ANSWERS

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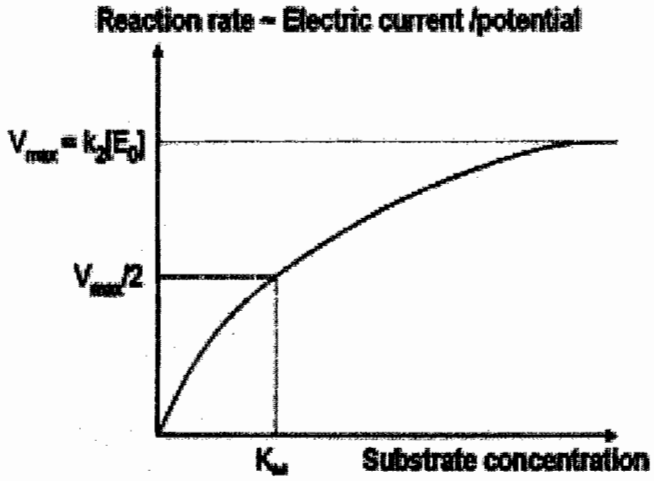
(a) This is the scheme for an oxidase enzyme, for example glucose oxidase. It was used in the first biosensor for glucose, where the enzyme was placed on an oxygen electrode and the concentration of glucose could be monitored by measuring the decrease in the current due to oxygen reduction at *ca.* -0.65 V *versus* Ag/AgCl. Alternatively, glucose concentration could be correlated with production of hydrogen peroxide, which can be oxidized at a potential of *ca.* +0.6 V *versus* Ag/AgCl.

(b) The dose response curve arises from solving the pathway in scheme 1 :



(c) The reduced oxidase enzyme (eg glucose oxidase, GOx) normally forms a complex with an electron acceptor (such as O_2), regenerating the active, oxidized form of the enzyme, as in scheme 1. An enzyme **mediator** is an artificial co-substrate, that replaces O_2. It is a redox couple that gives efficient and rapid electron transfer to or from the enzyme.

A mediator is used to avoid interference by other things that may be present in the sample, eg ascorbic acid, uric acid, acetaminophen. Hydrogen peroxide is oxidized at a higher potential than many interferents and thus at the potential required to perform the peroxide assay, the current measured potentially leads to falsely high readings. Accordingly, a key consideration is to lower the measuring potential below that for the interferents (ideally within the optimal potential window of −100 to 0 mV versus SCE, where oxidation of most electrochemical interferents is avoided). Mediators with lower redox potential allow this.

An ideal redox mediator has to display stable oxidized and reduced forms, and fast reaction rates both with the enzyme and on the electrode interface.

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

This gives a maximum current response I_{max}, when [S] » K_M.

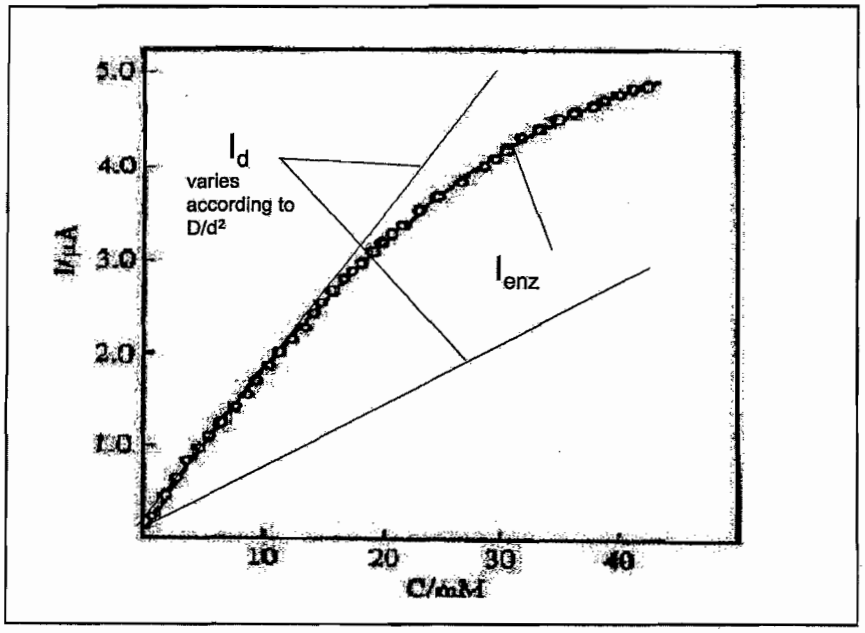
$$I_{max} = nFdk_2[E_o]$$

so that the diffusion limited current could be described by:

$$\frac{I_d}{I_{max}} = \frac{D[S]}{d^2 k_2[E_o]}$$

and the enzyme kinetic current:

