1 (a) Explain the assumptions behind the Michaelis Menten model for enzyme kinetics. Write down the chemical reactions involved and introduce the relevant kinetic constants. [30%]

Answer: The simplest (and generic) form of enzyme catalysis is the following:

$$\mathrm{S}+\mathrm{E} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{C} \xrightarrow{k_2} \mathrm{P}+\mathrm{E}$$

All these steps are assumed to be elementary reactions.

There are usually two types of assumptions that can be made regarding the relative kinetics of the reactions.

- the equilibrium on the left side is fast, and that $\mathrm{C} \xrightarrow{k_2} \mathrm{P}+\mathrm{E}$ is the rate limiting reaction,
- the reaction $\mathrm{C} \xrightarrow{k_2} \mathrm{P}+\mathrm{E}$ is much faster than the substrate/enzyme binding.

Both assumptions lead to similar experimental characteristics.

(b) Find the expression for the product creation rate V as a function of the substrate concentration S_0, the Michaelis Menten constant K_M and the maximal product creation rate V_{max}. [30%]

Answer: The differential equations governing this system are:

$$\begin{aligned}
\frac{d[\mathrm{S}]}{dt} &= k_{-1}[\mathrm{C}] - k_1[\mathrm{S}][\mathrm{E}] \\
\frac{d[\mathrm{E}]}{dt} &= (k_{-1}+k_2)[\mathrm{C}] - k_1[\mathrm{S}][\mathrm{E}] \\
\frac{d[\mathrm{C}]}{dt} &= k_1[\mathrm{S}][\mathrm{E}] - (k_2+k_{-1})[\mathrm{C}] \\
\frac{d[\mathrm{P}]}{dt} &= k_2[\mathrm{C}]
\end{aligned}$$

We would like to relate the overall rate of the reaction V (i.e. the rate of production of P) to the substrate concentration. If we assume that the transformation from C to P is fast, it is therefore appropriate to use the steady-state assumption on C, i.e. that $\frac{d[\mathrm{C}]}{dt} = 0$.

$$k_1[\mathrm{S}][\mathrm{E}] = (k_2+k_{-1})[\mathrm{C}] \Rightarrow \quad \frac{[\mathrm{S}][\mathrm{E}]}{[\mathrm{C}]} = \frac{(k_2+k_{-1})}{k_1} \equiv K_M$$

K_M is called the Michaelis constant.

Assuming that the total amount of enzyme molecules is constant ($[\mathrm{E}]+[\mathrm{C}] = E_0$), we obtain:

$$\begin{aligned}
\Rightarrow \quad [\mathrm{C}] &= E_0 \frac{[\mathrm{S}]}{[\mathrm{S}]+K_M} \\
\Rightarrow \quad V &= k_2 E_0 \frac{[\mathrm{S}]}{[\mathrm{S}]+K_M} = V_{max} \frac{[\mathrm{S}]}{[\mathrm{S}]+K_M} \qquad (1)
\end{aligned}$$

Answers deriving the same expression but using the fast equilibrium approach were also accepted.

(c) The data presented in Table 1 were measured for a particular enzyme-catalysed reaction by monitoring the product formation rate at various substrate concentrations, keeping the enzyme concentration constant. Calculate the values of V_{max} and K_M. What would V be for substrate concentrations equal to 2.5×10^{-5}M and 5×10^{-5}M? What would V be if the enzyme concentration is doubled for a substrate concentration equal to 5×10^{-5}M? [40%]

Substrate concentration (Molar)	Product formation rate V (10^{-9} moles liter^{-1} min^{-1})
6.25×10^{-6}	15.0
7.50×10^{-5}	56.25
1.00×10^{-4}	60
1.00×10^{-3}	74.9
1.00×10^{-2}	75

Table 1

Answer:

We can see here that V saturates for substrate concentrations lager than 10^{-3}M.

