Chapter 11
Piezoelectric Sensors

Although optical and electrochemical transducers are the two most popular transducers for biosensors, piezoelectric transducers have also gained some popularity in the past couple of decades. Like optical and electrochemical transducers, piezoelectric transducers can be used as-is as physical sensors (to sense mass), or used with bioreceptors as biosensors (to quantify biomolecules). Collectively they are called *piezoelectric sensors*.

11.1 Piezoelectricity

All piezoelectric sensors work on the principle of piezoelectricity. In the late nineteenth century, the Curie brothers (the younger brother, Pierre, was Marie Curie's husband) found that an electrical voltage was generated when they compressed or stretched *quartz*. This is called *piezoelectric effect*. This effect is reversible, meaning that quartz can be lengthened or shortened when an electrical voltage is applied. *Piezoelectricity* refers to the material's ability to exhibit this piezoelectric effect.

Other materials can also exhibit piezoelectricity, but quartz is still the most popular and the most used material for piezoelectric sensors. Quartz (SiO_2) has a unique, tetrahedron crystal structure as shown in Fig. 11.1. There are four oxygen molecules in a single tetrahedron (SiO_4), where all four oxygen molecules are shared with nearby tetrahedra (thus it becomes SiO_2). These tetrahedra are stacked up in a highly ordered manner to create a much larger crystal structure.

Like any other materials, the distribution of polarity is not uniform throughout its crystal structure, creating *dipoles*. As typical quartz is *monocrystal*, indicating all tetrahedra are ordered in one direction, thus the dipoles are also ordered in one direction (Fig. 11.2 top).

Some other materials have *polycrystal* structure, meaning that the directions of dipoles are not in one direction. It is possible to use this polycrystal material as

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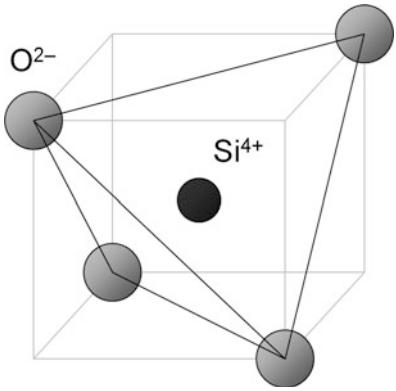


Fig. 11.1 A quartz tetrahedron

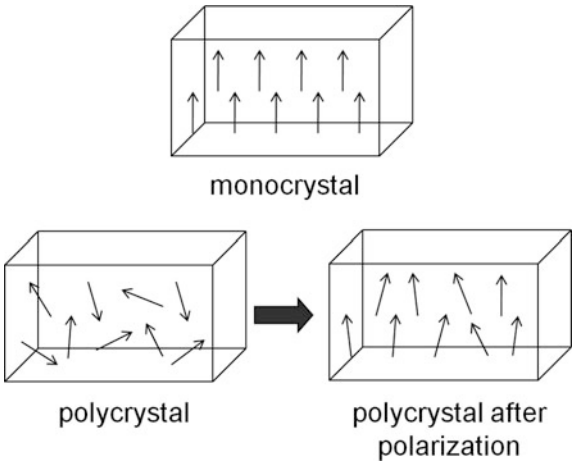


Fig. 11.2 Dipoles are well aligned in monocrystal but random in polycrystal. The dipoles in polycrystal can be aligned through polarization

piezoelectric sensor by applying an electrical voltage to the polycrystal to align the dipoles in one direction, called *polarization* (Fig. 11.2 bottom).

To use the monocrystal quartz as a piezoelectric sensor, it is important to cut the crystal in a certain angle so that dipoles are aligned parallel to the electrical voltage. The most widely used angle is 35° 10′, shown in Fig. 11.3, called *AT-cut*.

Once the dipoles are aligned parallel to the electrodes, as shown in Fig. 11.4, piezoelectric effects can be observed. When a quartz crystal is compressed, the dipole itself is also compressed, creating an electrical voltage that has the same direction of the dipole. When the quartz crystal is stretched, the dipole is also stretched, creating a negative electrical voltage.

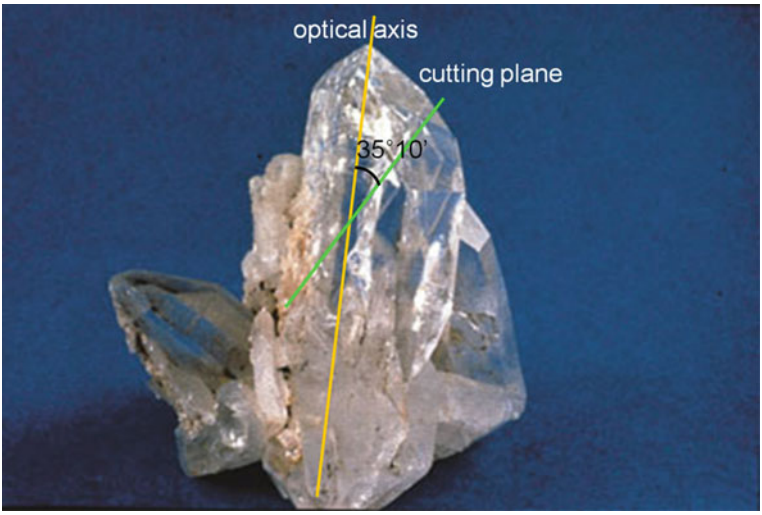


Fig. 11.3 AT-cut quartz crystal. Image adapted from USGS (public domain). Accessed in October 2015 from http://commons.wikimedia.org/wiki/File:Quartz_Crystal.jpg

