Version AK/2

EGT2
ENGINEERING TRIPOS PART IIA

Thursday 21 April 2016 9.30 to 11

Module 3G2

MATHEMATICAL PHYSIOLOGY

*Answer not more than **three** questions.*

All questions carry the same number of marks.

*The **approximate** percentage of marks allocated to each part of a question is indicated in the right margin.*

*Write your candidate number **not** your name on the cover sheet.*

STATIONERY REQUIREMENTS
Single-sided script paper

SPECIAL REQUIREMENTS TO BE SUPPLIED FOR THIS EXAM
CUED approved calculator allowed
Engineering Data Book

10 minutes reading time is allowed for this paper.

You may not start to read the questions printed on the subsequent pages of this question paper until instructed to do so.

1 (a) Explain what competitive inhibition is in the context of enzyme kinetics. [20%]

Answer: In competitive inhibition, the inhibitor and the substrate would typically bind the enzyme on the same site, so that only one can be bound at a given time. The inhibitor therefore sequester some of the enzyme.

Comment from examiner: Please note that it is insufficient to say that both substrate and inhibitor can bind to the enzyme.

(b) Write a set of reactions with their rate constants to model competitive inhibition, and derive an expression for the rate of product formation V. [30%]

Answer:

$$\begin{aligned} \mathrm{S}+\mathrm{E} &\underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{C}_1 \xrightarrow{k_2} \mathrm{P}+\mathrm{E}. \\ \mathrm{E}+\mathrm{I} &\underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \mathrm{C}_2 \end{aligned}$$

The conservation of the number of enzyme molecules implies that $[\mathrm{E}]+[\mathrm{C}_1]+[\mathrm{C}_2]=E_0$. The quasi steady state assumption says that the concentrations of the two complexes C_1 and C_2 do not change, which yields two equations:

$$\begin{aligned} k_1[\mathrm{S}][\mathrm{E}] &= (k_2+k_{-1})[\mathrm{C}_1] \\ k_3[\mathrm{I}][\mathrm{E}] &= k_{-3}[\mathrm{C}_2] \end{aligned}$$

we define new combinations of the rate constants:

$$\begin{aligned} \frac{[\mathrm{S}][\mathrm{E}]}{[\mathrm{C}_1]} &= \frac{(k_2+k_{-1})}{k_1} \equiv K_M \\ \frac{[\mathrm{I}][\mathrm{E}]}{[\mathrm{C}_2]} &= \frac{k_{-3}}{k_3} \equiv K_I \end{aligned}$$

Alternatively, it is possible to use the fast equilibrium assumption, and obtain similar results except that $K_M = \frac{k_{-1}}{k_1}$. Both approaches are correct.
Using the conservation equation, we get:

$$\begin{aligned} [\mathrm{S}](E_0-[\mathrm{C}_1]-[\mathrm{C}_2]) &= K_M[\mathrm{C}_1] \\ [\mathrm{I}](E_0-[\mathrm{C}_1]-[\mathrm{C}_2]) &= K_I[\mathrm{C}_2] \end{aligned}$$

Rearranging gives the following equation for the C_1

$$[\mathrm{S}](E_0-[\mathrm{C}_1]-\frac{[\mathrm{I}](E_0-[\mathrm{C}_1])}{K_I+[\mathrm{I}]}) = K_M[\mathrm{C}_1]$$

yielding the expressions:

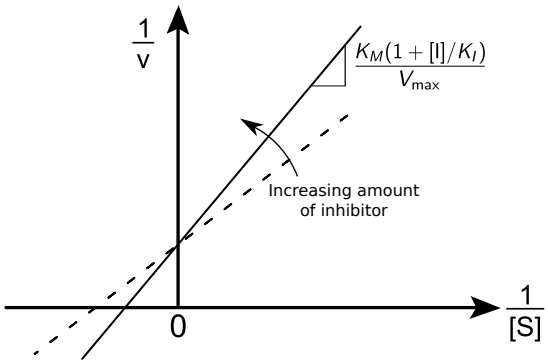
$$[\mathrm{C}_1] = \frac{[\mathrm{S}]E_0K_I}{[\mathrm{S}]K_I + [\mathrm{I}]K_M + K_IK_M}$$
$$[\mathrm{C}_2] = \frac{[\mathrm{I}]E_0K_M}{[\mathrm{S}]K_I + [\mathrm{I}]K_M + K_IK_M}$$