Hence, $V_{max} = 75 \cdot 10^{-9}$ moles litre^{-1} min^{-1}

To solve for K_M, we just need to pick a pair of values from the table and substitute in equation 1.

We then find $K_M = 2.5 \times 10^{-5}$ M

If the substrate concentration [S] is equal to K_M, then the velocity is $V_{max}/2$.

$V([S] = K_M) = 37.5 \times 10^{-9}$ moles litre^{-1} min^{-1}

Using equ. 1, we find:

$V([S] = 5 \cdot 10^{-5}\text{M}) = 50 \cdot 10^{-9}$ moles litre^{-1} min^{-1}

Finally, we can see from equ. 1 that doubling the enzyme concentration increases the product creation rate in the same proportion. Therefore:

$V([S] = 5 \cdot 10^{-5}\text{M}$ & doubled enzyme concentration$) = 100 \cdot 10^{-9}$ moles litre^{-1} min^{-1}

2 (a) Explain what the tube and discharge hematocrit represent. [20%]

Answer: The tube hematocrit, Hct_T, is the hematocrit measured from a snapshot of the blood flowing in the tube; the discharge hematocrit, Hct_D, is measured from the composition of the blood that leaves the capillary.

(b) Considering a perfectly cylindrical vessel of radius R, write down the expressions of the tube and discharge hematocrits as a function of the local volume fraction of red blood cell $h_{ct}(r)$ and the velocity profile $u(r)$, where r is the radial position in cylindrical polar coordinates. [30%]

Answer:

$$\mathrm{Hct}_T = \frac{\int_0^R 2\pi r h_{ct}(r) dr}{\pi R^2} \tag{2}$$

$$\mathrm{Hct}_D = \frac{\int_0^R 2\pi r h_{ct}(r) u(r) dr}{\int_0^R 2\pi r u(r) dr} \tag{3}$$

(c) In a simple model, the local hematocrit $h_{ct}(r)$ takes the following values:

$$\begin{cases} h_{ct}(r) = \mathrm{H}_{ct_0} & \text{for } 0 \leq r \leq R - \delta \\ h_{ct}(r) = 0 & \text{for } R - \delta < r \leq R \end{cases}$$

where H_{ct_0} is a positive constant.

(i) What does δ represent and what would be its approximate value? [10%]

Answer: The length δ is the width of the red blood cell (RBC) depletion zone due to their finite size. It can be assumed to be similar to the radius of a RBC, of the order of a few microns.

(ii) Assuming that the velocity profile corresponds to a simple Poiseuille flow, derive an expression for the ratio of the tube and discharge hematocrits and sketch this as a function of the tube radius. [40%]

Answer: In the case of a Poiseuille flow, the flow profile has the form $u(r) = C(1 - r^2/R^2)$ where C is a constant. By substituting the expression of $u(r)$ in the definitions of the tube and discharge hematocrits, we get:

$$\mathrm{Hct}_T = \mathrm{Hct}_0 \frac{\pi(R-\delta)^2}{\pi R^2} = \mathrm{Hct}_0 (1 - \delta/R)^2$$

$$\mathrm{Hct}_D = \mathrm{Hct}_0 \frac{\int_0^{R-\delta} 2\pi r C(1-\frac{r^2}{R^2})dr}{\int_0^R 2\pi r C(1-\frac{r^2}{R^2})dr} = \mathrm{Hct}_0 \left(2\left(1-\delta/R\right)^2 - \left(1-\delta/R\right)^4\right)$$