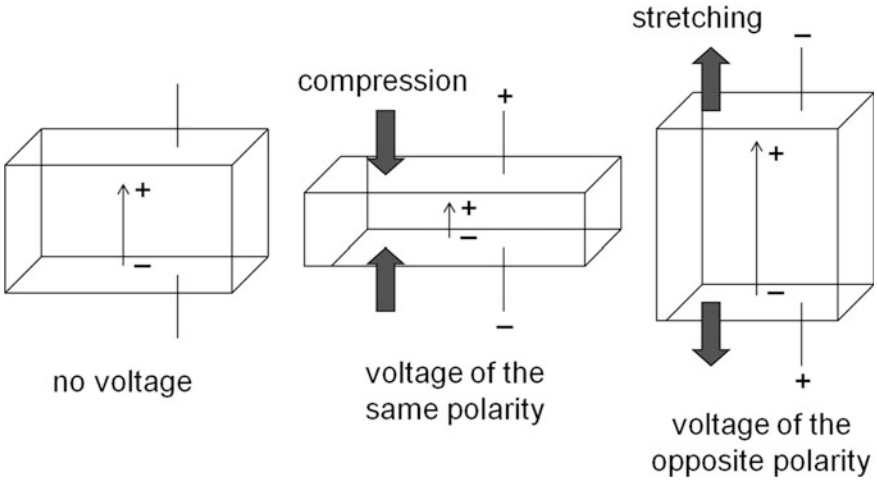


Fig. 11.4 Piezoelectric effect of an AT-cut quartz crystal

Obviously this effect is reversible. When an electrical voltage is applied with the same direction of the dipole, the quartz crystal is compressed. When an electrical voltage is applied with the opposite direction of the dipole, the quartz crystal is stretched. If an alternating current (AC) voltage is applied, the crystal should repeat the compression–stretching cycle.

11.2 Pressure Sensors

The more common pressure sensor would be the capacitor-type pressure sensor that was briefly described in Chap. 1, but the piezoelectric crystal can also be used as a pressure sensor. When one side of the piezoelectric crystal is exposed to a certain pressure, the crystal can be compressed (with positive pressure) or stretched (with negative pressure). This compression or stretching should generate positive or negative voltage, which can be used as a pressure sensor.

The piezoelectric pressure sensor can also be used as an accelerometer, by attaching a mass to the one side of a piezoelectric crystal. When the accelerometer is accelerated or decelerated, the force $F = ma$ is applied to the crystal, which generates a voltage. With the known mass m, acceleration a can be determined.

11.3 Crystal Oscillators

When an electrical voltage is applied to the quartz crystal, the material is deformed due to the piezoelectric effect. When the voltage is removed, the quartz returns back to its original shape, which also generates electrical voltage (the reverse of the above). Thus the quartz crystal can temporarily store electrical potential and release them later, just like the resistor-inductor-capacitor circuit (RLC circuit). As RLC circuit is capable of generating *harmonic oscillation* at a certain *resonant frequency* (refer to the other electronics textbook for more details), the quartz crystal has the same ability.

The quartz crystal is shock-excited by an applied voltage, and it mechanically vibrates (oscillates) at a resonant frequency to generate AC voltage. To sustain this oscillation, it is necessary to provide another shock-excited voltage before the oscillation is dampened. The positive feedback loop of an op-amp can do this function. Unlike the negative feedback loop that we learned previously, positive feedback loop generates the voltage that goes to their extremes, e.g., saturated voltages of +11 or −11 V for LM741 or LM324, which provides the required shock-excited voltages necessary to maintain oscillation. This situation is exactly identical to that of LC or RLC oscillator.

The frequency signal generated from a quartz crystal is generally in the MHz range, which is about the same as an LC or RLC oscillator. Quartz crystals that generate a fixed frequency signal are called *crystal oscillators*. The real advantage of crystal oscillator is its accuracy, with the stability of 0.01–0.001 %; LC oscillators have the stability of 0.01 % at best. The resonant frequency is not affected by environmental parameters, such as external temperature or the magnitude of shock-excited applied voltage. The first application of a crystal oscillator was wristwatch, clock, and radio. The circuit necessary to sustain the crystal oscillation (op-amps or transistors) is

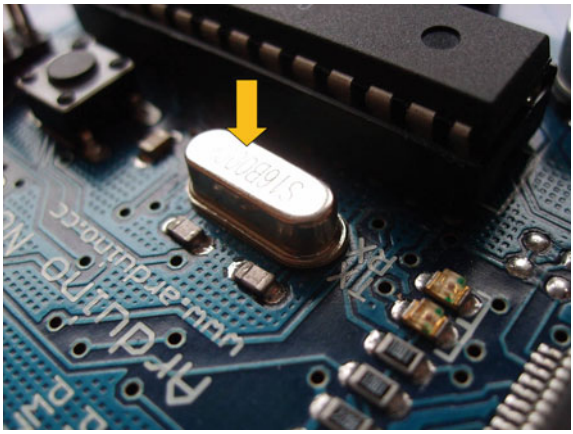


Fig. 11.5 A quartz crystal oscillator in a computer board

available as integrated circuits (ICs), such as 74S124. These days, crystal oscillators are omnipresent in almost all electronic appliances, especially for computers and microprocessors to run at certain clock speed (Fig. 11.5).

