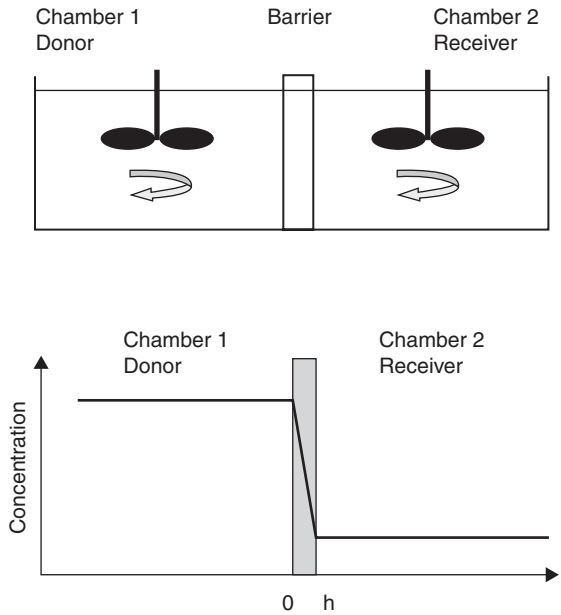

Figure 3.2.2 Top: a two-compartment system for transepithelial flux measurements. A barrier (a tissue or an artificial membrane) is bathed in stirred solutions. Bottom: concentration gradient in the two-compartment system. Normally perfect stirring is assumed, i.e. it is assumed that there are no concentration gradients within each of the two compartments and that the whole concentration drop occurs across the barrier.

or $P_{\text{effective}}$ (P_{eff}) are used when dealing with experimentally obtained permeability coefficients, in order to underscore their vulnerability to bias due to experimental conditions. Assuming that the concentration gradient, $C_{\text{donor}} - C_{\text{receiver}}$, across the barrier is linear and constant, i.e. time independent, Fick's law can be simplified to the equation shown in Equation 3.2.4 and further simplified if it is assumed that the drug substance concentration in the donor compartment is constant and that the concentration in the receiver chamber is virtually zero as compared to the donor concentration. The concentration gradient thus becomes equal to the concentration of the drug substance in the donor chamber at time zero, as shown in Equation 3.2.5:

$$J = P\, C_{\text{donor}} \Leftrightarrow P = \frac{J}{C_{\text{donor}}} \tag{3.2.5}$$

It follows from Equations 3.2.4 and 3.2.5 that the flux is proportional to the concentration gradient and that the permeability is simply the constant that relates flux and concentration gradient. It follows that the

permeability of a drug substance in a given barrier can be estimated from a simple flux experiment at a given concentration gradient, and that the determined permeability value can readily be compared with other permeability values obtained at other concentration gradients. The obtained permeability value can thus be compared with permeability values obtained from a similar experimental setup and, on basis of previous experience, the drug substance in question could be categorised in terms of permeability.

However, a number of experimental considerations must be made, before the estimated permeability value is valid.

1 The concentration gradient should be constant or nearly constant throughout the experiment. Normally, experiments are performed under sink conditions, i.e. the concentration in the receiver compartment is initially zero and is assumed to increase insignificantly during the time course of the experiment. The acceptable increase in $C_{receiver}$ depends on the required precision, but we suggest that the concentration gradient should not change more than 10% during the experiment. The change in concentration gradient is easily measured, either by measuring the concentration in the donor and receiver chamber before and after the experiment, or by using the measured flux value to estimate how much compound has moved during the experiment. If, in fact, the concentration gradient changes considerably, data can be processed assuming non-steady-state conditions, see Section 3.2.4.
2 The concentration gradient should be the only gradient present across the tissue. This implies that concentrations of all other compounds than the drug should be kept similar in the donor and the receiver compartment, that no hydrodynamic gradient should exist and that no electrical gradient should be present when permeabilities of charged drug compounds are measured.
3 It should be ensured that the transport of drug is purely passive. This is normally performed by investigating drug transport at a range of concentration gradients. If transport is purely passive, a linear relationship between flux and concentration gradient will be observed. For active transport see Chapter 3.4.
4 Unstirred water layers surrounding the barrier in question should be minimised. In experimental practice, this is normally accomplished by choosing the highest possible stirring rate, i.e. a rate that minimises concentration gradients in the donor and receiver compartment but does not damage the barrier (properties).