Hence: $\mathrm{Hct}_T/\mathrm{Hct}_D = \frac{1}{2-(1-\delta/R)^2}$

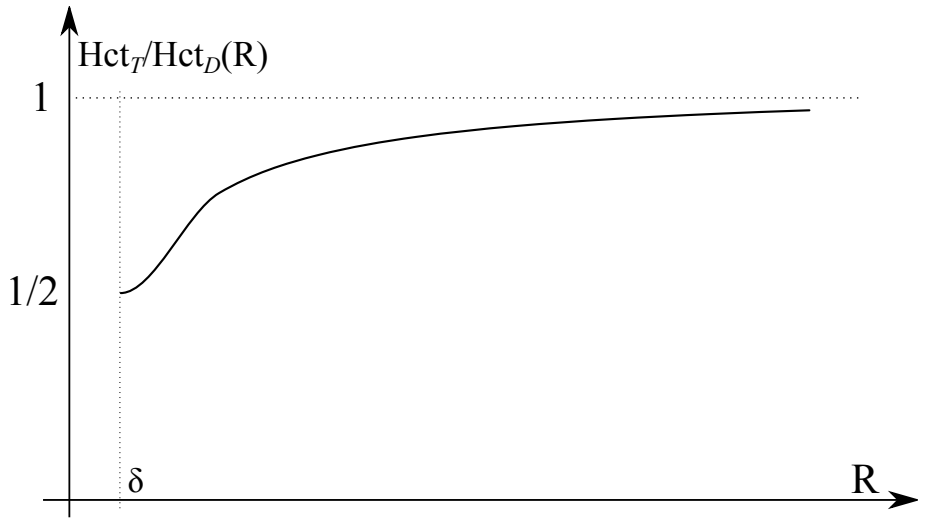

Fig. 1

3 (a) In what physical units are the following quantities measured? (use combinations of the following standard SI units: m, s, mol, kg, C, V, A, Ω, S, F)

– Diffusion coefficient

Answer: m^2/s

– Concentration

Answer: $\mathrm{mmol}/\mathrm{dm}^3$

– Electrovalency

Answer: unitless

– Permeability

Answer: m/s

– Membrane time constant

Answer: s

– Gating variables in the Hodgkin-Huxley model

Answer: unitless

– Membrane capacitance in the Hodgkin-Huxley model (assuming membrane currents are measured in $\mu\mathrm{A}/\mathrm{cm}^2$, membrane potential in mV and time in *s*)

Answer: $\mathrm{nF}/\mathrm{cm}^2$

– Axial resistance in the cable equation

Answer: $\mathrm{k}\Omega\mathrm{cm}$

– Input resistance

Answer: $\mathrm{k}\Omega$

– Propagation speed of the action potential

Answer: mm/s

Answer: Equivalent combinations of units will also be accepted. [20%]

(b) In an experiment, the sodium current through the membrane of a cell is measured while holding the membrane potential at a fixed value. The normal values of the intracellular and extracellular concentrations of sodium are $c_\mathrm{i} = 50$ mM and $c_\mathrm{e} = 437$ mM, respectively. Before the experiment, both quantities are lowered to $1/100^{\mathrm{th}}$ of these normal values. The experiment is conducted at room temperature, $T = 293$ K. Answer the following questions with regard to this experiment using the following physical constants:

$R = 8.314$ J/(molK) and $F = 96485$ C/mol. Make sure you provide the appropriate physical units with your answers.

(i) What is the Nernst potential of sodium under these conditions?

Answer:

$$V_{\text{Nernst}} = \frac{RT}{F} \ln\left(\frac{c_e}{c_i}\right) = 54.7 \text{ mV}$$

[10%]

(ii) What is the sodium current at the Nernst potential?

Answer: The sodium current at the Nernst potential is $I = 0$ nA cm^{-2} (by definition).

[5%]

(iii) The sodium current is measured at 0 mV and is found to be $I = -5.47$ nA cm^{-2}. What is the sodium conductance of the membrane?

Answer:

$$\begin{aligned} I &= g\,(V - V_{\text{Nernst}}) \\ g &= \frac{I}{(V - V_{\text{Nernst}})} = 0.001 \frac{\text{S}}{\text{m}^2} = 0.1 \frac{\mu\text{S}}{\text{cm}^2} \end{aligned}$$

Comment: we used the formula for the long channel limit, but the result should be the same for any other channel model since the channel current at 0 mV is only governed by the Nernst-Planck equation and so it is independent of the particular model of the channel that is used.

