Impedance and Biosensors

Impedance, an electrical property, is widely and often discussed in the realms of physics and electrical engineering. However, this subject rarely makes its way into the discussion of biologists and food safety experts. However, with the growing need for the rapid and efficient detection of food borne pathogens, such as the pathogenic O157:H7 strain of *E. coli* common in the United States a number of detection methods have been explored and suggested, including the use of impedance measurements as a detection method for the presence of certain target bacteria. The integration of two branches of science have not only resulted in a practical application for everyday life, but also, the use of impedance as a detection method has solved many problems that exist within current detection methods. To understand the role that impedance is able to play in the field of biosensor detection, it is first necessary to understand the concept of impedance, specifically the nature of impedance with respect to physics and electricity. Then it can better be seen how such a concept is integrated into another, seemingly unrelated, branch of science.

The impedance measurement of a circuit begins with the construction of an RLC series circuit, where R represents the resistor component of the circuit, L represents the inductor component of the circuit, and C represents the capacitor component of the circuit. All components are connected in series. Then an alternating current (AC) is passed through the circuit so that the current and voltage across each element experiences the same frequency. An AC voltage as a function of time appears sinusoidal graphically and can be expressed as:

$$V(t)= V_m \sin(\omega t+\phi)$$

Where V(t) is the voltage as a function of time, V_m is the amplitude of the wave, ω is the angular frequency (in radians/second), t is the time in seconds, and ϕ is the phase angle relative to $V_m\sin(\omega t)$, called the reference sinusoid. A phase angle that is positive represents a wave that occurs earlier in time than that of the reference wave; this type of wave is referred to as a "leading" wave. A negative phase angle represents a wave that occurs later in time than the reference wave; this type of wave is referred to as a "lagging" wave. The frequency of the sinusoidal wave is related to the angular frequency such that:

$$f=1/T=\ \omega/2\pi$$

where *f* is the frequency (in 1/seconds)and T is the period of the wave, or the time it takes the wave to complete one full cycle (from crest to crest, for example) in seconds (Alciatore and Histand, 2007). To simplify further calculations, it will be said that the current passing through the RLC series circuit is the reference sinusoid, such that $V(t)= V_m\sin(\omega t)$. The current through each component experiences the same amplitude and phase. However, the phase and amplitude of the voltage across each component differs. Across the resistor, the voltage does not differ by any phase angle from the current so that when substituting into the equation for voltage as a function of time and using Ohm's Law where ΔV=IR, the voltage as a function of time for the resistor becomes:

$$V(t)_R=IR\sin(\omega t)=\Delta V_R\sin(\omega t)$$

The voltage across the inductor is a leading wave in comparison to the current by a phase angle of 90˚(π/2) such that:

$$V(t)_L = IX_L \sin(\omega t + \pi/2) = \Delta V_L \cos(\omega t)$$

Where X_L is the component of inductance in relation to voltage. The voltage across the capacitor is a lagging wave in comparison to the current by -90˚(-π/2) such that the voltage as a function of time for the capacitor becomes:

$$V(t)_C = IX_C \sin(\omega t - \pi/2) = -\Delta V_C \cos(\omega t)$$

Where X_c is the component of the inductance in relation to voltage (Serway and Jewett, 2008).

These three voltages can be added together to equal the voltage supplied to the circuit. These voltages can be added using vector addition to generate a resultant vector that represents the maximum voltage of the circuit. The voltage due to the resistor ΔV_R can be drawn as a vector on the horizontal, as it does not differ by any phase angle from the current. The voltages due to the capacitor and inductor of the circuit can be represented as one vector, since each differs by an angle of π/2 from the current in opposite directions. The resultant vector is $\Delta V_L - \Delta V_C$, and it extends vertically from the origin and is perpendicular to the vector ΔV_R. By placing these two vectors tail to tail, and drawing the resulting rectangle, the diagonal of the rectangle represents the maximum voltage of the circuit. The magnitude of this ΔV_{max} vector can be determined by using the Pythagorean theorem such that:

$$\Delta V_{max} = (\Delta V_R + (\Delta V_L - \Delta V_C))^{1/2} = \{(I_{max}R)^2 + (I_{max}X_L - I_{max}X_C)^2\}^{1/2}$$

Therefore, $\Delta V_{max} = I_{max}(R^2 + (X_L - X_C)^2)^{1/2}$ (Serway and Jewett, 2008). This radical comes to represent Z, the impedance of the circuit. By substituting this new value into Ohm's law, $\Delta V = IZ$ (Alciatore and Histand, 2007). By these equations, it can be seen that impedance depends upon

resistance, inductance, capacitance, and frequency. Since frequency has been previously discussed in relation to impedance, resistance, inductance, and capacitance must now be defined.

