2008 IIB 4G2 BIOSENSORS DR AA SESHIA

Biosensors Exam Crib

Question 1 Answer

a. This is an oxidase enzyme. The first example of such an oxidase enzyme biosensor was 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. The same construction could be used for lactate. Alternatively, the substrate concentration can be correlated with production of hydrogen peroxide, which can be oxidized at a potential of *ca.* +0.6 V *versus* Ag/AgCl.

A further possibility is to use a mediator. The reduced oxidase enzyme (here lactate oxidase, LOD) normally forms a complex with an electron acceptor (such as O_2), regenerating the active, oxidized form of the enzyme, as given in the scheme. 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. 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.

A mediator is used to avoid interference by other things that may be present in the sample, eg ascorbic acid, uric acid, acetaminophen. In measuring lactate during exercise, all these interferents are highly possible. 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. If a mediator is chosen with a lower redox potential that can still interact with the enzyme, then the measuring potential can be 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).

b. Given: $I_{enz} = \frac{nFdk_2[E_o][S]}{K_M + [S]}$ and given $V_{max}/2$ is rate at substrate conc of Km;

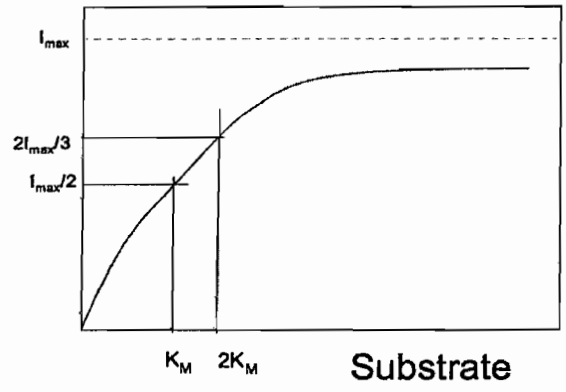
thus $I_{max}/2$ is current at substrate conc. of Km.

At [S]=Km $I_{enz} = \frac{nFdk_2[E_o]}{2} = \frac{I_{max}}{2}$

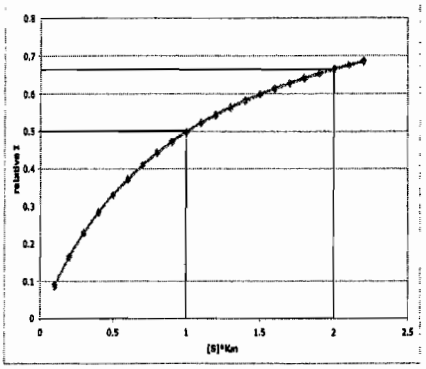
$I_{max} = nFdk_2[E_o]$

At [S] =Km $I_{enz} = \frac{I_{max}}{2}$; At [S]=2Km $I_{enz} = \frac{2I_{max}}{3}$

current



Sketch & excel plot for comparison!



Useful analytical range is below Km, since it is usually approximated to a linear fit over a narrow calibration range with the highest sensitivity. At higher concentration the sensitivity becomes lower and if a linear calibration is extended into this region, the current reading obtained will predict too low a concentration. Useful analytical range generally seems to be of the order of 0.5Km units, with the calibration concentration centred in this range.

c. Km values given in the Qu are: LOD =5.7; M1-LOD =15; M2-LOD =2 mM Response for 2mM and 7mM lactate are recorded

For M2-LOD with Km=2mM, V_{max} would be twice the signal obtained at 2mM. But signal due to 7mM lactate is greater than this for both sensors, so Sensor I and Sensor II are unlikely to be M2-LOD.

Conversely for M1-LOD, both 2mM and 7mM are much less that Km and would be on the more linear part of the calibration curve. For Sensor I the 7mM response < 3.5 times 2mM response. For Sensor II the 7mM response ~ 3.5 x 2mM response, so predict this sensor uses M1-LOD.