11.4 Quartz Crystal Microbalance (QCM)

The most popular biosensor application of piezoelectric sensors would be quartz crystal microbalance (QCM). QCM is essentially a crystal oscillator that is described in the previous section, utilizing an AT-cut quartz crystal (Fig. 11.6). As described previously, the resonant frequency f is not affected by environmental parameters. However, in 1959, a German physicist Günter Sauerbrey found that this resonant frequency could be decreased when a mass is loaded to one side of a quartz crystal (practically speaking, on the surface of an electrode, as both sides of a quartz crystal must be deposited with metal films to apply voltage). This phenomenon can intuitively be explained in the following manner: suppose an athlete is doing an exercise by moving quickly from the left to the right and coach measures the frequency of such movement per given time. If the coach asks the athlete to do the same exercise but with a heavy backpack, the frequency of such exercise would obviously be decreased.

Typical resonant frequency of QCM is around 5 MHz. Quartz crystal oscillates at this region of frequency in the *thickness shear mode*, as illustrated in Fig. 11.7. For this reason, QCM is often referred as thickness shear mode resonator (TSM resonator). This thickness shear mode oscillation generates a surface acoustic wave that travels through the film of a loaded mass.

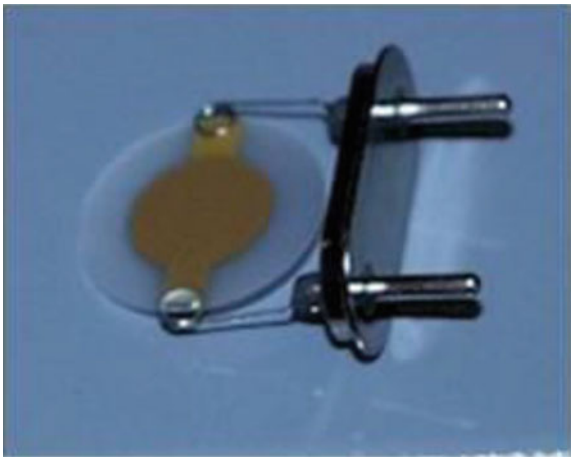


Fig. 11.6 A quartz crystal for QCM. Gold electrodes are deposited on front and back of an AT-cut quartz crystal

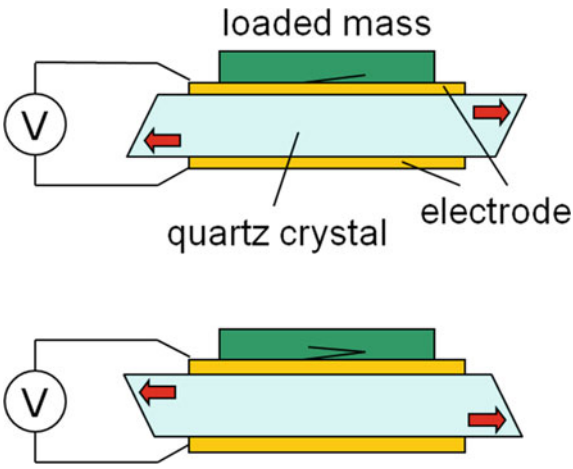


Fig. 11.7 Upon applying voltage, a quartz crystal oscillates to generate a surface acoustic wave into the layer of a loaded mass. With the loaded mass, the quartz crystal oscillates slower than normal, creating a frequency shift

Sauerbrey was able to determine the exact linear relationship between this frequency change Δf and the loaded mass Δm, called *Sauerbrey equation*:

$$\Delta f = -\frac{2f_0^2}{A\sqrt{\rho_q \mu_q}}\Delta m \tag{11.1}$$

where

f_0 resonant frequency (Hz)
Δf frequency change (Hz)
Δm loaded mass (g)
A piezoelectrically active area (=electrode area) of a crystal (cm^2)
ρ_q density of quartz (=2.648 g/cm^3)
μ_q shear modulus of AT-cut quartz crystal (=2.947 × 10^{11} $g/cm\ s^2$)
v_q transverse wave velocity in quartz (m/s)

Note that all parameters in Eq. 11.1 are constant for a given quartz crystal except for the loaded mass Δm; therefore, the change in frequency is a function of (or proportional to) the change in mass. For the following laboratory, we will use 5 MHz crystals ($f_0 = 5 \times 10^6$ Hz) with an active area of 0.4 cm^2. Then Eq. 11.1 can be simplified to

$$\begin{aligned} \Delta f(\mathrm{Hz}) &= -0.0565(\mathrm{Hz/ng}) \times \Delta m(\mathrm{ng}) \quad \text{or} \\ \Delta m(\mathrm{ng}) &= -17.7(\mathrm{ng/Hz}) \times \Delta f(\mathrm{Hz}) \end{aligned} \tag{11.2}$$

The resonant frequency and its change can accurately be measured with a frequency counter. Since modern QCM sensors have a resolution of 1 Hz or sometimes down to 0.1 Hz, the theoretical resolution of mass detection can be on the nanogram scale. Sub-nanogram mass detection may become possible by increasing the resonant frequency (f_0) to a higher value.