Resistance is defined as the "ratio of the potential difference across a conduction to the current in the conductor" (Serway and Jewett). That is: $R= \Delta V/I$ where R is the resistance, ΔV is the change in voltage across the resistor and I is the current. Resistance can also be expressed such that $R= l/\sigma A$, where *l* is the length of the conductor, A is the cross sectional are of the conductor through which the current travels, and σ is the conductivity of the conductor. This conductivity constant reflects the ratio of the current density in the conductor to the generated electric filed. This constant varies for each conductor material (Serway and Jewett, 2008).

Inductance can really be considered a constant in the calculation of emf (electromotive force). An inductor is a loop of wire within a circuit. An emf and a current are induced in the loop when magnetic flux through the loop changes as a function of time. The emf of a circuit due to self inductance is expressed as: $emf= -L(dI/dt)$, where L is the inductance of the loop. This inductance depends upon the geometry and number of turns of the loop such that the inductance of the loop is: $L= (N \phi_m)/I$, where N is the number of turns in the loop, ϕ_m is the magnetic flux, and I is the current through the loop. Inductance is measured in Henry (Serway and Jewett, 2008).

A capacitor is composed of two conductors such that when the capacitor is charged, the conductors carry charges equal in magnitude but opposite in sign. This results in a potential difference, ΔV, between the two conductors. The capacitance of a capacitor is the "ratio of the

magnitude of the charge on either conductor to the magnitude of the potential difference between the conductors" (Serway and Jewett, 2008). That is: $C = Q/\Delta V$, where C is the capacitance of the circuit, Q is the magnitude of the charge on either conductor, and ΔV is the potential difference between the two conductors. Capacitance is measured in Farads.

Therefore, a circuit must include a capacitor, an inductor, and a resistor connected in series for impedance to be measured. Impedance can be calculated by measuring the resistance (in Ohms), the capacitance (in Farads) and the inductance (in Henry) of the circuit. The resultant impedance value is measured in Ohms. By changing the capacitance, inductance, resistance, or the frequency of the alternating current passing through the circuit, a change in the impedance of the circuit will be exhibited.

Impedance, now understood, can then be integrated into the field of biology and food safety as an effective measuring method for the detection of such pathogenic bacteria as *E. coli* O157:H7. This particular strain, common in the United States, is becoming increasingly important to detect as it can cause significant health problems in humans, resulting even in death. It has been known to cause hemorrhagic colitis, severe inflammation and bleeding in the colon, and Hemolytic Uremic Syndrome (HUS), which is linked to prolonged kidney problems and kidney failure. These bacteria are transmitted to humans via improperly cooked meats and raw vegetables such as tomatoes and alfalfa sprouts (Tortura et al., 2007). Because these foods are commonly consumed, it is of the utmost importance to detect the presence of the bacteria in these foods to prevent human infection and product loss due to recalls.

Traditionally, methods for detection of *E. coli* bacteria have included plating of the bacteria, PCR (polymerase chain reaction), and ELISA (enzyme-linked immunosorbent assay) (Tortura et al., 2007). However, these methods can be particularly time consuming, require extremely skilled technicians, lack repeatability for a specific sample, and cannot be specifically designed to test for the presence of only one serotype of bacteria. Therefore, these methods have become impractical for rapid detection of pathogenic bacteria in the food industry. Thus, the industry has seen a rise in the use of biosensors for the detection of such pathogens. With the development of biosensors, it is possible to design a sensor coupled with a specific protocol and antibody that measures a signal (such as impedance or chemiluminescence) generated in the presence of only one strain of bacteria. Most biosensors require the labeling of secondary antibodies so that when the antigen is captured by the antibody, an optical or electrochemical signal is detected (Yang, 2004). Such is the case in the use of chemiluminescence, in which luminol is used as a label that can be seen with a fiber optic light when the antibody and antigen interaction occurs (Varshney, 2006). While this method provides a low detection limit for the number of bacteria that produces a detectable signal, the protocol for this method can be particularly complex and time consuming. Further, detection relies on optical observation alone, a method that cannot be easily converted into a quantitative measurement. Due to these disadvantages, methods have been explored to detect bacteria without the use of labeled secondary antibodies. These label-free immunosensors include quartz crystal microbalance (QCM), surface Plasmon resonance (SPR), and impedance detection, and they afford inexpensive and rapid detection capabilities (Yang, 2004).

There are two basic types of impedance detection: non-Faradiac and Faradiac. Faradiac measurements require the presence of a redox probe, while non-Faradiac measurements do not. When a redox probe is used in such Faradiac measurements, the impedance measurements are analyzed by using the Nyquist plot where parameters relate impedance to the resistance of the solution and the charge transfer. When the non-Faradiac method is used, impedance is based solely on the detection of bacterial cells, and a Bode plot is used to analyze the impedance measurements. This plot graphs the impedance (measured in Ohms) as a function of frequency (measured in Hertz), and it is this method that will be discussed (Varshney, 2006). The basic way to take such measurements is with the use of an impedance analyzer that conducts a "sweep" of the material being measured, and measures the impedance of the sample at a range of frequencies. Repeated measurements of a sample using this method can allow the development of a portable biosensor that is fixed at a particular frequency. This frequency is chosen based upon where the impedance measurement of the control sample and the impedance measurement of the bacterial sample exhibit the greatest difference in impedance when measured using the sweep method. The development of a portable sensor further increases the appeal and practicality of impedance as a detection method.