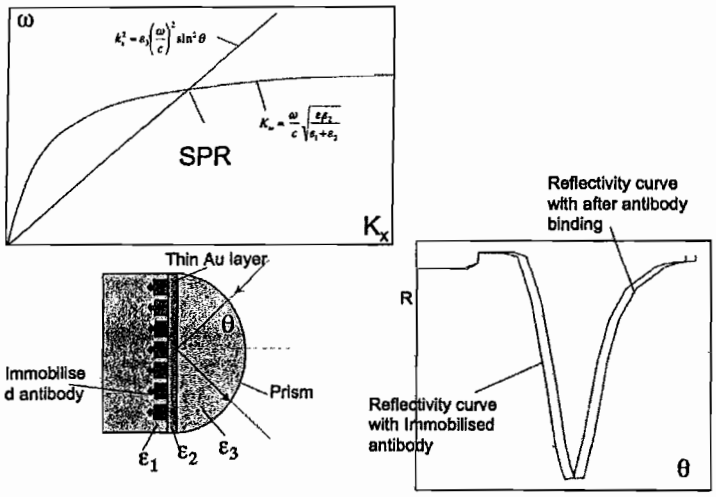
For LOD, 2mM<Km, but 7mM>Km, so this is consistent with the response seen for Sernsor I.

d. For measurement of lactate in exercise, measurements around the threshold level of 3.5-4.0mM are required. For the wild type enzyme LOD this is towards the upper end of the linear range (Km 5.7), but is useful for following the gradual increase in lactate from rest to threshold lactate. It will not measure very high levels

under extended exercise. For this purpose M1-LOD with a nearly linear range up to ~15mM would be more suitable. To follow the full range both enzymes are needed. M2-LOD will saturate at 2mM, below the threshold potential, so this enzyme does not seem to be very suitable for measurement of blood lactate during exercise.

Question 2 answer

a. Surface plasmon resonance experiments measure optical thickness (dielectric x thickness). 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_1, which is measured as a change in resonance angle or resonance wavelength.



In the Kretschman configuration the light is incident in a medium of higher dielectric constant ($\varepsilon_3 > \varepsilon_1$), which is in contact with a thin metal film, the plasmon resonance can be excited on the opposite interface –figure above.

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

$$\frac{\omega}{c}\sqrt{\varepsilon_3}\sin\theta_0 = \frac{\omega}{c}\sqrt{\frac{\varepsilon_1\varepsilon_2}{\varepsilon_1+\varepsilon_2}}$$

see dispersion relation sketch.

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

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:

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

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

At resonance, a minimum in reflected light intensity will be observed, and this SPR angle can thus be determined by measuring the intensity of the reflected light, and

plotting it as a function of incidence angle. 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 ε_1. The field only extends as far as the evanescent field penetration depth at the surface of the metal film, so the change in SPR is only sensitive to binding occurring on the surface within the evanescent field. This is important because it means that it is insensitive to things in the bulk solution, so the assay becomes selective and doesn't require labels.
When the antigen binds to the antibody, the position of the resonance minimum will change.

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

b. from:
$$R = \frac{[S]R_{\max}}{[S]+K_D}(1-e^{(k_a[S]+k_aK_D)t})$$

for long t

$$R = \frac{[S]R_{\max}}{[S]+K_D}$$

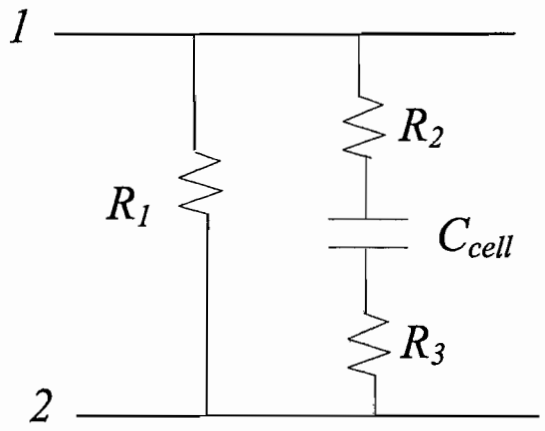
$$for [S]=K_D; R=\frac{R_{\max}}{2}$$

$$for [S]=\frac{K_D}{2}; R=\frac{R_{\max}}{3}$$

$$for [S]=2K_D; R=\frac{2R_{\max}}{3}$$

Thus, for the sensorgrams given here with [S]=150nM, curve B is close to $R_{max}/2$ and thus belongs to X2 which showed $K_D = 0.192\ \mu M$. X1 showed a $K_D = 0.043\ \mu M$ and belongs to curve A. X3 showed a $K_D = 0.658\ \mu M$ and belongs to curve C.