Thus the overall rate of the reaction is:

$$V = k_2[\mathrm{C}_1] = \frac{k_2[\mathrm{S}]E_0}{[\mathrm{S}] + K_M(1 + [\mathrm{I}]/K_I)}$$
$$V = \frac{V_{\max}[\mathrm{S}]}{[\mathrm{S}] + K'_M} \text{ with } K'_M = K_M(1 + [\mathrm{I}]/K_I)$$

(c) Use a graphical representation to illustrate the effect of the inhibitor concentration on the product formation rate. [10%]

Answer: The intercept with the y-axis, $1/V_{max}$ is unchanged, but the slope increases.



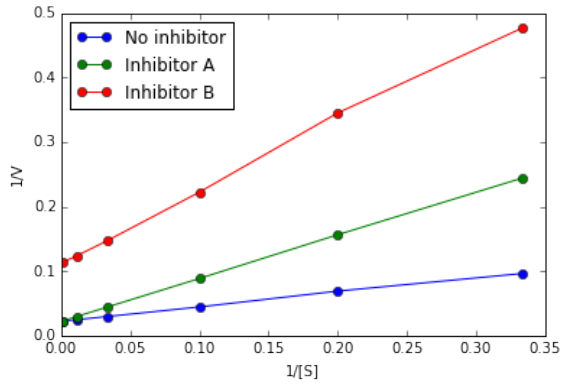
(d) The following data show how the rate of product formation V depends on the concentrations of substrate S and inhibitor I for two particular enzyme reactions. In each case, indicate if it is consistent with a competitive inhibition model, and if it is, extract as much information as you can about the equilibrium constants.

(i)

	[S] (μmol L^{-1})	3	5	10	30	90	900
No inhibitor	V (μmol L^{-1} min^{-1})	10.4	14.5	22.5	33.8	40.5	44.5
[I]=0.01 μmol L^{-1}	V (μmol L^{-1} min^{-1})	4.1	6.4	11.3	22.6	33.8	44.4

[20%]

Answer: The following figure shows all the data in a Lineweaver-Burke plot. It looks straight enough and seems consistent with a simple enzyme model.



It is apparent from the table (and the graph) that inhibitor 1 preserves V_{max}, as expected from competitive inhibition.
We find that $V_{max} \approx 44.5$ µmol L^{-1} min^{-1}.
From the table alone, one can see that V is halfed for [S] ≈ 10 µmol L^{-1}. This is also when [S] = K_M, hence $K_M \approx 10$ µmol L^{-1}.
The same argument applied to the case with the inhibitor shows that the speed is halfed when [S] = $K_M(1 + [I]/K_I) \approx 30$ µmol L^{-1}.
Similar values could be extracted from the slopes and intercepts of the graphs with and without inhibitor, with a comparable level of precision.
From the data above, one finds that $I]/K_I \approx 2$, hence $K_I \approx 5 \cdot 10^{-3}$ µmol L^{-1}.

(ii)

	[S] (µmol L^{-1})	3	5	10	30	90	900
No inhibitor	V (µmol L^{-1} min^{-1})	10.4	14.5	22.5	33.8	40.5	44.5
[I]=0.01 µmol L^{-1}	V (µmol L^{-1} min^{-1})	2.1	2.9	4.5	6.8	8.1	8.8

[20%]

Answer: It is clear already from the table that inhibitor B has a different value for V_{max} and could not match a competitive inhibition model.