[10%]

(iv) What is the permeability of the membrane for sodium?

Answer:

$$\begin{aligned} g &= P \frac{F^2}{RT} \frac{c_e - c_i}{\ln c_e/c_i} \\ P &= g \frac{RT}{F^2} \frac{\ln c_e/c_i}{c_e - c_i} = 146.5 \frac{\text{pm}}{\text{s}} \end{aligned}$$

See also comment above. [15%]

(v) The sodium current is also measured at half the Nernst potential and is found to be $I = -2.26$ nA cm^{-2}. How can it be possible that the magnitude of this current is less than half of that measured at 0 mV, ie. that the sodium current does not appear to be a linear function of the membrane potential?

Answer: The experiment is conducted at extremely low concentrations at which the sodium channels likely not behave as simple ohmic elements (because for the long channel limit, in which they do, to be valid ionic concentrations need to be high) and instead they obey the Goldman-Hodgkin-Katz equation (valid at low concentrations) which predicts a non-linear dependence on membrane potential, and in particular it predicts lower current magnitudes than a linear dependence would. [20%]

(vi) Normal sodium concentrations are restored both inside and outside the cell. What are the values of the sodium current at the following values of the membrane potential:

– Nernst potential;

– half the Nernst potential;

– 0 mV?

Answer: According to both the short and long channel limits (and intuition), currents scale linearly with concentrations (because the same forces act on more or less ions thus carrying more or less current), and the normal ionic concentrations are 100 times larger than those used in the original experiment, so that would predict the following sodium currents:

– at Nernst potential: $I = 0\ \frac{\text{nA}}{\text{cm}^2}$

– at half the Nernst potential: $I = -226\ \frac{\text{nA}}{\text{cm}^2}$

– at 0 mV: $I = -547\ \frac{\text{nA}}{\text{cm}^2}$.

However, because ionic concentrations are much higher now, the channel currents may not obey the GHK equations any more and instead may be linear functions of the membrane potential (see also answer to previous question), in which case the sodium current at the Nernst potential and at 0 mV remains as given above (because at these membrane potentials it is the same regardless the channel model used) but at half the Nernst potential it will be $I = -547/2\ \frac{\text{nA}}{\text{cm}^2} = -273.5\ \frac{\text{nA}}{\text{cm}^2}$ [20%]

4 At the end-stage of renal disease, over 90% of the blood filtration function is typically lost. Dialysis is one of the main treatments for such disease. Hemodialysis consists in connecting the bloodstream to a dialysis machine in order to remove toxic solutes accumulating in the blood. The key principle involved in hemodialysis is a transfer across a membrane of the solute from the blood to a second solution, called a dialysate. The main objective in the machine design is to maximise the removal of solute from the blood in each pass. In this question, the filtration rates obtained with two simple designs are quantified and compared.

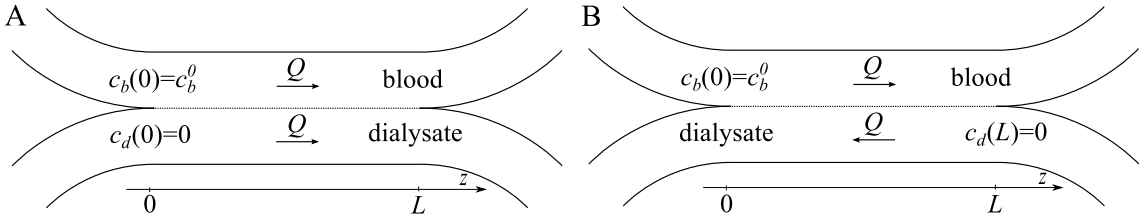

Fig. 2

As shown in figure 2, the flow rates in the blood and dialysate vessels are assumed to be identical and equal to Q. The concentrations of a particular solute along the vessels are $c_b(z)$ in the blood vessel and $c_d(z)$ in the dialysate vessel. The solute concentration in blood entering the dialyser is c_b^0 and the solute concentration in the dialysate entering the machine is negligible. The flux per unit length $\phi(z)$ of solute across the membrane is due to passive diffusion and controlled by the following relationship: $\phi(z) = K(c_b(z) - c_d(z))$, where K is the membrane permeability.