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



(e) I_d is linearly proportional to [S] (concentration), whereas I_{enz} shows a typical Michaelis Menten type curve. A linear approximation is often used for a biosensor, but this is only valid across a narrow concentration range below the K_M. At higher concentration the sensitivity becomes lower and if the linear calibration is extended into this region, the current reading obtained will predict too low a concentration.

(a) The evanescent field is decaying in the z direction with a penetration depth d_p,

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

which infers that the attenuation of the signal will be greatest at the interface and zero in the bulk. This is useful in designing a sensor since the reaction taking place on the sensor surface within the evanescent field can be detected, whereas the bulk sample solution outside the evanescent field does not influence the signal.

(b) Surface plasmon are surface electromagnetic waves that propagate parallel along a metal/dielectric interface. They are excited by an exterior electric field of the right energy, on a plasmon boundary, which causes discontinuities in the normal component of the electric field across the interface. When coupling occurs, oscillating surface charges are produced that propagate along the surface. These oscillations couple with E_Z, the field extending in the z direction, and for a surface plasmon wave existing between two media of dielectric constant ε_1 and ε_2, the wave is confined at the interface with dispersion relation $\mathbf{K}_\omega$ in the propagtion direction and $\mathbf{k}_{Z1}$ and $\mathbf{k}_{Z2}$ in the $+z$ and $-z$ directions, where continuity across the interface requires that:

$$\frac{\mathbf{k}_{z1}^2}{\varepsilon_1^2} = \frac{\mathbf{k}_{z2}^2}{\varepsilon_2^2}$$

so that an expression for $\mathbf{K}_\omega$ can be obtained

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

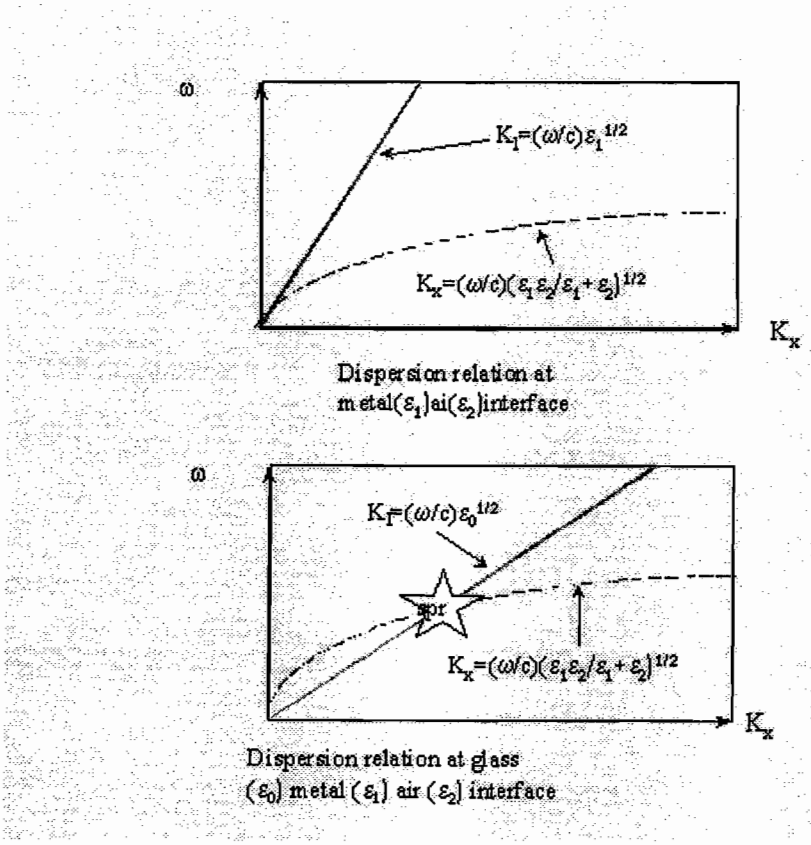
The conditions for SPR excitation are dependent on several factors, including: characteristics of the metal film, the incident light, and the thickness and refractive index of the molecular layer in contact with the metal sensing surface. The binding of biomolecules at the metal surface results in the change of the refractive index on the sensor surface, thereby changing the value of ε_2, which is measured as a change in resonance angle or resonance wavelength. Thus in immunoassay an antibody can be immobilised on the surface of the SPR Au layer and the binding of antigen will be detected as a change in refractive index, without requiring a label.