Permeability data may be used for a number of purposes. The permeability constant obtained in a cell model or an *in vitro* tissue model can be used to predict the bioavailability of a given drug substance, or permeabilities of a series of related drug candidates can be compared for selection of a drug candidate with a high permeability. Permeability comparisons must, however, be done with caution. Table 3.2.1 shows the permeabilities of a number of compounds in the Caco-2 cell model and intestinal tissue, respectively. It is evident that the relative ranking of compounds is meaningful, but the absolute values vary between models.

The permeability is a constant that consists of a number of model-specific variables, i.e. the diffusion coefficient of the drug in the barrier (D), the thickness of the barrier (h) and the partition coefficient of the drug into the barrier (α) (Equation 3.2.6, see also Chapter 2.1 for a discussion on partition coefficients).

$$P = \frac{\alpha D}{h} \tag{3.2.6}$$

When comparing permeability data obtained in different experimental setups such as for example intestinal epithelial cell culture models and *in situ* perfusion models, the lipid composition may vary between the two systems, causing different values of D and α. The thickness of the barrier,

Table 3.2.1 Permeabilities of drug and reference compounds in Caco-2 cells and human intestine

Compound	*Permeability in Caco-2 cells, apical–basolateral* ($cm\,s^{-1}$)	*Intestinal permeability in rat (r) or human(h)* ($cm\,s^{-1}$)	*Bioavailability (%)*
Mannitol	0.069×10^{-5}	0.3×10^{-5} (r)	Low
Atenolol	0.13×10^{-5}	1.5×10^{-5} (h)	45
Ranitidine	0.01×10^{-5}	1.5×10^{-5} (r)	~50
Terbutaline	$\sim 0.1 \times 10^{-5}$	3.0×10^{-5} (h)	65
Ondansetron	1.8×10^{-5}	n/a	100
Metoprolol	3×10^{-5}	15×10^{-5} (h)	100
Antipyrine	5×10^{-5}	50×10^{-5} (h)	100

Notes: Values are taken from Gan *et al.* (1993); Collett *et al.* (1999); Rege *et al.* (2001); Lennernas (1998); Brusewitz *et al.* (2007); Laitinen *et al.* (2003).
n/a, not available.

h, i.e. the height of the cell layer can also vary. Variations in the thickness of the unstirred water layers surrounding the tissues can furthermore cause differences in permeability values obtained in the two models. In order to underline that permeability estimates can be prone to errors, it is quite common to use the notation $P_{\text{effective}}$ (P_{eff}) or P_{apparent} (P_{app}) when referring to permeability estimates from flux experiments.

3.2.3 Unstirred water layers

In simple two-compartment systems, barrier permeabilities, estimated from flux measurements, are determined assuming that the concentrations in compartments 1 and 2 are constant throughout the individual compartments. The only concentration gradient present will thus be the gradient across the barrier. However, this simplification does not always hold true. In some cases the permeability measurements are dependent on the stirring conditions. This phenomenon is due to the presence of unstirred water layers close to the tissue, and it is caused by local concentration gradients in the solutions surrounding the tissue. Unstirred water layers can be viewed as two additional barriers in the transport pathway, a barrier in compartment 1 and a barrier in compartment 2 (see Figure 3.2.3).

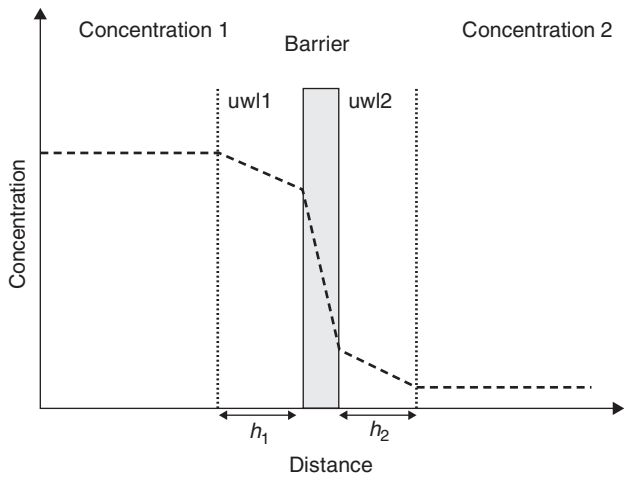

Figure 3.2.3 Unstirred water layers (uwl) can be present close to the tissue barrier due to imperfect stirring of the experimental solutions. The unstirred water layers can be regarded as two extra barriers, with thickness h_1 and h_2, in series with the tissue barrier.