2 Certain organisms, such as the colonial algae Volvox, have all their cells arranged on the surface of a sphere, with only a single cell in the thickness of the layer. To survive, these cells need to collect oxygen and nutrients from their environment. In this question, we investigate if diffusion is sufficient as a transport mechanism to keep the organism healthy. We will focus on oxygen transport.

(a) Consider such a spherical organism with radius R. Assume the cells at the surface consume an amount ρ of oxygen per unit time and area. What is the total amount of oxygen consumed by the whole organism per unit time? [10%]

Answer: We just need to integrate ρ over the surface of the sphere. The amount needed per unit time is: $Q = \rho 4\pi R^2$

(b) What is the maximal amount of oxygen that the organism can collect per unit time in the steady state through diffusion alone, as a function of R, the coefficient of diffusion D of oxygen in the organisms environment (mostly water), and the concentration of oxygen c_0 away from the organism? [35%]

Answer: In the previous question, we calculated what is needed for the organism to survive. Because the oxygen can only reach the cells through diffusion from the external environment, there must be a gradient of concentration at the viscinity (but outside) of the sphere, leading to a net flux balancing the consumption. To find the flux, we must find the concentration profile and use Fick's law. To find the concentration profile we need to solve the diffusion equation outside of the organism, in steady regime.

Diffusion equation in spherical polar (see databook for operators):

$$\frac{1}{r^2}\frac{\partial}{\partial r}\left(r^2\frac{\partial c}{\partial r}\right) = 0$$

The general solution takes the following form: $c = \frac{A}{r} + B$.

What are the boundary conditions? We know that away from the organism, the concentration is c_0, so $c(\infty) = B = c_0$.

What about the second boundary condition? Intuitively, the larger the concentration drop at the viscinity of the organism, the larger the gradient and the larger the flux. So the maximum flux is achieved when $c(R) = 0$, which is sufficient to find the solution. If one wants to follow a more mathematical approach instead, we can write the flux as $J(r) = -D\frac{\partial c}{\partial r} = DA/r^2$. A must be negative for oxygen to go towards the centre. Since we want the flux inwards to be maximum, A has to be as negative as possible, while keeping $c(R) = \frac{A}{R} + c_0 \geq 0$. This implies that $A \geq -c_0 R$. The best solution is therefore when $A = -c_0 R$. Hence $c(r) = c_0\left(1 - \frac{R}{r}\right)$.

The flux at the surface of the organism is $J(R) = -Dc_0 R/R^2$. The total diffusive flux integrated over the surface is $Q_d = 4\pi R^2 \|J(R)\| = 4\pi R^2 \cdot Dc_0 R/R^2 = 4\pi D c_0 R$.

Comment from examiner: Very few students answered this question properly. Many tried to solve the reaction-diffusion equation within the sphere, which cannot provide any information about the supply from the environment.

(c) Under what condition is diffusive transport sufficient to keep the organism properly oxygenated? [10%]

Answer: The maximum diffusive flux must be larger than the need of the organism for it to be properly oxygenated: $Q_d > Q$.

$$4\pi D c_0 R > \rho 4\pi R^2$$

This set essentially a constraint on the maximal size of the organism:

$$R < \frac{Dc_0}{\rho}$$

(d) Write the expression for the oxygen concentration at the surface of the organism when $R = \frac{1}{2}\frac{Dc_0}{\rho}$. [35%]