11.5 Viscoelasticity Consideration in QCM

When deriving his equation, Sauerbrey made a very important, yet limiting assumption: the loaded mass is ideally rigid and exhibit no viscoelasticity. In actual situation, however, all materials should exhibit a certain degree of viscoelasticity. The frequency change is no longer a sole function of the loaded mass Δm; it is also a function of viscoelasticity of the loaded mass. If you use the Sauerbrey equation to estimate the loaded mass that is viscoelastic, you will overestimate the mass. Despite this limitation, Sauerbrey equation is widely used (and still used today) in estimating the mass loading regardless of its rigidity.

In modern QCM systems, however, an impedance analyzer is typically utilized, where the *impedance* $Z = R + jX$ (R = resistance; X = reactance) is scanned over a range of frequency f, to determine the resonant frequency. Impedance is essentially an extended version of resistance for AC circuits. In a DC circuit, there is no imaginary part of impedance and the impedance simply becomes the same as resistance. Similarly, conductance can be extended to AC circuits by defining the admittance $Y = G + jB$ (G = conductance; B = susceptance). Obviously $Z = 1/Y$.

As the mechanical oscillation of QCM generates AC voltage, either impedance or admittance should be monitored to get an accurate resonant frequency as well as to provide additional information related to its viscoelastic behavior (the latter will

be discussed later in this section). For simplification, only the real part of impedance Z or admittance Y can be used, i.e., resistance R or conductance G (Fig. 11.8). It is known that ΔR or ΔG is a stronger function of viscoelasticity of the loaded mass than of Δm; while Δf is a stronger function of Δm with weaker contribution from viscoelasticity. Obviously, R and G do not change for ideally rigid mass loading, i.e., $\Delta R = \Delta G = 0$. There have been numerous attempts to relate this ΔR or ΔG (with Δf) to a more quantifiable viscoelastic properties, such as the *complex viscosity* (η^*) or the *complex shear modulus* ($\mathbf{G}^*$; not to be confused with conductance G). However, exact analytical solutions are available only for certain specific situations, such as the films with infinite thickness (i.e., surface acoustic wave decays completely within the film of loaded mass) and homogeneous liquid with no solute adsorption to the electrode surface of a quartz crystal. In most cases, there are more unknown parameters than the number of available equations, leading to an infinite number of possible solutions.

Despite this difficulty, people have found that the ratio $\Delta R/\Delta f$ is a good qualitative measure of viscoelasticity, though not linearly proportional to it, which people have found useful in many biosensor applications. The QCM equipment shown in this chapter's laboratory is capable of monitoring both ΔR and Δf.

An alternative parameter for viscoelasticity is the *quality factor* Q (Fig. 11.8):

$$Q = f/w \tag{11.3}$$

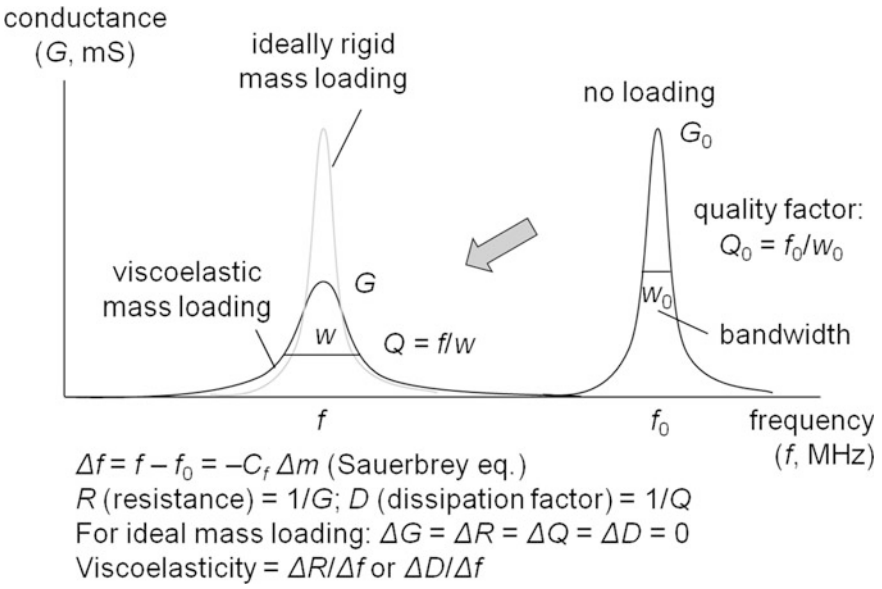


Fig. 11.8 Admittance Y or conductance G is scanned over frequency f to determine frequency change Δf and conductance change ΔG. Similarly, ΔR can be measured. Quality factor Q and dissipation factor D can be used instead of G and R

where

f resonant frequency
w bandwidth (width of a peak at its half height, $G/2$)

Q has a strong correlation with conductance G, because a higher peak gives larger G and smaller w (thus larger Q). Q itself is rarely used in QCM as the bandwidth determination is more difficult than measuring G or R. However, there is an easier way to measure Q through evaluating *dissipation factor* D (Fig. 11.8):

$$D = 1/Q = w/f \tag{11.4}$$

Note that D has a strong correlation with resistance R. Dissipation factor D can be measured by shock-excite the quartz crystal with an applied voltage and measuring its oscillation decay (i.e., no positive feedback loop to sustain its oscillation). By monitoring the exponential decay of oscillation, it is possible to obtain the bandwidth of the original oscillation. The QCM instrument *QCM-D* (trademark of Q-Sense AB, Gothenburg, Sweden) is capable of measuring the dissipation factor. Similar to the conventional QCM, the ratio $\Delta D/\Delta f$ can also be used as a qualitative measure of the viscoelasticity, though still not linearly proportional to it. QCM-D often provides more stable and more reproducible results than conventional QCM, as the energy of crystal oscillation does not accumulate in the loaded mass. In conventional QCM, however, such energy accumulation typically leads to constant drifting of resistance R (and conductance G and quality factor Q) and frequency f.