Steady-state flux across an unstirred water layer can be described by the simplified Fick's expression, and an equation can easily be set up describing the measured permeability as a function of the real barrier permeability and the permeabilities in the two unstirred water layers (Equation 3.2.7):

$$\frac{1}{P_{\text{eff}}} = \frac{1}{P_{\text{uwl1}}} + \frac{1}{P_{\text{barrier}}} + \frac{1}{P_{\text{uwl2}}} \Leftrightarrow \frac{1}{P_{\text{eff}}} = \frac{h_1}{D_{\text{uwl1}}} + \frac{1}{P_{\text{barrier}}} + \frac{h_2}{D_{\text{uwl2}}} \quad (3.2.7)$$

where P_{eff} is the actual estimated permeability across the tissue and the unstirred water layers, $P_{\text{uwl1}}/P_{\text{uwl2}}$, h_1/h_2 and $D_{\text{uwl1}}/D_{\text{uwl2}}$ are the permeabilities, thickness and diffusion coefficients of uwl1 and 2, respectively, and P_{barrier} is the true permeability of the tissue barrier.

However, the permeabilities, P_{uwl1} and P_{uwl2} are not readily determined, and therefore the real permeability is not easily derived. A simpler approach for dealing with unstirred water layers is to determine the permeability (P_{eff}) at a range of different stirring rates, and since the estimated permeability will approach the true permeability asymptotically, the true permeability can be estimated from a mathematical fit of the obtained data. An alternative approach is to choose an experimental setup and just compare P_{eff} values under identical conditions, while knowing that they might be underestimated due to the presence of the unstirred water layers.

This possible influence of unstirred water layers must be kept in mind when absolute permeabilities are compared between different experimental setups with different stirring rates and different barrier structures.

3.2.4 Fluxes across a barrier under non-steady-state conditions

In experiments with highly permeable compounds, the transport of a drug candidate from the donor compartment to the receiver compartment cause a first-order decrease of the concentration of drug candidate in the donor chamber and an accompanying increase in its concentration in the receiver chamber (see Figure 3.2.4). In this situation, the concentration gradient cannot be considered constant, and the permeability cannot be calculated directly from Equations 3.2.4 or 3.2.5.

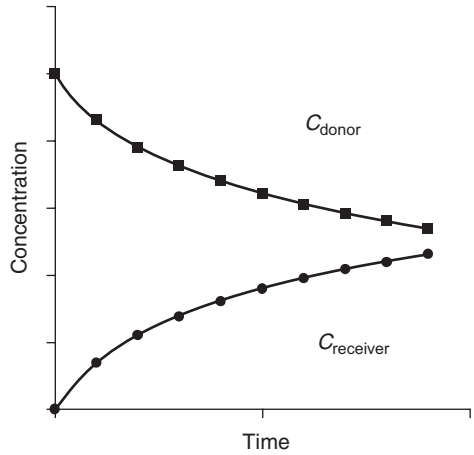

Figure 3.2.4 Non-stationary diffusion of a solute from a donor compartment to a receiver compartment, in a two-compartment system with equal volumes of solution on both sides of the barrier, and initially only solute present in the donor compartment. Both C_{donor} and $C_{receiver}$ will approach the same value asymptotically.

This is observed when flux of a lipophilic drug candidate is measured across a barrier tissue for a sufficiently long time period. The problem can be solved in two ways, either by keeping the volumes of the donor and receiver compartments very large, i.e. by constantly flushing the donor and receiver compartments with fresh experimental solutions (a flow-through system), or alternatively by treating the obtained data using a set of equations that take the changing concentration gradient into account. When a significant flux occurs across a barrier with a constant permeability, the concentration of drug candidate in the donor chamber will decrease with one-phase exponential decay, and the concentration in the receiver chamber will increase with one-phase exponential association. When the concentration of drug is plotted in a log diagram against time, the slope of the curve will be related to the permeability.

