ENGINEERING TRIPOS PART IIB

Tuesday 6 May 2008 2.30 to 4

Module 4G2

BIOSENSORS

*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.*

There are no attachments.

STATIONERY REQUIREMENTS
Single-sided script paper

SPECIAL REQUIREMENTS
Engineering Data Book
CUED approved calculator allowed

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

1 At rest, the blood level of lactate is low at about 0.5-1.6 mM but as the intensity of exercise increases the lactate level rises. The plot in Fig. 1 shows the lactate level against the exercise intensity: it rises gradually at first but then becomes very steep as the exercise intensity (workload) increases. Eventually exhaustion occurs at which time the lactate level might be 10 or 20 times the resting level. For most individuals the exercise level at which the curve becomes very steep is constant. It is referred to as the `lactate threshold' at a level of about 3.5-4.0 mM. If the curve is plotted during various phases of training it will be seen that it shifts to the right when there is improved performance implying higher intensity exercise at the same or lower lactate levels.

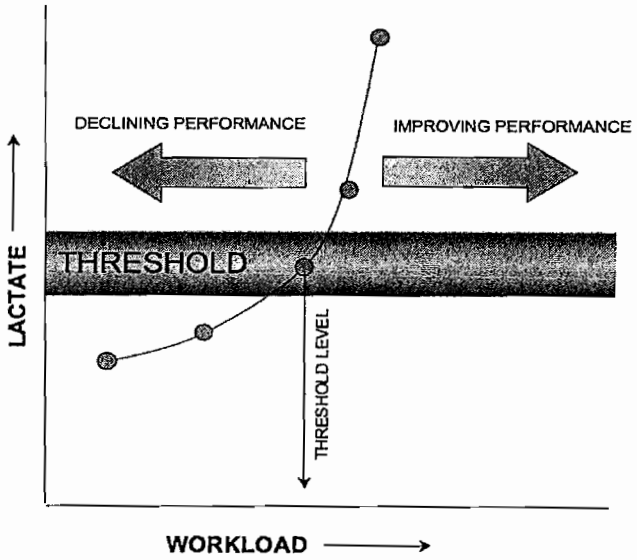

Fig. 1

You are given the flavoenzyme, L-lactate oxidase (L-LOD), and two L-lactate oxidase mutant enzymes, m1-LOD and m2-LOD to construct a family of lactate biosensors. The enzyme pathway for LOD and its mutants is:

$$L-lactate + LOD_{ox} \rightarrow pyruvate + LOD_{red}$$
$$O_2 + LOD_{red} \rightarrow H_2O_2 + LOD_{ox}$$

The mutant enzymes cause a change in the K_M for the enzyme. The K_M values are 5.7mM, 15 mM and 2 mM respectively.

(a) Describe an enzyme-based, lactate electrode amperometric biosensor that

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could form the sensing element for a blood lactate self-monitor kit, discussing the role of

(i) the enzyme

(ii) the mediator [30%]

(b) The rate of an oxidase enzyme catalysed reaction is given by

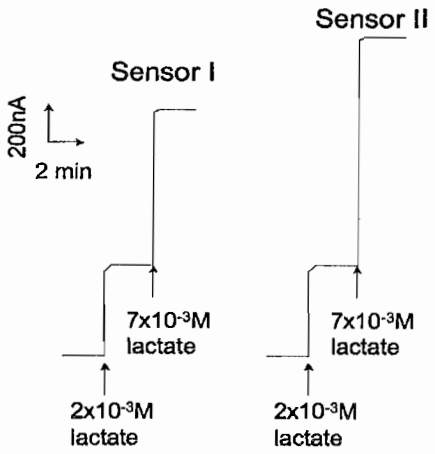
$$\frac{\mathrm{d}[\mathrm{S}]}{\mathrm{d}t} = \frac{k_2[\mathrm{E_o}][\mathrm{S}]}{K_\mathrm{M} + [\mathrm{S}]}$$

[S] is the enzyme substrate concentration, $[\mathrm{E_o}]$ the total enzyme concentration and K_M is the Michaelis Menten constant. K_M is a characteristic of the enzyme and is the substrate concentration at $V_{max}/2$, where V_{max} is the maximum rate. At the simplest level, the current at an enzyme electrode becomes

$$I_{enz} = \frac{nFdk_2[\mathrm{E_o}][\mathrm{S}]}{K_\mathrm{M} + [\mathrm{S}]}$$