Answer: We are looking for the concentration $c(R)$ for which the diffusive surface flux balances the need of an organism of size R smaller than the critical size obtained above. The general solution of the diffusion equation is: $c(r) = \frac{A}{r} + c_0$ (with A negative).
The expression for the total flux then become: $Q_d = 4\pi R^2 \cdot D\frac{-A}{R^2} = -4\pi DA$.
Equating Q_d to the needs of the organism Q:

$$Q_d = -4\pi DA = Q = \rho 4\pi R^2 = \rho 4\pi \left(\frac{1}{2}\frac{Dc_0}{\rho}\right)^2 = \frac{\pi D^2 c_0^2}{\rho}$$

$$A = -\frac{Dc_0^2}{4\rho} = -\frac{Rc_0}{2}$$

$$c(R) = -\frac{Rc_0}{2R} + c_0 = \frac{c_0}{2}$$

(e) The radius R of *Volvox carteri* ranges from about 100 µm to 500 µm. Is diffusive transport sufficient to supply the cells with oxygen? If it isn't, suggest other mechanisms that might help. The following figures might inform your answer:
$\rho = 10^{14}\text{cm}^{-2}\ \text{s}^{-1}$, $c_0 = 10^{17}\text{cm}^{-3}$ and $D = 2 \cdot 10^{-5}\ \text{cm}^2\ \text{s}^{-1}$. [10%]

Answer: $\frac{Dc_0}{\rho}$ is of the order of 200 μm. The largest *Volvox carteri* would therefore not be able to get their oxygen by diffusion. Transport of oxygen therefore needs to be increase by other mechanisms such as advection, or migration/swimming.

3 (a) What are the units of measurement (if any) for the following physical quantities?

- (diffusion) flux
- diffusion coefficient
- electrovalency
- electric field
- Faraday constant
- universal gas constant
- Nernst potential
- ionic concentration
- dielectric constant
- channel permeability

[20%]

Answer: Multiple equivalent solutions are possible that translate to the same combination of basic SI units (potentially with some prefixes).

- mol / (m^2 s)
- m^2/s
- 1 (unitless)
- V/m (or N/C)
- C/mol
- J / (mol K)
- V
- mol / m^3
- C/(V m) or F/m
- m/s

(b) This question is about the Nernst potential.

(i) Derive the value of the Nernst potential of an ion, defined as the membrane potential (electric potential difference between the two ends of a channel) when the flux of the ion is zero everywhere inside the channel. Start from the Nernst–Planck equation describing the flux of an ion:

$$J(x,t) = -D\left(\frac{\partial}{\partial x}c(x,t) + \frac{zF}{RT}\,c(x,t)\,\frac{\partial}{\partial x}\phi(x,t)\right)$$

where x is one-dimensional space, t is time, J is the flux, ϕ is the electric potential, F is the Faraday constant, R is the universal gas constant, T is absolute temperature, D is the diffusion coefficient, c is the concentration, and z is the valence of the ion. In your derivation, you can use the following definitions: let L denote the length of the channel, $V(t) = \phi(L,t) - \phi(0,t)$ denote the membrane potential, and $c_{\mathrm{i}}(t) = c(0,t)$ and $c_{\mathrm{e}}(t) = c(L,t)$ denote the concentration of the ion at the intra- and extra-cellular end of the channel, respectively. [20%]

Answer:

$$\frac{\partial}{\partial x}c(x,t) + \frac{zF}{RT}\,c(x,t)\,\frac{\partial}{\partial x}\phi(x,t) = 0$$
$$\frac{\partial}{\partial x}\phi(x,t) = -\frac{RT}{zF}\,\frac{\frac{\partial}{\partial x}c(x,t)}{c(x,t)}$$
$$\frac{\partial}{\partial x}\phi(x,t) = -\frac{RT}{zF}\,\frac{\partial}{\partial x}\ln c(x,t)$$

By integrating both sides of the last equation wrt. x between 0 and L, we obtain

$$\phi(L,t) - \phi(0,t) = -\frac{RT}{zF}\,(\ln c(L,t) - \ln c(0,t)) = -\frac{RT}{zF}\,\ln\frac{c(L,t)}{c(0,t)}$$
$$V(t) = \frac{RT}{zF}\,\ln\frac{c_{\mathrm{e}}(t)}{c_{\mathrm{i}}(t)}$$

which is the Nernst potential of the ion.