The calculation of the permeability from non-stationary fluxes demands knowledge of C_{donor} at time zero ($C_{donor,\ t=0}$), the volumes of the donor and receiver compartments (V_{donor} and $V_{receiver}$), the tissue area (A) and the concentration of at least one (but ideally 3–5) receiver samples at different time intervals (C_t). From these input values, other values can be derived, for use in the calculation, such as the total mass of compound in the system (m_{total}), which is equal to the mass initially added to the donor compartment ($m_{i,\ t=0}$) and can be calculated as $C_{donor,\ t=0} \times V_{donor}$. Then

the following equations can be applied:

$$C_\infty = \text{the final concentration in both compartments} = \frac{m_{i,t=0}}{V_{donor} + V_{receiver}} \tag{3.2.8}$$

$$\frac{C_\infty - C_{receiver,t}}{C_\infty} = e^{-kt} \tag{3.2.9}$$

$$k = AP\frac{C_{donor} + C_{receiver}}{C_{donor} + C_{receiver}} \tag{3.2.10}$$

The constant k can be isolated mathematically or found by plotting time and concentration data in a logarithmic plot, and P can be isolated easily from Equation 3.2.10.

When applying this treatment to experimental data, it must be verified that mass balance exists, as lipophilic drugs often adsorb to pipettes and glass and plastic surfaces.

3.2.5 Fluxes of a charged solute in the presence of an electrical potential gradient

The estimation of a permeability value as described in Equations 3.2.4 and 3.2.5 implies that the only factor responsible for mass transport across the barrier in question is the concentration gradient of the solute. If an electrical potential gradient is present across the tissue and the solute is charged, the driving force for mass transport is a combination of the electrical and chemical gradients, and the estimated permeability will be influenced by the electrical potential gradient (see Figure 3.2.5).

The presence of electrical fields generated by an electrically active tissue can be dealt with in two ways, either by setting up the barrier tissue in an Ussing chamber setup, allowing voltage clamp of the barrier and thereby reducing the electrical potential to zero by applying current from an external current source, or by measuring the electrical potential gradient during the experiment and estimating the permeability by using Equation 3.2.11 (also known as the Nernst–Planck equation):

$$J = zP\nu\frac{C_2 - C_1 e^{z\nu}}{1 - e^{z\nu}} \tag{3.2.11}$$

where J is the flux of charged compound from compartment 1 to compartment 2, z is the charge of the substrate, P is the permeability of the compound, C_1 and C_2 are the concentrations of the compound in

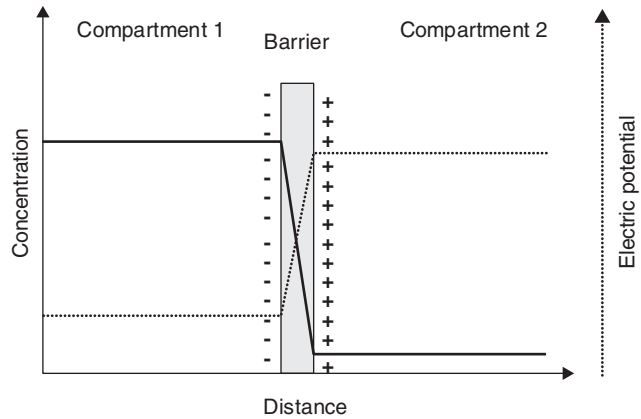

Figure 3.2.5 Flux of a charged solute across a barrier with an electric potential difference between compartment 1 and compartment 2. If an electrical potential exists across a barrier, it will impose a force on charged drug molecules. The direction of the force will depend on whether the compound is positively or negatively charged. When drug transport is studied across electrically active epithelia or across cell membranes, or when currents carried by an electrophoretic process should be predicted, electrical fields must thus be taken into consideration.

compartments 1 and 2, respectively, and v is the normalised electrical potential difference across the barrier (Equation 3.2.12):

$$v = \frac{V\mathrm{F}}{\mathrm{R}T} \tag{3.2.12}$$

In this equation, v is the potential difference across the barrier (with compartment 1 as reference), F is Faraday's number, R is the gas constant and T is the absolute temperature. Using Equations 3.2.11 and 3.2.12, one can either calculate the permeability of a charged compound in the presence of an electric field, by measuring values of flux, J, and potential, V, and then calculating P from Equation 3.2.11, or calculate how much compound will be moved by a given electrical field across a tissue with a given permeability, by inserting the potential V and the permeability in the equation.