Derive an expression for the maximum current response I_{max} and hence give I_{enz} at K_M and $2K_\mathrm{M}$ in terms of I_{max}. Sketch a plot of I_{enz} versus [S] and comment on the useful analytical range. [30%]

(c) The signal obtained from two of the LOD enzymes is shown below. Identify the mutant (LOD, m1-LOD or m2-LOD) used for each sensor and explain your reasoning. [20%]



(d) From the information given above discuss the suitability of the LOD, m1-LOD and m2-LOD to measure blood lactate levels during exercise in recreational exercise and for the elite athlete. [20%]

2 Immunoassay exploits the unique specificity of an antibody binding to an antigen to selectively recognise and determine antigens as analytes. It can be described according to the equilibrium:

$$S + L \underset{k_d}{\overset{k_a}{\Leftrightarrow}} SL$$

Where S = sample/analyte and L = antibody (ligand), so that

$$\frac{k_d}{k_a} = K_D = \frac{[S][L]}{[SL]}$$

Surface Plasmon Resonance (SPR) has often been used for immunoassay to measure the binding between the analyte (S) and a surface bound antibody (L).

(a) What parameter does SPR measure? Describe how SPR can be configured in the Kretschman format to perform immunoassay? [50%]

(b) An expression for dR/dt (R is the SPR signal at time t expressed in resonance units RU), which describes the rate of association between [S] and [L] is given by:

$$\frac{dR}{dt} = k_a[S](R_{max} - R) - k_d R$$

with total available ligand L immobilized on the SPR surface, ready for binding of analyte S, represented by R_{max}

$$R = \frac{k_a[S]R_{max}}{k_a[S] + k_d}\left(1 - \exp\left(-(k_a[S] + k_d)t\right)\right)$$

$$R = \frac{[S]R_{max}}{[S] + K_D}\left(1 - \exp\left(-(k_a[S] + k_d)t\right)\right)$$

A series of monoclonal antibodies (MABs) have been isolated and could be new antiviral agents with activity against hepatitis C virus (HCV) proteins. SPR is being used to provide a rapid screen for binding of these agents. A test protein is immobilized to the SPR chip and sensorgrams are obtained for three of the best candidate MABs. The

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binding curve sensorgrams are given in Fig. 2. K_D for the three candidate MABs were obtained from the sensorgrams

(i) MAB X1 showed a $K_D = 43$ nM

(ii) MAB X2 showed $K_D = 192$ nM

(iii) MAB X3 showed a $K_D = 658$ nM

From the equations given above, derive an expression to predict what R will be at long t (before dissociation) for [S]= $K_D/2$; [S]= K_D; [S]= $2K_D$ and hence assign each MAB to curve A, B or C, giving your reasoning. [50%]

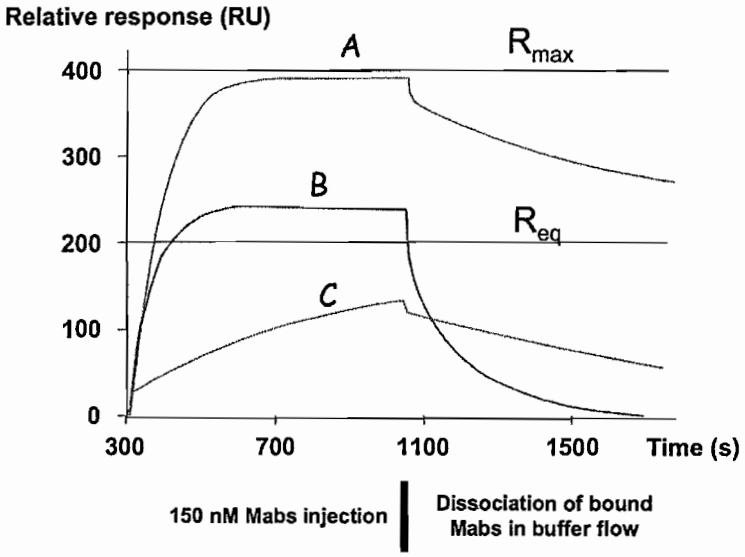

Fig. 2

3 An impedimetric device is proposed in an application for cell sorting. The device consists of two plane parallel electrodes separated by a gap through which the analyte solution is flown through as shown in Fig. 3. The electrodes have a length L and width W into the page. You can treat the solution as an electrical resistor of resistivity, ρ_s. You may assume that the cell can be treated as a dielectric material of dielectric constant ε_c and rectangular dimensions as shown in the figure. The width of the cell into the page is assumed to be larger than the electrode width.