To take such impedance measurements of a biological sample, an electrode must be involved. Until recently, macroelectrodes consisting of metal rods or wires in a liquid medium were utilized in biosensors, but these did not afford high sensitivity and thus did not allow low detection limits. Therefore, microelectrodes have been developed to solve such problems. One particular type of microelectrodes, interdigitated array microelectrodes (IDAM), in which

microelectrode pairs are interlocked like fingers, provides an even greater advantage of an increased signal-to-noise ratio. This decreases problems with interference due to nearby electrical devices while impedance measurements are being conducted (Varshney et al., 2007).

While these measurements could be conducted by placing the sample solution on top of the IDAM, in what would be considered an open electrode, efficiency and sensitivity can be increased by coupling the electrode with a microfluidic cell to create an integrated microfluidic chip. In such a chip, the electrode array (usually of gold or titanium oxide) is embedded at the bottom of a micro channel. This channel is closed to the atmosphere, reducing risk of contamination, and allows the sample to be pumped into the channel on top of the electrode array, where it is contained for the duration of the measurement process. This design is optimal for a number of reasons. First, it allows the precise handling of a very small sample volume. It increases detection sensitivity and serves to concentrate the number of bacterial cells contained in the sample into a very small area. Most importantly, it causes the bacterial cells of the sample to concentrate themselves in the “active layer” of the electrodes. The active layer is the area above the electrodes where the electric field is the strongest, producing the best impedance signal. Because this layer usually extends only a few micrometers above the surface of the electrodes, this feat is hard to accomplish when utilizing an open electrode, but with a microfluidic chip, the channel can be designed such that its depth is approximately the height of the active layer (Varshney et al., 2007).

Generally, impedance measurements are made by immobilizing antibodies on the microelectrode surface to capture the bacteria contained in the sample that is pumped through

the chip. However, this method provides a low capture efficiency of the bacteria, and therefore does not produce the highest impedance signal desired. Capture efficiency can be increased by utilizing methods that do not require antibody immobilization on the electrode surface. One such method incorporates the use of yet another concept widely explored in physics, magnetism. In this method, magnetic beads are coated with antibodies that capture the target bacteria. These beads, when used in the preparation of the sample can serve to isolate the target bacteria from a biological sample to such a small volume that is desired for the microfluidic chip being used. This small volume increases the sensitivity of detection for the sample (Varshney et al., 2007).

Now that all required components for such an impedance measurement have been discussed, a sample protocol for the preparation of an *E. coli* O157:H7 bacterial sample to time of measurement can be presented. First, preliminary steps are taken to prepare the control and bacterial samples by binding the coated nanobeads to the anti-*E. coli* antibodies. The magnetic nanobeads are coated in strepatvidin and the antibodies are labeled with a complex called biotin. Biotin and streptavidin exhibit a strong affinity for one another, comparable nearly to the strength of a covalent bond, and therefore binding the antibody to the bead is easily accomplished simply by mixing. Then, one sample is mixed with *E. coli* bacteria contained in a nutrient broth to create the bacterial sample, while the remaining sample is mixed with nutrient broth absent of bacteria to create the control sample. Both samples are then repeatedly exposed to a magnet and waste drawn off after each exposure. This serves to concentrate the bacteria complexed to the antibodies on the nanobeads into subsequently smaller and smaller volumes, in the desire to increase the resulting impedance signal. Once

both the control sample and bacteria sample have been prepared to a desired volume suitable for the channel of the microelectrode being used, impedance measurements can be taken. The impedances of water and buffer samples are also taken so as to compare to the control and bacteria samples. When any measurement is taken, the sample is pumped through the chip at a uniform rate and the solution is allowed to spread evenly over the microelectrode array. The impedance analyzer (or the portable impedance meter) connects to the two external copper leads of the chip (at either end of the microelectrode array), and an impedance measurement is generated for that sample. Each substance pumped through the chip should generate a unique impedance due to the specific electrical properties of each substance. The impedance of the bacterial sample should be particularly distinguishable from that of the control sample due to the "insulating effects of the cell membranes" of the bacteria present in the sample (Yang, 2004). Therefore, with repeated experimentation to determine the common observed impedance difference of the bacteria sample and the control, it can be determined if the target bacteria are present in an unknown sample by comparing its impedance with those of the control, buffer, and water samples.

By utilizing impedance, a measurement traditionally discussed in physics and electronics, biology and food safety become integrated into the realm of engineering where scientific concepts become useful, tangible tools in the modern world. Impedance not only serves as an adequate detection method for pathogenic bacteria, but serves to produce an optimal method that provides increased detection sensitivity, lower detection limits, repeatability, practicality, speed, and specificity to the target bacteria. This demonstrates the

necessity for the melding of different branches of science to produce optimal, practical techniques for managing the safety of our modern world.

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