3.2.6 Use of flux ratios to analyse transport mechanisms

The transport mechanism of a compound across a given tissue barrier can be analysed in terms of flux ratios, in order to investigate whether the transepithelial transport is active, i.e. energised by ATP-consuming

pumps, or passive (see Section 3.3.1). The flux ratio equation deals with unidirectional fluxes of radiolabelled compounds (Ussing, 1949; Dawson, 1976). A unidirectional flux can be defined as a flux from one compartment to another, across a barrier, measured by radiolabel, without considering counterflux of the same compound. If a charged compound does not interact with other compounds and its transepithelial transport is solely driven by the electrochemical potential difference across the barrier, then the ratio between the unidirectional fluxes can be described as follows:

$$\frac{J^{1-2}}{J^{2-1}} = \left[\left(\frac{C_1}{C_2}\right) e^{\left(\frac{-zFV}{RT}\right)}\right] \tag{3.2.13}$$

where J^{1-2} and J^{2-1} are the fluxes from compartment 1 to 2 and vice versa, V is the potential difference between compartment 1 and 2 ($V_2 - V_1$), C_1 and C_2 are the concentrations in the respective compartments, z is the charge of the compound, F is Faraday's number, R is the gas constant and T is the absolute temperature. What Equation 3.2.13 actually states is that the ratio between the unidirectional fluxes should equal the concentration ratio times a factor describing the electric gradient across the barrier. It follows from the equation that for a non-charged solute, this factor becomes 1, and the flux ratio will thus equal the concentration ratio. A flux-ratio analysis is thus a very simple method of investigating whether a transepithelial transport process is passive or energised.

3.2.7 Conclusions

Mass transport across a barrier, e.g intestinal epithelium or other pharmaceutically relevant barriers, can be caused by diffusion or migration. Transport by simple diffusion can be measured as flux, and characterised by a permeability for the transported solute. The permeability can be calculated using the measured flux and the concentration gradient. Permeabilities can be compared between a series of related compounds in order to select drug candidates in a screening process, or used to predict *in vivo* bioavailability of a given drug substance. Care must be taken in experimental design and when interpreting permeabilities, in order to distinguish permeabilities of compounds with carrier-mediated flux components from permeabilities of compounds that are solely driven by diffusion.

3.2.8 Examples

Example 3.2.1: calculation of the permeability of a drug compound in the Caco-2 cell intestinal model

The apical to basolateral (equivalent to lumen–blood) flux, J^{l-b}, of a new drug candidate **x**, has been measured across 21-day-old Caco-2 cell monolayers grown on permeable filter support. **x** has been added to the apical solution at a concentration of 50 mM. Samples have been taken from the basolateral solution at intervals of 15 min. The concentration of the compound has been measured using radiolabelled **x**. At time zero and at the end of the experiment, donor samples have been taken (see Table 3.2.2).

Experimental parameters:
Volumes of the experimental solutions: apical volume = 0.5 ml, basolateral volume = 1.0 ml. Sample volume, receiver solution (basolateral) = 100 µl; sample volume, donor solution (apical) = 20 µl. Cross-sectional area of tissue = 1 cm^2.

Calculations:
In order to obtain the flux across the tissue, the amount of substance which has moved per time unit must be calculated. This is done, first by calculating the amount (mass) of substance present in the receiver solution at time = t, by multiplying the sample concentration by the volume of experimental solution, i.e.:

$$C_n \times V_r = \text{mass} \tag{3.2.14}$$

However, since substance has been removed from the receiver solution, every time a sample has been taken, a correction must be introduced. The total mass that has crossed the barrier at a given time is thus:

$$\text{Mass}_{\text{total}} = V_s \left(\sum_{n=1}^{n} C_{n-1} \right) + C_n \times V_r \tag{3.2.15}$$

where $C_1, C_2 \ldots C_n$ are the sample concentrations in samples $1, 2 \ldots n$, V_r is the volume of the receiver solution and V_s is the volume of the sample.

The data can be plotted, either in a plot of accumulated **x** versus time (Figure 3.2.6, graph 1) or as a plot of flux of **x** versus time (Figure 3.2.6, graph 2). Both these types of plots have advantages and drawbacks. Graph 1 simply displays the mass of substance per area which has crossed the cell monolayer at a given time. The flux can then be found as the slope

Table 3.2.2 A theoretical data set, showing concentration values sampled at different time points, and the derived flux values

Sample number	*Time, t (min)*	*[Drug x] in donor sample (mM)*	*[Drug x] in receiver sample, (mM)*	*Mass of Drug x in receiver solution (nmol)*	*$Mass_{total}$ (nmol)*	*Flux (nmol $(cm^2\ min)^{-1}$)*
1	0	50	0	0	0	0
2	15	–	0.02	20	20	1.33
3	30	–	0.07	70	72	3.47
4	45	–	0.15	150	159	5.80
5	60	–	0.25	250	274	7.67
6	75	–	0.35	350	399	8.33
7	90	–	0.44	440	524	8.33
8	105	–	0.51	510	638	7.60
9	120	48	0.58	580	759	8.07

of the linear part of the relation. In this example, the flux is 0.0081 µmol $(cm^2\ min)^{-1}$ or 8.1 nmol $(cm^2\ min)^{-1}$. The intercept with the x axis is called the *lag time* and in the example shown this is 25.5 min. The lag time is obtained by setting $f(x) = 0$.