Figure illustrating this.

(c) The surface plasmon can be excited by incident light of the same frequency and $\mathbf{k}_x$ component. For light incident on a metal (ε_2) from a dielectric ε_1 the $\mathbf{k}_x$ component will be:

$$\mathbf{k}_x^2 = \varepsilon_1\left(\frac{\omega}{c}\right)^2 \sin^2\theta$$ where θ is the angle of incidence

but this is always less than the SPR dispersion relation, so there is no excitation. See sketch of dispersion relations.



This can be overcome by coupling the evanescent field across different dielectric materials. For example, in the Kretschman configuration the light is incident in a medium of higher dielectric constant ($\varepsilon_0 > \varepsilon_1$), which is in contact with a thin metal film, the plasmon resonance can be excited on the opposite interface

The resonance condition of the light in the prism with the surface plasmon at metal (2) | air (1) interface (Kretschmann) is

$k_{0x} = k_{spx}$

$$\frac{\omega}{c}\sqrt{\varepsilon_0}\sin\theta_0 = \frac{\omega}{c}\sqrt{\frac{\varepsilon_1\varepsilon_2}{\varepsilon_1+\varepsilon_2}}$$

see dispersion relation sketch.

The evanescent electrical field associated with the plasma wave travels for a short distance (~300 nm) into the medium from the metallic film. Because of this, the resonant frequency of the surface plasma wave (and thus θspr) depends on the refractive index of this medium. If the surface is immersed in an aqueous buffer (refractive index ~1.0) and protein (~1.33) binds to the surface, this results in an increase in refractive index, which is detected by a shift in the θspr. At resonance, a minimum in reflected light intensity will be observed, and the SPR angle can thus be determined by measuring the intensity of the reflected light, and plotting it as a function of incidence angle.

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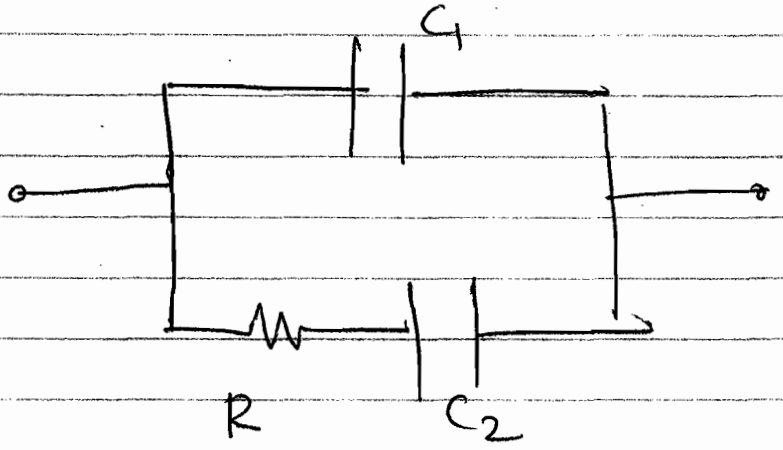
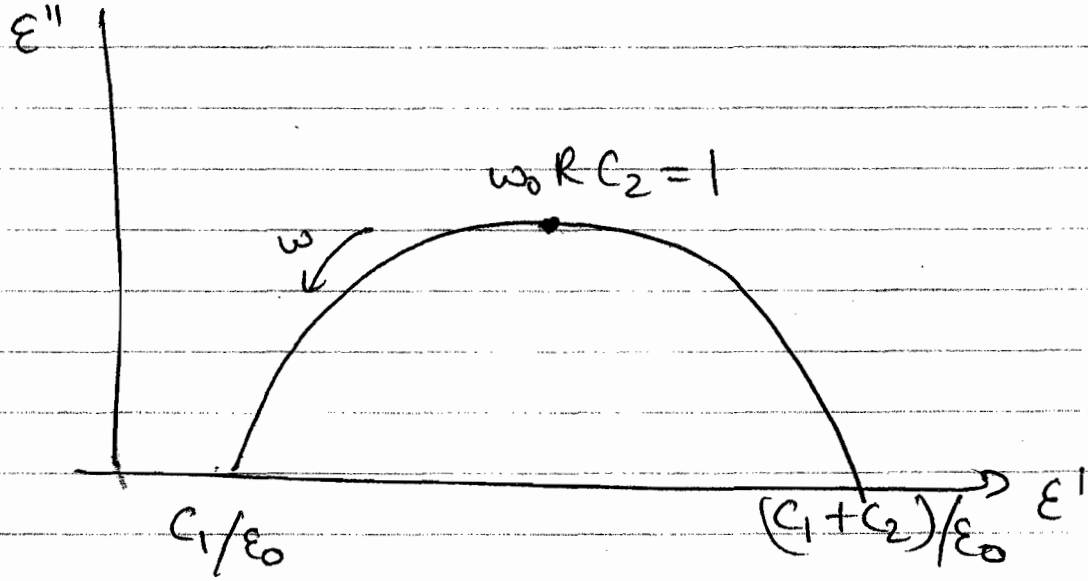
(a) An ~~experiment~~ technique to study the absorption of electrical energy by the (biological) system as a function of frequency. Measures of energy absorption include "impedance" and "admittance" of the sample sandwiched between 2 parallel plate electrodes. In certain systems, the permittivity variation with frequency gives useful insight into dielectric properties → dielectric spectroscopy