(a) Consider first the case of the co-current flow geometry depicted in figure 2A, in which the blood and dialysate flow alongside each other, in the same direction, over a length L.

(i) Show that $\frac{\mathrm{d}c_b}{\mathrm{d}z} = -\frac{\mathrm{d}c_d}{\mathrm{d}z} = -\frac{\phi}{Q}$ [15%]

Answer: Consider a small element dz along the vessels. The conservation equation on the blood vessel side gives:

$$Q\,dt\,c_b(z) = Q\,dt\,c_b(z+dz) + \phi(z)\,dz\,dt \implies \frac{\mathrm{d}c_b}{\mathrm{d}z} = -\frac{\phi}{Q}$$

Similarly, considering mass conservation on a dialysate side, we find:

$$Q\,dt\,c_d(z) + \phi(z)\,dz\,dt = Q\,dt\,c_d(z+dz) \implies \frac{\mathrm{d}c_d}{\mathrm{d}z} = \frac{\phi}{Q}$$

(ii) Write down a first order differential equation for $\phi(z)$ and find its solution. Find the expression of $c_b(z)$ and $c_d(z)$ and sketch their graphs. [20%]

Answer: By differentiating $\phi(z) = K(c_b(z) - c_d(z))$ and substituting in the results from section a.i, we get:

$$\frac{\mathrm{d}\phi}{\mathrm{d}z} = K\left(\frac{\mathrm{d}c_b}{\mathrm{d}z} - \frac{\mathrm{d}c_d}{\mathrm{d}z}\right) = -\frac{2K}{Q}\phi$$

Its solution is:

$$\phi = \phi_0 \exp(-z/\lambda) \text{ with } \lambda = \frac{Q}{2K} \text{ and } \phi_0 = Kc_b^0$$

From the result of a.i, we have:

$$\frac{\mathrm{d}c_b}{\mathrm{d}z} = -\frac{\mathrm{d}c_d}{\mathrm{d}z} = -\frac{c_b^0}{2}\frac{1}{\lambda}\exp(-z/\lambda)$$

By integrating and using the boundary conditions at $z = 0$, we find:

$$c_b(z) = \frac{c_b^0}{2}\left(1 + \exp(-z/\lambda)\right) \text{ and } c_d(z) = \frac{c_b^0}{2}\left(1 - \exp(-z/\lambda)\right)$$