(ii) Demonstrate with a derivation that the converse of the previous situation may not always hold, i.e. the flux of an ion may not be zero everywhere at the moment when the membrane potential reaches its Nernst potential. [20%]

Answer:

$$\begin{aligned}
V(t) &= \frac{RT}{zF}\,\ln\frac{c_{\mathrm{e}}(t)}{c_{\mathrm{i}}(t)} \\
\phi(L,t) - \phi(0,t) &= -\frac{RT}{zF}\,(\ln c(L,t) - \ln c(0,t)) \\
\int_0^L \frac{\partial}{\partial x}\phi(x,t)\,dx &= -\int_0^L \frac{RT}{zF}\,\frac{\partial}{\partial x}\ln c(x,t)\,dx \\
\int_0^L \left(\frac{\partial}{\partial x}\phi(x,t) + \frac{RT}{zF}\,\frac{\frac{\partial}{\partial x}c(x,t)}{c(x,t)}\right) dx &= 0 \\
\int_0^L \frac{\frac{\partial}{\partial x}c(x,t) + \frac{zF}{RT}\,c(x,t)\,\frac{\partial}{\partial x}\phi(x,t)}{c(x,t)}\,dx &= 0 \\
\int_0^L \frac{J(x,t)}{c(x,t)}\,dx &= 0 \qquad (1)
\end{aligned}$$

Thus, in this situation, rather than the flux being zero, all what we can say is that the integral of the relative flux along the channel is zero.

(iii) In order to prove that the flux of an ion is zero at the Nernst potential, one must take the steady state limit. What defines this steady state limit, what are the relevant boundary conditions, what are the assumptions underlying these boundary conditions, and why are these assumptions justified? [20%]

Answer: We use the term steady-state for an ion channel to refer to a condition when we keep the concentration at the two ends of the channel constant, and similarly, we also hold the voltage between the two ends of the channel (the membrane potential) constant. In such a condition, after waiting long enough (formally, asymptotically, i.e. in the infinite time limit), ionic concentrations and the electric potential inside the channel do not change any more. From these it follows that in steady state, the flux is constant along the channel, although not necessarily zero.
There are two assumptions.

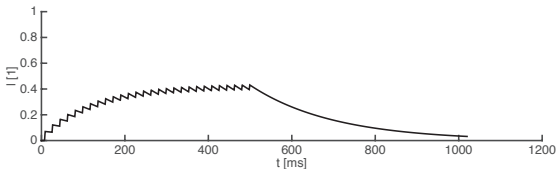
A. The concentration of the ion at the two ends of the channel remains constant despite the flux of the ion not necessarily being zero. This is justified because there are mechanisms (such as ion pumps) in place in the membrane that actively maintain ionic concentrations in the extra- and intracellular space (i.e. at the two ends of the channel), and because the actual absolute number of ions passing through the channel is diminishingly small compared to the number of ions at the two ends, such that concentrations at the two ends hardly change (or rather, they change only very slowly, see also separation of time scales argument below).

B. The membrane potential is constant for an infinite amount of time. Although this is not formally true, it can be justified by a separation of time scales argument. This means that the time scale for reaching equilibrium flux is much shorter that the time scale on which the membrane potential is changing. The former time scale is roughly given by the time it takes for the ion to diffuse from one end of the channel to the other, and is on the order of $0.1\,\mu$s, while the latter time scale is typically on the order of 1 ms, so indeed, there is a several orders of magnitude separation of time scales.

(c) Beside the usual currents of the Hodgkin–Huxley model responsible for action potential generation, some neurons also include a so-called A-type potassium current. The single gating variable, a, of this A-type current activates slowly with depolarisation, and deactivates even more slowly with hyperpolarisation, such that it could be modelled simply as having a steady-state value a_∞ that switches from 0 to 1 at a threshold voltage of 50 mV, and a time constant τ_a that is 200 ms and 20 ms below and above this threshold, respectively.