Graph 2 in Figure 3.2.6 displays the same data set, but with the flux instead of accumulation on the y axis. Thus the bar at the x value 15 min is the mean flux during time 0–15 min, the bar at 30 min is the mean flux at 15–30 min etc. The flux, or transport rate, reaches steady state after

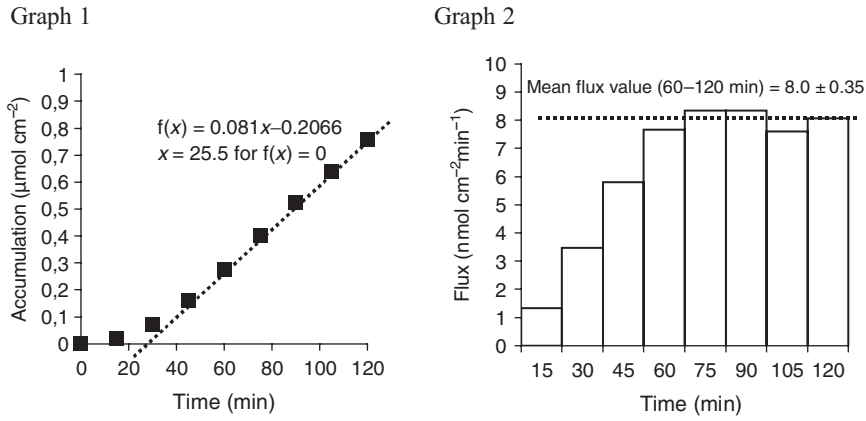

Figure 3.2.6

~60 min, as judged by visual inspection of the data. The steady-state flux can thus be determined as the mean of the flux values obtained in the steady-state period (60–120 min). The value 8.0 nmol $(cm^2\ min)^{-1}$ is obtained from the present data set, a value corresponding fairly well with the determination from graph 1. When plotted in a graph like graph 2, parameters such as the time of steady-state and possible depletion of donor compound will be more visible to the investigator, whereas graph 1 demands fewer calculations and yields the lag-time.

The permeability can now be calculated using Equation 3.2.5 (thus ignoring the slight concentration drop in the donor chamber during the experiment):

$$P = \frac{J\,\frac{\text{nmol}}{\text{cm}^2\ \text{min}}}{C_{\text{donor}}\,\frac{\text{mmol}}{\text{l}}} = \frac{8\ \text{nmol l}}{50\ \text{mmol cm}^2\ \text{min}} = 0.16\,\frac{10^{-9}\ \text{l}}{10^{-3}\ \text{cm}^2\ \text{min}}$$

$$= 0.16\,\frac{10^{-9}\ 10^{3}\ \text{cm}^3}{10^{-3}\ \text{cm}^2\ \text{min}} = 0.1610^{-3}\,\frac{\text{cm}}{\text{min}} = 9.6\ 10^{-3}\,\frac{\text{cm}}{\text{h}} = 2.6\ 10^{-6}\,\frac{\text{cm}}{\text{s}} \qquad (3.2.16)$$

Example 3.2.2: calculation of the permeability of a drug in the Caco-2 cell intestinal model under non-steady-state conditions

The apical to basolateral (equivalent to lumen-blood) flux, $J^{\text{l}-\text{b}}$, of a new drug compound **y**, has been measured across 21-day-old Caco-2 cell monolayers grown on permeable filter support. As in the previous example, **y** has been added to the apical solution at a concentration of 50 mM. Samples have been taken from the basolateral solution at varying intervals, see below. The concentration of the compound has been measured using radiolabelled compound **y**. At time zero and at the end of the experiment, donor samples have been taken (see Table 3.2.3).

Experimental parameters:
Volumes of the experimental solutions: apical volume = 0.5 ml, basolateral volume (at $t = 0$) = 1.0 ml. Sample volume, receiver solution (basolateral) = 10 µl; sample volume, donor solution (apical) = 10 µl. Cross-sectional area of tissue = 1 cm^2.