11.6 Flow Cell QCM as Biosensor

QCM can be used in both air and liquid (mostly water) environment. For the latter, the quartz crystal is typically modified or functionalized with the material of choice, and a solution of biomolecules (typically proteins, DNAs and cells) is introduced onto it. Only one side of a quartz crystal is used for surface modification/functionalization. Occasionally, a bare electrode can also be used without any surface modification/functionalization, where the most typical electrode material for QCM is gold (Au). A *flow cell* is constructed around this side of a quartz crystal oscillator, with an inlet and an outlet, through which a solution of biomolecules is pumped. Figure 11.9 illustrates the schematic of this flow cell for QCM.

The QCM has the ability to measure mass deposition/adsorption in nanogram scale, which is three or four orders of magnitude better than any electronic balance. The result can be obtained in real time, such that monitoring the real-time kinetics of biomolecular adsorption to certain surfaces is achievable with extremely high sensitivity. In addition, QCM can also monitor the viscoelasticity change of the adsorbed biomolecules in real time through monitoring $\Delta R/\Delta f$ or $\Delta D/\Delta f$ (though it cannot provide quantitative information). This is an important advantage of using

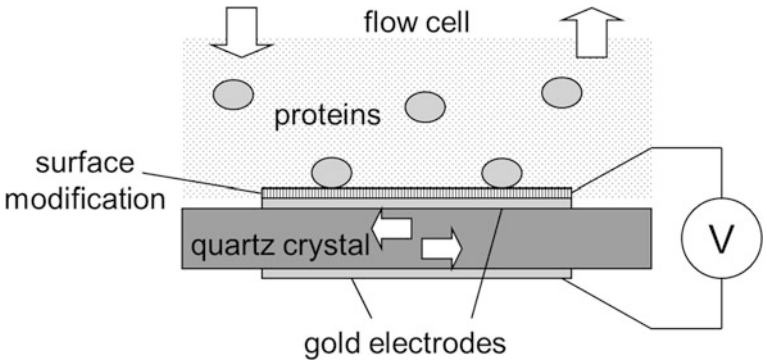


Fig. 11.9 A flow cell for QCM in studying biomolecular adsorption to synthetic surfaces

QCM for biological applications. In the following laboratory, we will have a flow cell QCM to study protein adsorption on the bare gold electrode surface.

If the surface modification/functionalization is made with bioreceptors, like enzymes, antibodies, or nucleic acids, the QCM turns into a biosensor. This particular situation will be discussed in detail in Chap. 13 Immunosensors.

11.7 Laboratory Task 1: Quantifying BSA Adsorption on QCM Sensor

The quartz crystal microbalance (QCM) is among the most popular types of piezoelectric sensors. Mass loading onto one side of the electrodes causes the crystal to oscillate at a lower frequency. This decrease in frequency is linearly proportional to the loaded mass, which can be represented by the Sauerbrey equation (Eqs. 11.1 or 11.2). In this laboratory, we will evaluate the adsorption of a model protein molecule, bovine serum albumin (BSA), on a sensor surface.

In this task, you will need the following:

- QCM (QCM200 from Stanford Research Systems, Fig. 11.10) with a flow cell
- 5 MHz quartz crystals (for QCM200)
- Support stand and clamp for QCM
- A *syringe pump*
- 1 mL syringes and tubings
- Electronic balance, weighing paper, laboratory spatula
- Distilled and/or deionized water
- Beakers, magnetic stir bars, and magnetic stirrer
- Pipettes and pipet tips (1000 μL)
- Acetic acid ($C_2H_4O_2$ or HOAc) and sodium acetate ($C_2H_3O_2Na$ or NaOAc)

Fig. 11.10 A complete QCM system with QCM200 digital controller (*top*), QCM25 crystal oscillator (*bottom middle*), crystal holder (white plastic piece connected to crystal oscillator), and three 5 MHz quartz crystals. Accessed in October 2015 from http://www.thinksrs.com/downloads/PDFs/Manuals/QCM200m.pdf, © Stanford Research Systems 2011, reprinted with permission

- Monobasic and dibasic potassium phosphate (KH_2PO_4 and K_2HPO_4)
- Bovine serum albumin
- Latex gloves, delicate task wipers (Kimwipes®).

– Acetate buffer: Take 5 mmol each of acetic acid ($C_2H_4O_2$ or HOAc) and sodium acetate ($C_2H_3O_2Na$ or NaOAc). Dissolve them into 100 mL of deionized (DI) water. This makes pH 4.76 100 mM acetate buffer (since pK = 4.76).
– Phosphate buffer: Take 5 mmol each of monobasic potassium phosphate (KH_2PO_4) and dibasic potassium phosphate (K_2HPO_4). Dissolve them into 100 mL of DI water. This makes pH 7.20 100 mM phosphate buffer (since pK = 7.20).
– BSA solution: Add DI water to the vial of protein standard (BSA; bovine serum albumin) to make 400 μg/mL BSA solution.
– Prepare 1 mL each of 10 mM acetate and 10 mM phosphate buffer solutions.
– Also prepare 1 mL each of 40 μg/mL BSA solutions in (1) 10 mM acetate buffer and (2) 10 mM phosphate buffer.