(i) Sketch and describe in words the behaviour of a in time when the cell is made to fire at around 60 Hz for several hundred milliseconds. For simplicity, assume that the maximal conductance of the A-type current is near zero, so that it has no visible effect on the membrane potential. [10%]

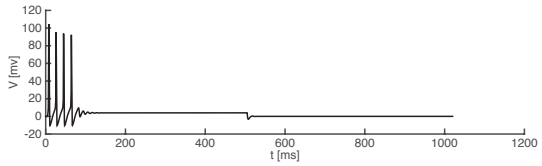
Answer:



At the time of spikes there will be rapid 'jumps' in a (governed by the faster, 20 ms activation time constant) which will slowly decay between spikes (governed by the slower, 200 ms deactivation time constant), but since at 60 Hz the inter-spike interval is much shorter than the time constant of decay, this decay will hardly be visible. This will result in a step-by-step accumulation of a until it reaches some 'steady' state value around which it hovers as spikes occur.

(ii) Sketch and describe in words the membrane potential trace of the cell in response to the stimulation described above when the maximal conductance of the A-type current is well above zero. [10%]

Answer:



The current hardly influences the shape of action potentials (except for some small decrease in their amplitude) as even its faster activation time scale (20 ms) is much slower than the time constants of the other channels (all below 10 ms). However, the accumulation of the A-type potassium conductance will make it increasingly harder for the cell to fire, such that the inter-spike-intervals will grow, and eventually the cell is going to stop firing altogether.

4 (a) Explain why capillaries have small holes called fenestrations. Give an approximate value for their diameter. [10%]

Answer: These holes allow water to go through the vessel wall, but not large globular proteins. These holes have a size of the order of a few tens of nanometres. Since globular proteins do not cross the vessel wall, they contribute to an osmotic pressure term that allows water to get back in at the other end of the capillary beds, where the hydrostatic pressure is lower.

(b) Consider a capillary of length L oriented along the x axis. The hydrostatic pressure inside the capillary is denoted $P_c(x)$, while the hydrostatic pressure in the surrounding tissue is P_i. The osmotic pressures in blood and tissue are π_c and π_i respectively. Write down the expression for the total pressure difference $\Delta P(x)$ that drives the movement of water through the capillary wall as a function of $P_c(x)$, P_i, π_c and π_i. Write a simple relationship that relates $\Delta P(x)$, the flux of water $\phi(x)$ moving through the vessel wall per unit of capillary length, and the permeability per unit length K_f. [15%]

Answer: One has to correct hydrostatic pressures with osmotic pressures on both sides, and subtract.

$$\Delta P(x) = K_f((P_i - \pi_i) - (P_c - \pi_c))$$

The fluid flux through the fenestration is proportional to the pressure drop. The proportionality coef is by definition K_f:

$$\phi_p = K_f \Delta P(x)$$
$$\phi_p = K_f(P_i - \pi_i - P_c + \pi_c)$$

(c) The flow rate of blood along the capillary is denoted $q(x)$. The influx at the entry $x = 0$ and exit $x = L$ are assumed to be identical, $q(0) = q(L) = Q$. The capillary hydrodynamic resistance, ρ, is defined by $\frac{dP_c}{dx} = -\rho q(x)$ and is constant along the capillary. Assume at this stage that P_i, π_c and π_i are homogeneous along the capillary.

(i) Derive the differential equation satisfied by the blood hydrostatic pressure $P_c(x)$ in this model. [15%]

Answer: Mass (or volume) conservation provides the following relationship:

$$q(x) + \phi_p(x)dx = q(x+dx)$$

Leading to:

$$\frac{dq}{dx} = \phi_p(x) = K_f(P_i - \pi_i - P_c + \pi_c)$$

Since the vessel has some hydrodynamic resistance:

$$\frac{dP_c}{dx} = -\rho q(x)$$

We can then eliminate q:

$$\frac{d^2P_c}{dx^2} = -\rho K_f(P_i - \pi_i - P_c + \pi_c))$$

Comment from examiner: A small number of students defined ϕ_q per unit area instead of per unit length, and ended up with slightly different expressions. This was accepted as long as it was correct and consistent.

