ENGINEERING TRIPOS PART IIB

Tuesday 8 May 2007 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 Scheme 1 shows the pathway for a class of enzymes encountered in a common biosensor.

$$E_{ox} + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E_{red} + P$$

$$E_{red} + O_2 \xrightarrow{k_3} E_{ox} + H_2O_2$$

Scheme 1

(a) Give an example of an existing biosensor that uses this class of enzyme and discuss what can be measured to obtain an estimate of the substrate concentration [S]. [10%]

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

$$\frac{d[S]}{dt} = \frac{k_2[E_o][S]}{K_M + [S]}$$

[S] is the enzyme substrate concentration, $[E_o]$ 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).

Derive the equation above from scheme 1 and sketch a plot of [S] *versus* rate of enzyme reaction showing $V_{max}$ and $K_M$. [20%]

(c) An enzyme electrode is constructed with a layer of enzyme of thickness $d$ deposited on an electrode (scheme 2).

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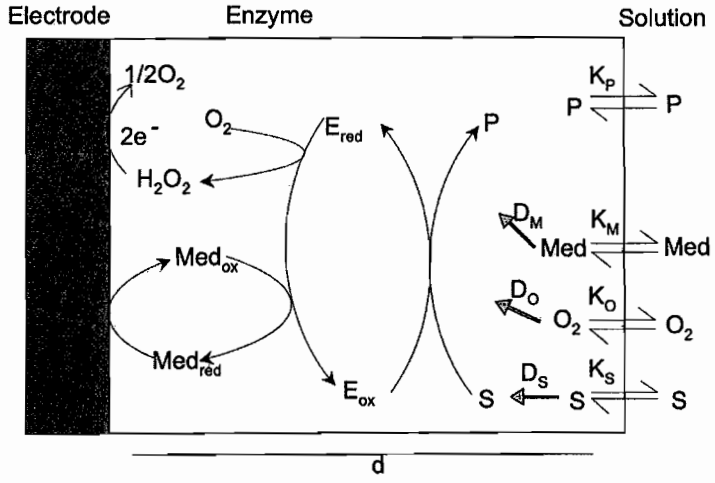


scheme 2

A current is measured at the electrode due to:

i. the oxidation of hydrogen peroxide or
ii. the oxidation of mediator (Med)

What is a mediator? Why is a mediator used in this biosensor? What are the characteristics of a good mediator? [20%]

(d) The steady state current at an amperometric electrode under diffusion control is:

$$I_d = \frac{nFD[S]}{d}$$

where $d$ is the diffusion layer thickness, F is Faraday's constant, D is the diffusion coefficient of the measured species and [S] is the substrate/analyte concentration. At the simplest level, the current at an enzyme electrode approximates to:

$$I_{enz} = \frac{nFdk_2[E_o][S]}{K_M + [S]}$$

Derive an expression for the maximum current response $I_{max}$ and hence obtain normalized expressions for $I_{enz}/I_{max}$ and $I_d/I_{max}$. [30%]

(e) Sketch plots of [S] *versus* $I$ for $I_{enz}$ and $I_d$. [20%]

2 Surface plasmon resonance (SPR) is an attenuated total reflection technique that has been widely exploited for biosensors and is presented as offering a 'label-free' assay. Under conditions of attenuated total reflection, as required for SPR, the angle of transmission is complex. This means that the transmitted wave only has real terms in the $z$ direction, so it is an evanescent wave, normal ($z$-direction) to the interface ($x$-direction) with the electric field $E$ expressed as

$$E = E_0 \exp\left\{\frac{2\pi n_1\left(\sin^2\theta - (n_2/n_1)^2\right)^{1/2} z}{\lambda}\right\}$$

Here subscripts 1 and 2 refer to the regions of material where light of wavelength $\lambda$ is incident and refracted and $n$ refers to the refractive index. The dispersion relation for the surface plasmon is given by

$$K_\omega = \frac{\omega}{c}\sqrt{\frac{\varepsilon_1\varepsilon_2}{\varepsilon_1 + \varepsilon_2}}$$

where $\varepsilon$ refers to the dielectric constant of the medium and c is the speed of light.

(a) What is the penetration depth for this evanescent wave and why is this useful to exploit in a biosensor? [30%]

(b) What is surface plasmon resonance and why can it be used to perform unlabelled immunoassay? [30%]

(c) The most common prism based system for SPR is the Kretschman configuration. Sketch and describe the Kretschman configuration and explain the conditions required for excitation of the surface plasmon, also sketching the dispersion relations. [40%]

3. An impedimetric device is proposed for use in a biosensor application. The device consists of two plane parallel electrodes of area $A$ separated by a gap $d$ through which an analyte solution is flown through.