3. (a) Equivalent circuit without double layer capacitance included:



Here, assuming that the cell has the same width into the page (or larger) than the electrodes:

$R_1 = \frac{\rho_s g}{(L-a)W}$ DC solution resistance when cell is fully enclosed by the two electrodes

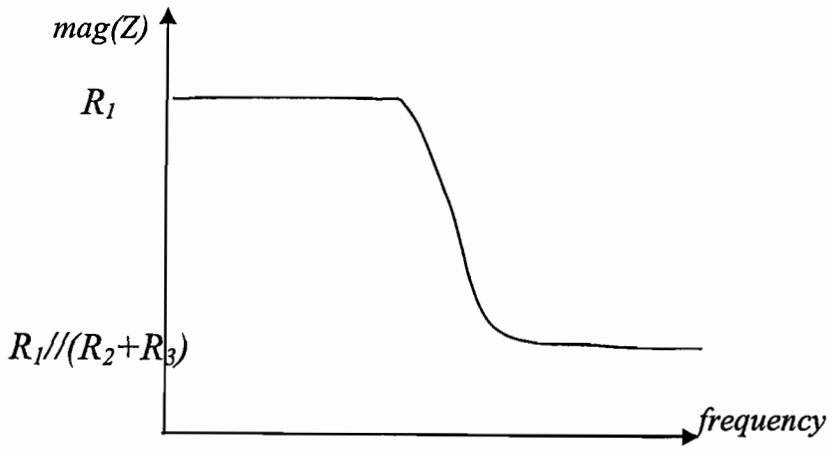
$R_2 + R_3 = \frac{\rho_s (g-b)}{aW}$

$C_{cell} = \frac{\varepsilon_c a W}{b}$ capacitance of the cell

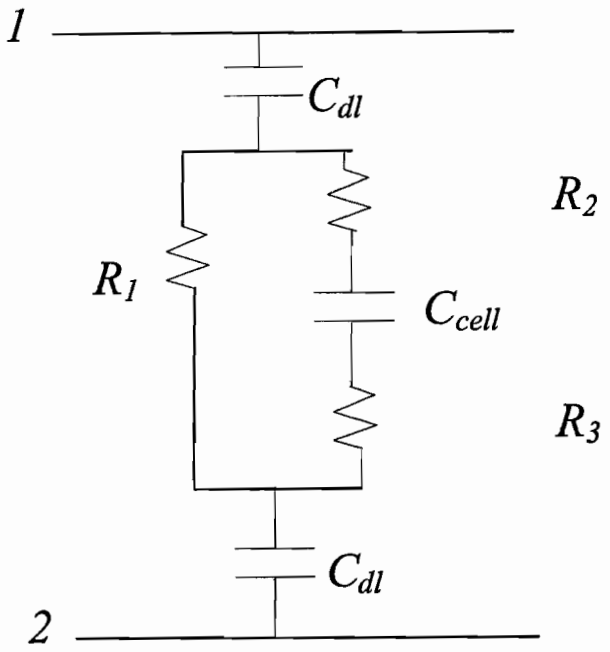
The net impedance (Z) between terminals 1 and 2 is given by:

$$Z = R_1 // \left(R_2 + R_3 + \frac{1}{j\omega C_{cell}} \right)$$

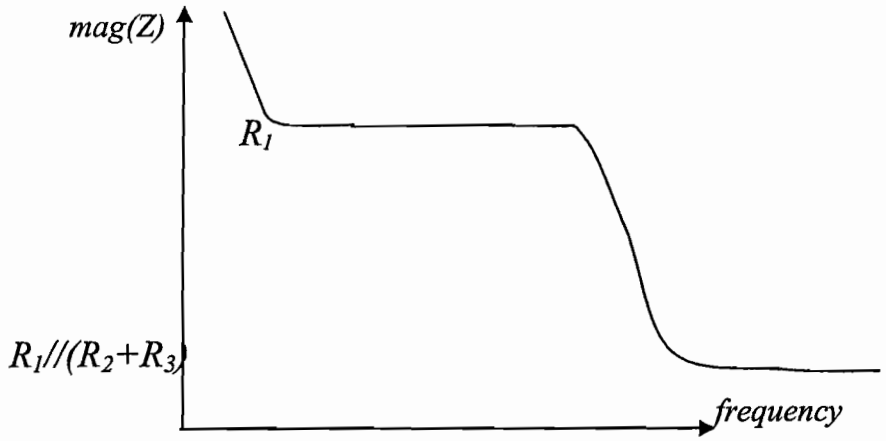
The frequency response is sketched as:



(b) With the double layer capacitance added, the equivalent electrical circuit is modified as:



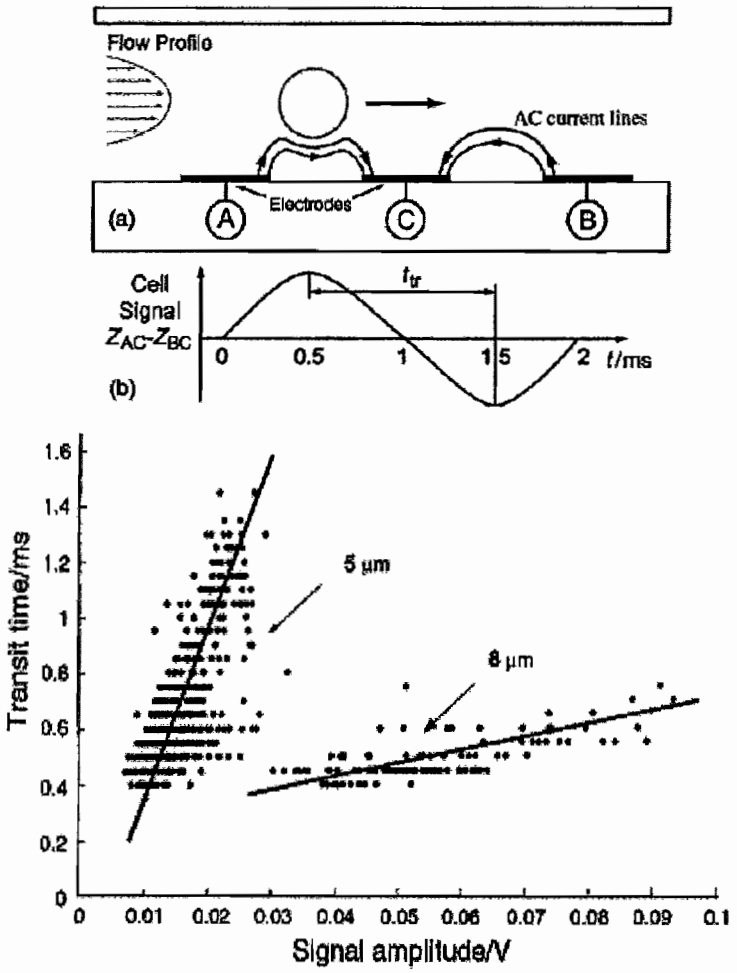
The impedance can be estimated and the modified frequency response is now sketched as:



The rollover frequencies (poles) can be easily obtained as well.

The origin of the double layer capacitance lies in the accumulation of ionic charge preferentially near a polarizable surface or a surface possessing fixed charge. While, the electrolyte solution as a whole remains electrically neutral a preferential accumulation of ions of one type near the electrodes occurs, diffusion and drift compete to set up a charge distribution at the surface that decays over a length scale defined by the Debye layer thickness.

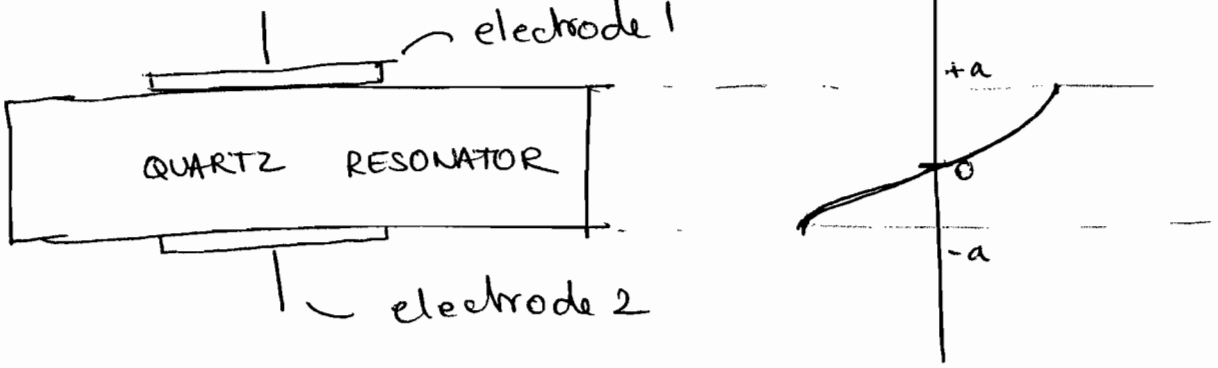
(c) Cells may be distinguished in a flow through microfluidic setup that is integrated together with pairs of electrodes as shown in the figure below. The larger cells move slower in solution as compared to the smaller cells. In addition, the impedance variation induced by cells with different electrical properties at a given frequency can be different. If these two quantities (transit time and impedance) are plotted on a set of axes, it may be possible to distinguish cells based on type as shown below.