(ii) Show that the following expression is solution of the problem:

$$P_c(x) = P_i + \pi_c - \pi_i - \frac{P_c(0) - P_c(L)}{2} \frac{\sinh\left(\sqrt{\rho K_f}\,(x - L/2)\right)}{\sinh\left(\sqrt{\rho K_f} L/2\right)}$$

[20%]

Answer: The expression can be differentiated twice and inserted in the differential equation to show that it is solution.

$$\frac{d^2P_c}{dx^2} = -\frac{P_c(0) - P_c(L)}{2} \sqrt{\rho K_f} \sqrt{\rho K_f} \frac{\sinh\left(\sqrt{\rho K_f}\,(x - L/2)\right)}{\sinh\left(\sqrt{\rho K_f} L/2\right)} = -\rho K_f(P_i - \pi_i - P_c + \pi_c))$$

(iii) Find the relationship between the entry flow rate Q and the pressure drop along the capillary $P_c(L) - P_c(0)$. [15%]

Answer: We need to differentiate P_c to find q and use the boundary conditions:

$$q(x) = \frac{P_c(0) - P_c(L)}{2\rho} \sqrt{\rho K_f} \frac{\cosh\left(\sqrt{\rho K_f}\,(x - L/2)\right)}{\sinh\left(\sqrt{\rho K_f} L/2\right)}$$

Using $q(0) = Q$, we get:

$$Q = \frac{1}{\rho} \frac{P_c(0) - P_c(L)}{2} \frac{\sqrt{\rho K_f}}{\tanh\left(\sqrt{\rho K_f} L/2\right)}$$

This could be more elegantly written as:

$$Q = \frac{1}{\rho} \frac{\sqrt{\rho K_f} L/2}{\tanh\left(\sqrt{\rho K_f} L/2\right)} \frac{P_c(0) - P_c(L)}{L}$$

(iv) What is the amount of water filtrating through the tissue? [10%]

Answer:

$$Q_f = Q - q(L/2)$$

$$Q_f = Q\left(1 - \operatorname{sech}\left(\sqrt{\rho K_f} L/2\right)\right)$$

(d) Is the approximation that $q(0) = q(L)$ physiologically realistic? What would happen in the tissue if $q(0)$ and $q(L)$ were different? [15%]

Answer: It is likely that in practice the amount going back to the circulatory system will be different as this relies on a very particular relationship between blood pressure, pressure drop and flow rate. There is in practice always an excess of plasma leaving the vessel, later collected by the lymphatic system.

Comments on Questions

Q1

Part (a) was straightforward and most students answered it properly. For part (b) students could choose either fast equilibrium or quasi-steady-state assumption, and both worked fine (although giving a slightly different meaning to the solution). Some students however announced one method, but used the other, so there seems to be some confusion about the meaning of these two methods. The other parts were generally well addressed with no systematic error.

Q2

This question, although relatively straight-forward once one realises how to approach it, confused most students who attempted it. All students did correctly the first part, but too many struggled with the second part. Instead of focussing on solving the diffusion equation in the exterior domain, many assumed that it had to be approached by a reaction-diffusion model inside the sphere, which could not lead to a meaningful solution. Considering the large number of students who failed to identify the right approach, the mark scheme has been adjusted. Marks were also awarded in order to reward partial solutions, approximate answers, or correct equations that were solved inside the sphere rather than outside.

Q3

All parts were well answered by some of the students, with the last part proving to be the most difficult - as expected as it required thinking beyond the lecture material.

Q4

The question was generally well answered. A few students did not think about using mass conservation for part (c), and a decent number were not comfortable enough with the differentiation of hyperbolic trig functions.

END OF PAPER