Why dissolve BSA in two different buffers?
We want to expose our BSA close to its isoelectric point (pH 4.8) and away from it (pH 7.2). The isoelectric point (or pI) of a protein is the pH at which the protein has an equal number of positive and negative charges. Figure 11.11 graphically depicts the surface charge distributions of BSA (left) and lysozyme (right) at pH 7.2. The net surface charge of BSA at pH 4.8 will be close to zero, while that at pH 7.2 will be negative.

– Get four 1 mL syringes.
– Load two 1 mL solutions (e.g., 10 mM acetate buffer only AND 40 μg/mL BSA in 10 mM acetate buffer) in two different 1 mL syringes (diameter = 5 mm). Be sure to eliminate air bubbles trapped inside.
– Install a syringe onto a syringe pump (Fig. 11.12).
– Install a crystal to the QCM200 system (Fig. 11.13).

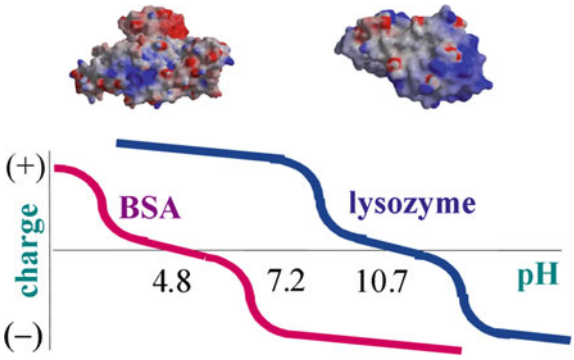


Fig. 11.11 *Top* surface charge distributions of BSA (*left*) and lysozyme (*right*) at pH 7.2. *Red* negative (−); *Blue* positive (+). *Bottom* net charge of BSA and lysozyme plotted against the medium pH

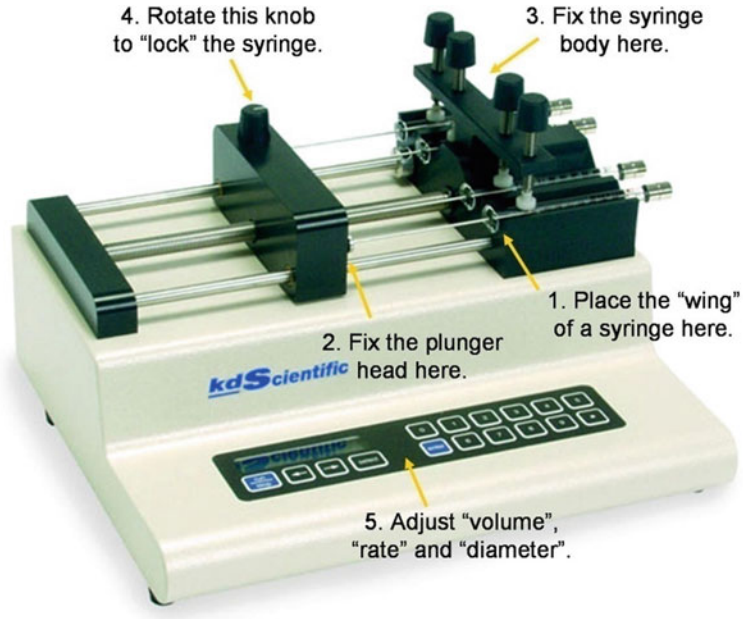


Fig. 11.12 Installing a syringe to a syringe pump

– Cap with a flow cell (Fig. 11.14).
– Experimental setup is shown in Fig. 11.15. Initially, you will need to connect the syringe containing the buffer solution to the QCM flow cell.
– The tubing between the syringe and the inlet of a flow cell should be made as short as possible.
– Turn on the power to the QCM system.

Fig. 11.13 A 5 MHz crystal is placed within the sensor holder of QCM200. In this mode, the QCM can be used as a "balance." To install a flow cell, the white plastic cap and O-ring should be removed. Accessed in October 2015 from http://www.thinksrs.com/downloads/PDFs/Manuals/QCM200m.pdf, © Stanford Research Systems 2011, reprinted with permission

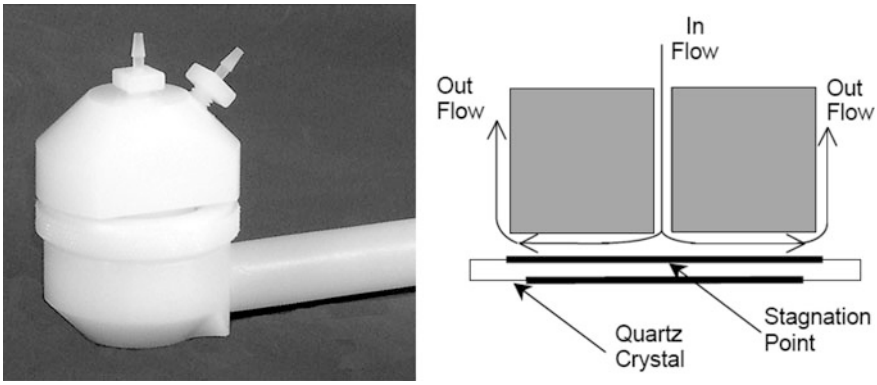


Fig. 11.14 A flow cell for the QCM200. The center connector is for in-flow and the side connector is for out-flow. Accessed in October 2015 from http://www.thinksrs.com/downloads/PDFs/Manuals/QCM200m.pdf, © Stanford Research Systems 2011, reprinted with permission