(b)





(c) $$\tau = \frac{4\pi \times 1.8 \times 10^{-3} \times (2 \times 10^{-9})^3}{4.14 \times 10^{-21}}$$

$$\tau = 4.37 \times 10^{-8} \text{ s}$$

$$f_c = (2\pi\tau)^{-1} = 3.64 \text{ MHz}.$$

(d) $$c = \frac{20 \times 2 \times 8.85 \times 10^{-12} \times 10^{3} \times 4.14 \times 10^{-21}}{6.02 \times 10^{26} \times (100)^2 \times (3.33 \times 10^{-30})^2}$$

$$c = 219 \text{ mg/mL}$$

(e) The advantages include handling of smaller sample volumes, integration both electronics, parallelisation to multiple simultaneous assays.

Q4

(a) The quartz crystal microbalance consists of a piezoelectrically excited quartz crystal configured as a sensitive mass balance by treating the surface of the sensor in a very specific manner. For a biosensing application for instance, a ligand may be immobilized to the surface of the crystal. Both electromechanical impedance and resonant frequency shift in response to a binding reaction on the surface can be measured to give an estimate of the analyte concentration, binding density rate constants.

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

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

Integrating :- with boundary condition $R(0) = 0$

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

(b) Details:

$$\int_0^R \frac{dR}{k_a[A]R_{max} - R(k_a[A] + k_d)} = \int_0^t dt$$

$$-\frac{\ln\left[k_a[A]R_{max} - R(k_a[A] + k_d)\right]}{k_a[A] + k_d}\Bigg|_0^R = t$$

(b) $$\ln \frac{k_a[A]R_{max}}{k_a[A]R_{max} - R(k_a[A]+k_d)} = (k_a[A]+k_d)t$$

$$\therefore 1 - \frac{R(k_a[A]+k_d)}{k_a[A]R_{max}} = e^{-(k_a[A]+k_a)t}$$

$$\text{or } R = \frac{k_a[A]R_{max}}{k_a[A]+k_d}\left(1 - e^{-(k_a[A]+k_a)t}\right)$$

as required. ⟶ (1)

(c) $$\therefore \Delta m/A = 10\ ng/cm^2 Hz \times 10\ Hz$$

$$= 100\ ng/cm^2$$

$$MW = 100\ kDa$$

$$\therefore \Delta N/A = \frac{100 \times 10^{-9}\ g/cm^2}{100 \times 1.67 \times 10^{24}\ g \times 1000}$$

$$= 5.99 \times 10^{11}\ /cm^2$$

Assuming even distribution on surface:
nearest neighbour spacing
$= 1.29\ \mu m$

(d) The rate constants can be obtained by fitting the experimental

curve plotted on a log-log plot to a straight line: used to obtain the slope $\Rightarrow k_a[A] + k_d$. The normal magnitude is $\frac{k_a[A] R_{max}}{k_a[A] + k_d}$.

Knowing R_{max} (max binding density of ligand) this can be worked out. In practice, a calibrati is required vs. sample concentration.