(d) Advantages of micromachining a flow cytometer: higher sensitivity to single cell geometry and material properties and therefore larger relative signals, ability to parallelise experiments on a small scale, ability to extract geometrical parameters for individual cells from electrical measurements.

Q4

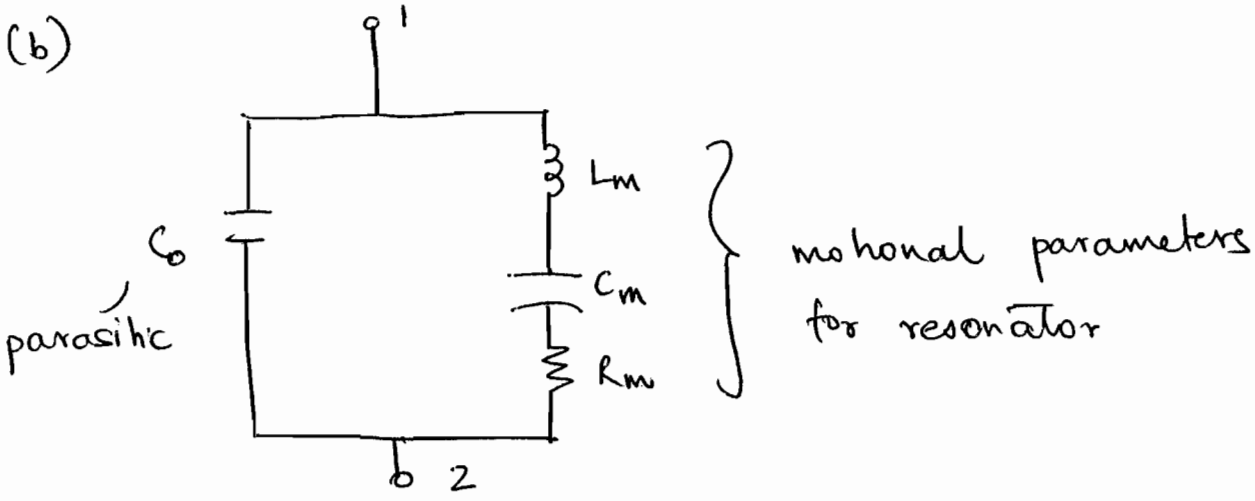
(a) Quartz is a piezoelectric material. A thickness shear mode is excited with gold electrodes patterned on either side of the structure (a)