(a) Explain briefly the terms, impedance spectroscopy and dielectric spectroscopy? [20%]

(b) Sketch an equivalent electrical circuit for a substance having a single relaxation time and a typical complex permittivity plot of the dielectric data obtained therefrom. [20%]

(c) A buffered saline solution consisting of a known globular protein is flown through the device. This protein molecule has a permanent dipole of a 100 D (1 D is $3.336 \times 10^{-30}$ C m) and is associated with a relaxation time $\tau$ in the electric field applied between the electrodes. The rotational relaxation time is given by:

$$\tau = \frac{4\pi\eta a^3}{k_B T}$$

where $\eta$ is the viscosity of the solution equal to $1.8 \times 10^{-3}$ kg m$^{-1}$ s$^{-1}$, $T$ is room temperature (300K), $k_B$ is the Boltzmann constant and $a$ is the radius of the globular protein known to be approximately 2 nm. Estimate this rotational relaxation time. Hence, estimate the 'characteristic' frequency at which the permittivity of the analyte solution dips. [20%]

(d) The observed dielectric shift for this analyte solution is found to be equal to 20 at the characteristic frequency. The dielectric shift $\Delta\varepsilon$ can be related to the protein concentration by the expression:

$$\Delta\varepsilon = Ncm^2 / 2\varepsilon_0 M k_B T$$

where $N$ is Avogadro's number, $\varepsilon_0$ is the permittivity of free space, $c$ is the protein concentration in mg/mL, $m$ is the molecular dipole moment in SI units, $M$ is the protein's molecular weight which is equal to 10 kDa. Estimate the protein concentration for this experiment. Sketch the observed dielectric shift response. [20%]

(e) The device is proposed to be built in a microfabricated format with integrated fluidic handling and sensing. Comment on the advantages of microfabrication for this technique. [20%]

(TURN OVER

4 (a) Explain briefly the application of a Quartz Crystal Microbalance (QCM) to biosensing. [20%]

(b) Immunoassay exploits the unique specificity of an antibody binding to an antigen to selectively recognize and determine antigens as analytes. It can be described very simply, according to the equilibrium

$$A + L \underset{k_d}{\overset{k_a}{\Leftrightarrow}} AL$$

where A = analyte and L = antibody (ligand), so that:

$$\frac{k_d}{k_a} = K_D = \frac{[A][L]}{[AL]}$$

QCM has often been used for immunoassay. An expression for d$R$/d$t$ ($R$=QCM signal at time $t$ in relative units), which describes the rate of association between [A] and [L] is given by:

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

with total available ligand immobilized on the QCM surface, ready for binding of antibody, represented by $R_{max}$. Show that the output signal can be written as:

$$R = \frac{k_a[A]R_{\max}}{k_a[A] + k_d}\left(1 - \exp\left(\left(k_a[A] + k_d\right)t\right)\right)$$ [30%]

(c) The QCM is used in an assay to detect the presence of an antigen in an unknown biological sample. The QCM surface is pre-coated with an antibody that binds specifically to the antigen and the remainder of the surface is passivated. Next the analyte solution is flowed through. The crystal operates at a fundamental resonant frequency of 5MHz and a frequency shift of 10 Hz is recorded at steady state as the analyte solution is flown through as shown in Fig. 1. The mass sensitivity of the crystal is assumed to be 10 ng cm$^{-2}$ Hz$^{-1}$. Given that the molecular mass of the analyte is 100 kDa, estimate the binding density of the analyte. Viscoelastic effects may be ignored for this calculation but you should comment on the impact of including a viscoelastic term in your model. [30%]

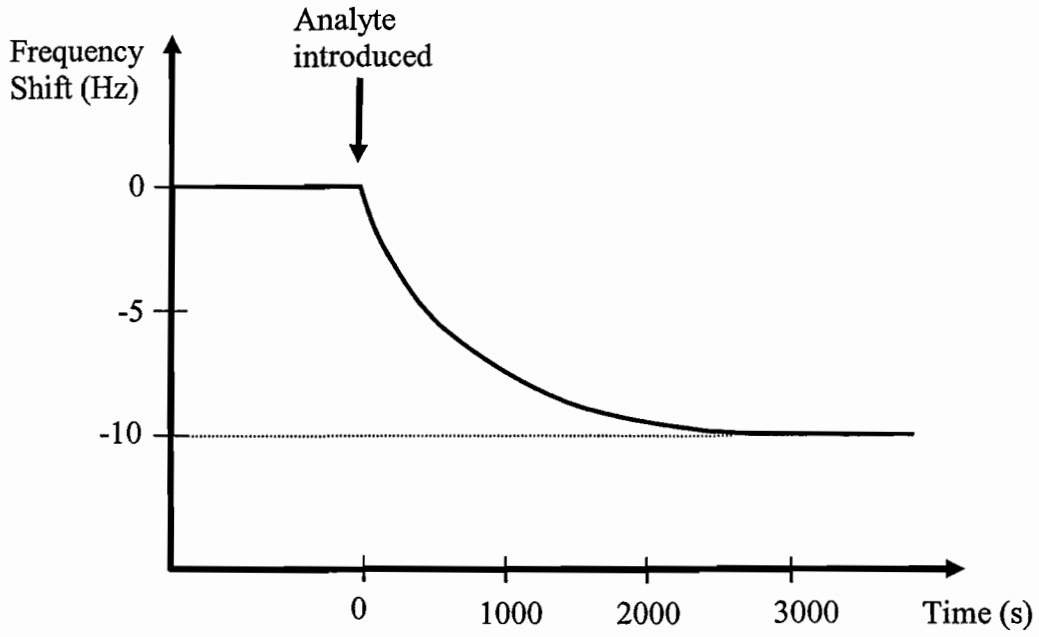


Fig. 1

(d) Explain how you would work out fundamental rate constants and analyte concentration from this experiment? [20%]

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