– Turn on the syringe pump and press "select" in order to set-up the diameter of the syringe, the flow rate, and the sample volume. The inner diameter of a 1 mL syringe is 5 mm. If you are using a different syringe and its inner diameter is unknown, you may want to measure the height (h) of a syringe and back-calculate the inner diameter using the following equation (for a 5 mL syringe) (Fig. 11.16):

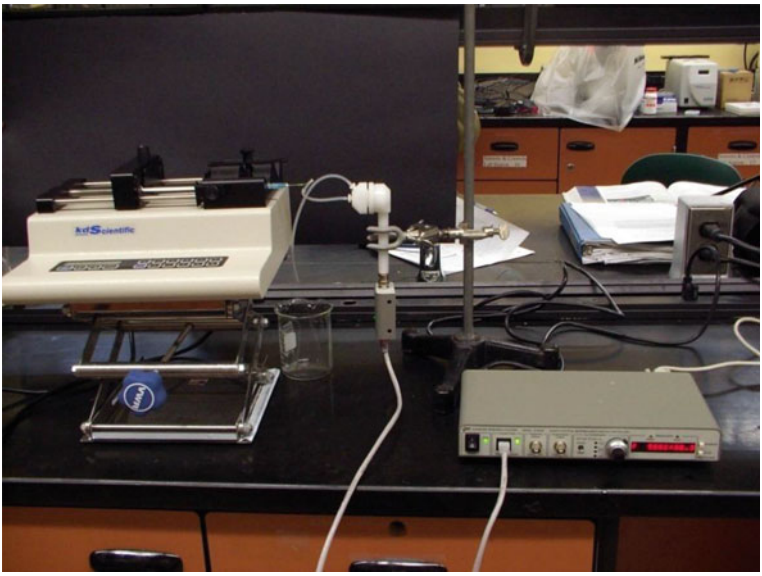


Fig. 11.15 Experimental setup of Task 1

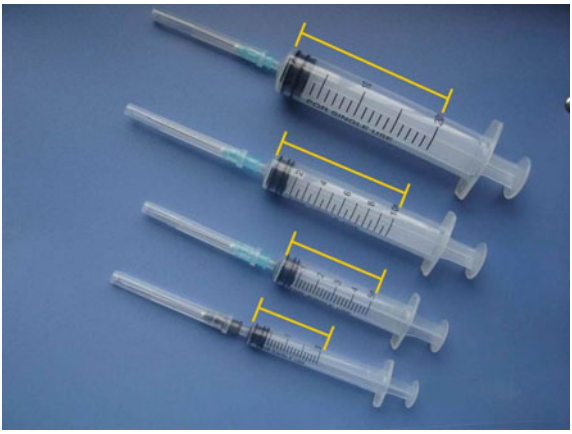


Fig. 11.16 Determining the inner diameters of disposable syringes

Table 11.1 QCM200 display

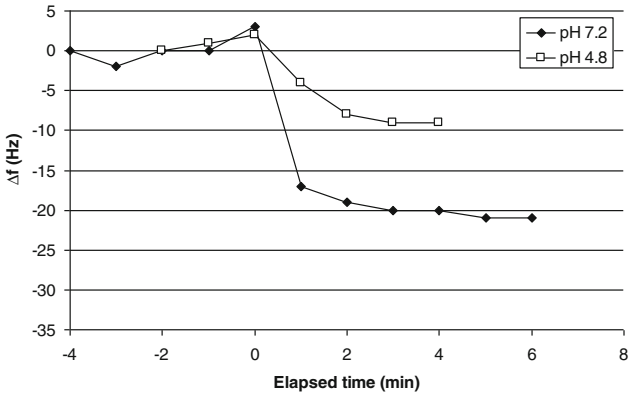
Parameter	Value	Symbol	Display
Frequency	Absolute frequency	F	Absolute frequency (Hz) = series resonance frequency of quartz crystal
	Relative frequency	f	Relative frequency (Hz) = absolute frequency − frequency offset
	Mass	m	Mass displacement (ng) = relative frequency/0.0566
Resistance	Absolute resistance	R	Absolute resistance (Ω) = series resonance resistance of quartz crystal
	Relative resistance	r	Relative resistance (Ω) = absolute resistance − resistance offset

$$5\ \text{mL} = 5\ \text{cm}^3 = 5000\ \text{mm}^3 = \frac{\pi D^2}{4} h \tag{11.5}$$