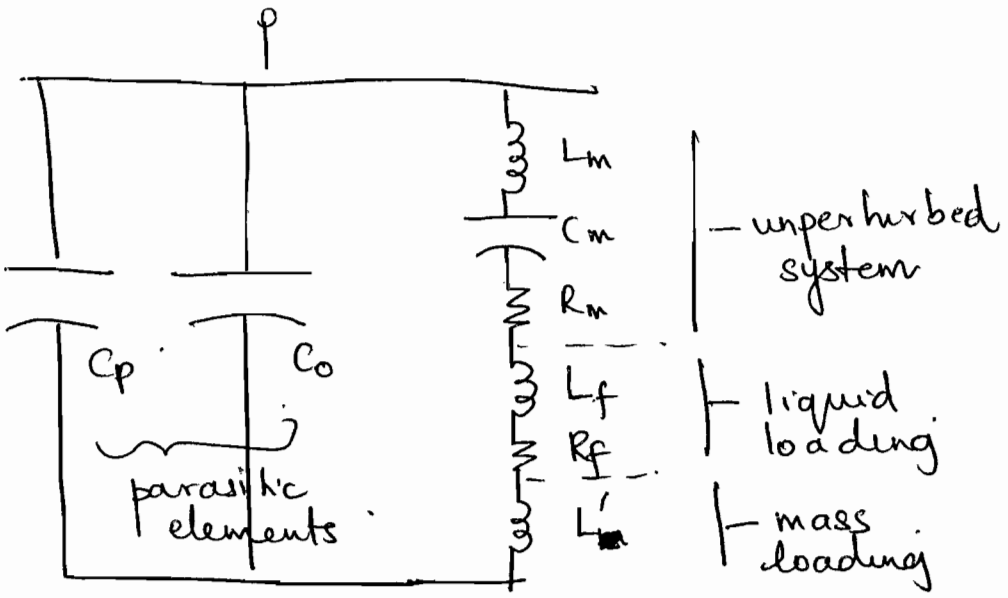
(a) (b)

(b) shows the fundamental mode displacement profile across the thickness of the crystal.

(b)



Under mass and liquid loading we have:



(c) $\frac{\Delta m}{A} = \frac{\Delta f}{2 f_o^2} \sqrt{\rho_q \mu_q}$

$= 4.06 \times 10^{-8}$ kg/m^2

$= 4.06 \times 10^{-12}$ kg/cm^2

$= 4.06$ ng/cm^2

(d) $\Delta f = -f_o^{3/2} \sqrt{\frac{\rho_l \eta_l}{\pi \rho_q \mu_q}}$

$= -(10^7)^{3/2} \sqrt{\frac{1000 \times 10^{-3}}{\pi \times 2200 \times 30 \times 10^9}}$

$= -2196$ Hz

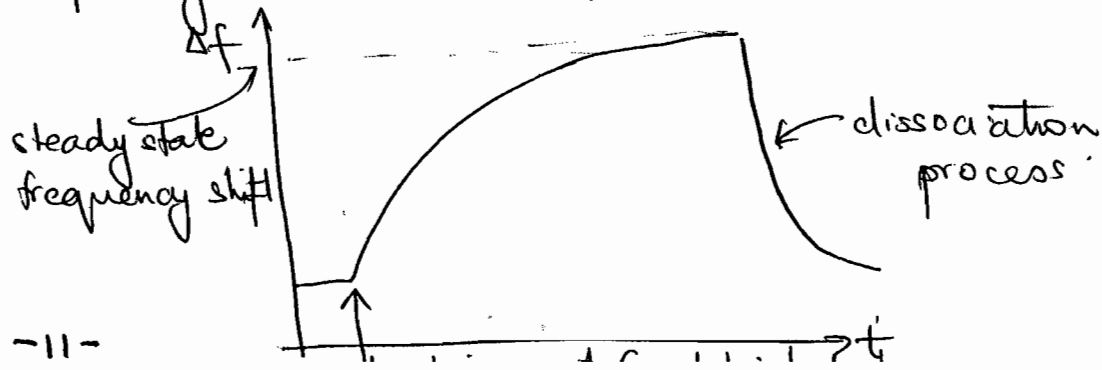
(substantially higher than recorded for mass loading above).

(e) A QCM may be employed as an immunosensor by binding an antibody (ligand) onto the surface of the QCM chip that binds very specifically to the target analyte of interest within the sample. i.e. the surface of the QCM is coated with an immobilized antibody. In presence of an analyte, an equilibrium reaction is set up where

$$A + L \rightleftharpoons AL$$

A = analyte; L = ligand; AL = bound complex consisting of antibody + analyte

A first order model may be used to describe the binding event such that the output signal (frequency shift at resonance) for the QCM can be used to extract the forward + reverse binding constants. Upon mass loading the frequency shift (Δf) output is:



The time constant for the binding process is proportional to $k_a[A]+k_d$ where k_a – foward reaction (association) constant; k_d – dissociation constant, $[A]$ – analyte concentration which is estimated from the magnitude of frequency shift at steady state. Through the dissociation process, the sample bound onto the surface of the crystal is removed by typically passing over a flow through buffer solution until the frequency shift reaches steady state. The time constant for this process is proportional to k_d. From these measurements it is possible to extract k_a, k_d.