(a) Assuming that there is no electrical double-layer formed at the interface between the electrodes and the solution, work out an equivalent electrical circuit model to estimate the impedance between the electrodes. Neglecting fringing field effects, calculate the net impedance between the two electrodes as a function of cell geometry and effective dielectric properties. Estimate the magnitude of the DC resistance as a function of cell size. Sketch the frequency response. [30%]

(b) Consider the double layer capacitance between the electrodes and the flow through solution as a capacitor of fixed magnitude per unit area C_{dl}. What is the origin of this capacitance? Draw a modified electrical circuit model including the double layer capacitance and estimate the impedance between the two electrodes. Sketch the frequency response. [30%]

(c) Explain how different cells may be differentiated as a function of transit time and relative impedance between the two electrodes measured at different frequencies. [20%]

(d) What are the advantages of micromachining a flow cytometer? Use the analysis in parts (a) and (b) to motivate your argument. [20%]

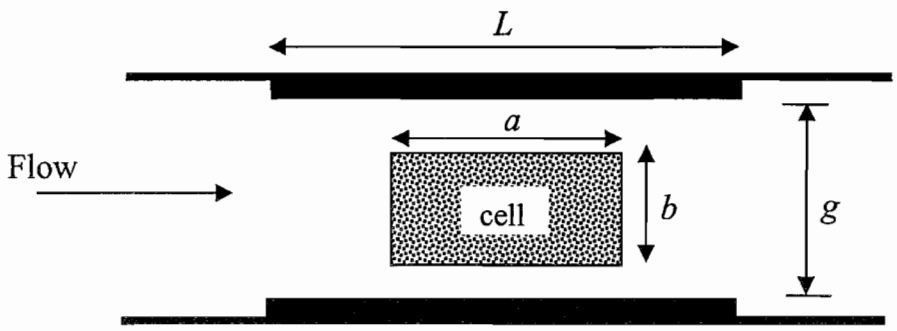

Fig. 3

4 (a) How is a quartz crystal microbalance (QCM) excited into a shear mode of vibration and plot the displacement profile for this mode across the thickness of the crystal. [20%]

(b) Draw the electrical equivalent circuit for an unloaded quartz crystal microbalance showing the parasitic parallel capacitor. Redraw this equivalent electrical circuit for the quartz crystal microbalance operating under mass loading and liquid loading. [20%]

(c) The frequency shift of a QCM due to elastic mass loading can be given by

$$\Delta f = -2f_0^2 \cdot \frac{\Delta m}{A\sqrt{\rho_q \mu_q}}$$

where f_0 is the nominal resonant frequency, Δm is the added mass, A is the active area of the crystal, ρ_q is the density of quartz equal to 2200 kg m^{-3} and μ_q is the shear modulus for quartz (30 GPa). Estimate the elastic mass loading per unit area that results in a 10 Hz frequency shift for a QCM operating at a fundamental mode of 10 MHz. [20%]

(d) The resonant frequency shift of a QCM due to contact with a viscous liquid may be written as

$$\Delta f = -f_0^{3/2}\sqrt{\frac{\rho_l \eta_l}{\pi \rho_q \mu_q}}$$

where ρ_l and η_l are the density and viscosity of the liquid in contact with the sensor. Estimate the resulting frequency shift induced due to liquid contact alone for a biosensing application in a liquid with density and viscosity equal to 1000 kg m^{-3} and 10^{-3} kg m^{-1} s^{-1}. [20%]

(e) Explain how the QCM may be used as an immunosensor and briefly explain how the rate constants associated with binding and disassociation processes may be extracted in the context of an immunosensing application. [20%]

END OF PAPER