Calculations:
In order to obtain the flux across the tissue, the amount of substance that has moved per time unit is calculated as in the previous example. The flux values indicate that a large percentage of the added dose has moved across

Table 3.2.3 A theoretical data set, showing concentration values sampled at different time points under non-stationary flux conditions

Sample number	*Time, t (min)*	*[Drug* **y***] in donor sample (mM)*	*[Drug* **y***] in receiver sample (mM)*
1	0	50	0
2	10	–	8
3	30	–	14
4	90	16.6	16.6

the barrier during the measurement period, therefore a non-steady-state flux analysis is performed.

The concentration of compound **y** at time infinity in both compartments is calculated, using Equation 3.2.8:

$$C_\infty = \frac{m_{i,t=0}}{V_{donor} + V_{receiver}} = \frac{50\,\text{mM}\,0.5\,\text{ml}}{0.5\,\text{ml} + 1\,\text{ml}} = 16.7\,\text{mM} \qquad (3.2.17)$$

The concentrations measured can be fitted to Equation 3.2.9:

$$\frac{C_\infty - C_{receiver,t}}{C_\infty} = e^{-kt} = \frac{16.7\,\text{mM} - C_{receiver,t}}{16.7\,\text{mM}} = e^{-kt} \qquad (3.2.18)$$

The k value can be estimated graphically or by isolation, and can be used to calculate the permeability according to Equation 3.2.10:

$$\begin{aligned} k = 62.7\,10^{-3}\,\text{min}^{-1} &= AP\,\frac{V_{donor} + V_{receiver}}{V_{donor}\,V_{receiver}} \\ &= 1\,\text{cm}^2\,P\,\frac{0.5\,\text{ml} + 1\,\text{ml}}{0.5\,\text{ml}\,1\,\text{ml}} \Leftrightarrow P = 62.7\,10^{-3}\,\text{min}^{-1}\,\frac{\text{cm}^3}{3\,\text{cm}^2} \end{aligned} \qquad (3.2.19)$$

obtaining a P value of $0.0209\,\text{cm}\,\text{min}^{-1}$ or $3.5 \times 10^{-4}\,\text{cm}\,\text{s}^{-1}$.

Example 3.2.3: flux ratio analysis

The unidirectional fluxes of a novel drug substance, testicine, have been measured in Caco-2 cell monolayers. The monolayers have been incubated with 1 mM of isotope-labelled testicine in both the apical and basolateral compartment, and steady-state fluxes have been determined. The apical and basolateral test solutions are identical. Testicine has a negative charge. The monolayers are mounted in an Ussing chamber, allowing for

measurement of the potential difference (V) from the apical to the basolateral side.

The folloving parameters are obtained during the experiment:

$$J_{\text{apical–basolateral}} = 15\,\text{nmol} \times \text{cm}^{-2} \times \text{min}^{-1}$$
$$J_{\text{basolateral–apical}} = 10\,\text{nmol} \times \text{cm}^{-2} \times \text{min}^{-1}$$
$$V = V_{\text{basolateral}} - V_{\text{apical}} = 10\,\text{mV} = 0.010\,\text{J C}^{-1}$$

Temperature $= 20\,^{\circ}\text{C}$

F, R and T have the values:

$$F = 96\,487\,\text{C mol}^{-1}$$
$$R = 8.3144\,\text{J (K mol)}^{-1}$$
$$\text{T}_{20\,^{\circ}\text{C}} = 293\,\text{K}$$

The experimentally determined flux ratio thus becomes:

$$\frac{J^{1-2}}{J^{2-1}} = \frac{15}{10} = 1.5 \tag{3.2.20}$$

and the calculated flux ratio becomes

$$\left(\frac{C^1}{C^2}\right) e^{\left(\frac{-zFV}{RT}\right)} = \left(\frac{1}{1}\right) e^{\left(\frac{-(-1)\ 96487\ \text{C mol}^{-1}\ 0.01\ \text{J C}^{-1}}{8.31144\ \text{J K}^{-1} \times \text{mol}\ 293\ \text{K}}\right)} = 1.49 \tag{3.2.21}$$

The flux ratio analysis thus indicates that no active transporters are involved in the transepithelial transport process, which appears to be driven solely by the transepithelial electrochemical gradient.

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