- Set the flow rate to 0.2 mL/min. This will inject the buffer into the QCM flow cell.
- Wait until both frequency (F) and resistance (R) are stabilized (Table 11.1). Once the crystal makes full contact with water, the resistance (R) should be stabilized around 400–500 Ω. (Note that water is a viscoelastic material, which makes the resistance reading much higher than air.) Less than 400 Ω may indicate the existence of air bubble(s) within a flow cell. Larger than 500 Ω may indicate the existence of viscoelastic mass contamination.
- Switch the syringe containing the BSA solution in buffer and inject the solution. Use a clip to hold off the tubing during the syringe exchange. If an air bubble is introduced during the syringe exchange, a sharp peak in frequency curve may be observed and the resistance may drop down significantly.
- Record the frequency and the resistance every 30 s. As the volume is set to 1 mL with a flow rate of 0.2 mL/min, the entire experiment should be finished in 5 min. Typical frequency shift for BSA is a few tens of Hz.
- Repeat the experiment at other pH (10 mM phosphate buffer only AND 40 μg/mL BSA in 10 mM phosphate buffer).
- Remove tubing, flow cell, and crystal holder. Rinse everything (except the crystal) rigorously with flowing DI water. Place the crystals into the crystal cleaning basket and rinse with DI water. Use wash bottles. Alternatively, you can use a brand new quartz crystal.
- Plot the frequency and resistance versus time.
- Evaluate "plateaued" Δf and plug into the Sauerbrey equation: $\Delta m = -C \cdot \Delta f$, where $C = 17.7$ ng/Hz.
- Divide the mass by the active electrode area of $\sim 0.40\ \text{cm}^2$. Convert this number to units of mg/m^2 or $\mu\text{g/cm}^2$.

Trial #1:

	t	-4	-3	-2	-1	0	1	2	3	4	5	6
pH 7.2	Δf	0	-2	0	0	3	-17	-19	-20	-20	-21	-21
pH 4.8	Δf			0	1	2	-4	-8	-9	-9		



Trial #2:

	t	-3	-2	-1	0	1	2	3	4	5	6	7	8
pH 7.2	Δf	0	1	2	3	-30	-25	-25					
pH 4.8	Δf				0	-8	-14	-17	-13	-13	-19	-20	-19

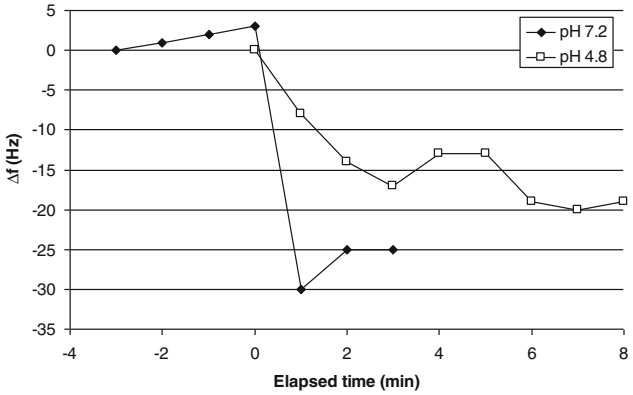


Fig. 11.17 Experimental data of Task 1 (trials #1 and #2): BSA adsorption on QCM sensor surface. More and faster adsorption is observed with pH 7.2, where the net charge of BSA is negative

- Adsorbed amounts of BSA (trial #1) (Fig. 11.17):
  pH 7.2: (17.7 ng/Hz) × (21 Hz)/(0.4 cm^2) = 929 ng/cm^2 = 9.29 mg/m^2
  pH 4.8: (17.7 ng/Hz) × (9 Hz)/(0.4 cm^2) = 398 ng/cm^2 = 3.98 mg/m^2.
- Adsorbed amounts of BSA (trial #2) (Fig. 11.17):
  pH 7.2: (17.7 ng/Hz) × (25 Hz)/(0.4 cm^2) = 1110 ng/cm^2 = 11.1 mg/m^2
  pH 4.8: (17.7 ng/Hz) × (19 Hz)/(0.4 cm^2) = 841 ng/cm^2 = 8.41 mg/m^2.

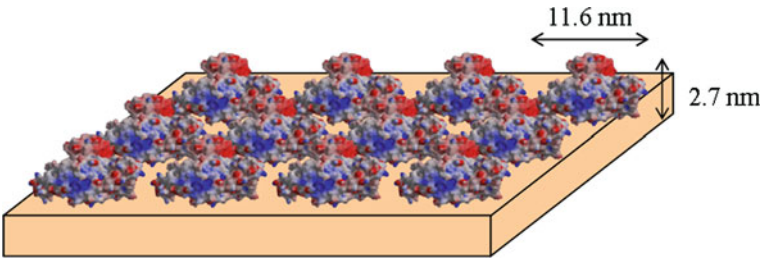


Fig. 11.18 Hexagonal, monolayer packing of BSA on gold surface

Question 11.1
More BSA adsorption and faster BSA adsorption was observed for the above experimental results. Explain this in terms of the net charge or the isoelectric point of BSA.

Question 11.2
Figure 11.18 shows BSA has dimensions of $11.6 \times 2.7 \times 2.7\ \text{nm}^3$. Assuming dense hexagonal side-on packing the theoretical adsorbed amount can be calculated by $\Gamma_{\text{theo}} = (\pi/3\sqrt{3}) \cdot (\delta/v)$, where $\pi/3\sqrt{3}$ is the packing factor, δ = 2.7 nm (the shortest dimension of BSA because the packing is side-on), and v = 0.733 mL/g (the specific volume of BSA, the inverse of its density). Be careful with unit conversions. Compare this with the experimental results obtained in Task 1. Why is there a discrepancy?

Question 11.3
Sketch the frequency shift behavior of lysozyme adsorption on the QCM sensor surface, in pH 7.2 (phosphate) and pH 10.3 (carbonate) buffer. Refer to Fig. 11.11 for the isoelectric point of lysozyme.

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Stanford Research Systems (2011) Operation and service manual—QCM200 and QCM25. http://www.thinksrs.com/downloads/PDFs/Manuals/QCM200m.pdf (Figs. 11.10, 11.13 and 11.14)