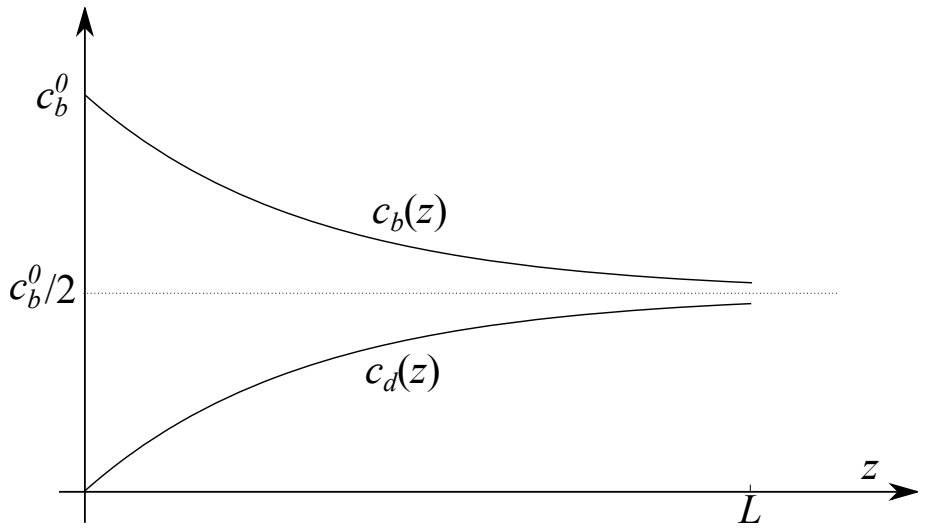

Fig. 3

(iii) The total mass transfer rate $\dot{M}$ is defined by: $\dot{M} = \int_0^L \phi \, \mathrm{d}z$. What is its maximum value, obtained when L tends to infinity? [10%]

Answer: When L tends to infinity, half of the solute crosses the membrane. Hence, $\dot{M} = Qc_b^0/2$. The same result can also be found by direct integration of ϕ.

(b) Consider next the case of the counter-current flow geometry depicted in figure 2B. Here, the blood and dialysate flow alongside each other, but in opposite directions, over a length L.

(i) Show that $\phi(z)$ is constant. [15%]

Answer: Considering the change of direction of the flow in the dialysate vessel, mass conservation in this new geometry provides the following equations:

$$Q\,dt\,c_d(z) = Q\,dt\,c_d(z+dz) + \phi(z)\,dz\,dt \implies \frac{\mathrm{d}c_d}{\mathrm{d}z} = -\frac{\phi}{Q}$$

We still have in the blood vessel $\frac{\mathrm{d}c_b}{\mathrm{d}z} = -\frac{\phi}{Q}$. The flux therefore satisfies:

$$\frac{\mathrm{d}\phi}{\mathrm{d}z} = K\left(\frac{\mathrm{d}c_b}{\mathrm{d}z} - \frac{\mathrm{d}c_d}{\mathrm{d}z}\right) = 0$$

Hence, ϕ is constant.

(ii) Show that: $\begin{cases} c_b^0 - c_b(L) = c_d(0) = \frac{\phi L}{Q} \\ c_b^0 - c_d(0) = c_b(L) = \frac{\phi}{K} \end{cases}$ [15%]

Answer: We consider first mass conservation between input and output of the device. Since ϕ is constant, the total mass transfer rate is ϕL. In the blood vessel, the solute flux going in is Qc_b^0. It must be equal to what goes out plus what have been transferred through the membrane, $Qc_b(L) + \phi L$. Considering the dialysate, the flux in is zero, therefore the flux out must be equal to what has been transferred, $Qc_d(0) = \phi L$.

The second equation simply corresponds to the expression of ϕ at the boundaries of the vessels: $\phi = K(c_b(0) - c_d(0)) = K(c_b^0 - c_d(0))$ and $\phi = K(c_b(L) - c_d(L)) = Kc_b(L)$.

(iii) What is the maximum value of $\dot{M}$ in this geometry, obtained when L tends to infinity? [15%]

Answer: We need to find $\phi(L)$ and then take the limit ϕL for L tends to infinity. In the previous equations, we found $c_b^0 - c_d(0) = \frac{\phi}{K}$ and $c_d(0) = \frac{\phi L}{Q}$. This leads to $\phi\left(\frac{1}{K} + \frac{L}{Q}\right) = c_b^0$. The expression for ϕL therefore writes:

$$\phi L = Qc_b^0 \frac{1}{1 + \frac{Q}{KL}}$$

$$\lim_{L\to+\infty} \dot{M} = \lim_{L\to+\infty} \phi L = Qc_b^0$$

This means that in a sufficiently long vessel, all the solute can be transferred to the dialysate.

(c) What is the most efficient dialysis geometry? Briefly justify your answer. [10%]

Answer: The counter-current flow geometry provides a total mass transfer rate twice as large in the limit of long vessels. It is therefore more efficient than the co-current flow geometry.

END OF PAPER