Can SPR be used to Interrogate the Double Layer of NaCl

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Abstract:

Solutions of reagent grade salt .1, .01, .001, .0001, .00001M Were ran through a SPR to see if SPR had enough resolution to detect the formation of the electric double layer. We found that NaCl did in fact return a detectable signal. The signal shape seams to depend on concentration. While more study may need to be done to get definite information it looks like SPR can interrogate the double layer.

Introduction:

The electric double layer is a result of the surface potential of any surface. Every surface has a charge even if it is very small so in order to balance out this charge two distinct layers form. First is the stern layer which is comprised of ions of the opposite charge that bind to the surface, this layer is immobile. The second layer known as the diffuse layer is created because of electron shielding provided by the stern layer. The diffuse layer has a higher concentration of counter ions, ions opposite in charge to the Stern layer.

Surface Plasmon Resonance is a sensitive instrument that responds to slight changes of index of refraction close to the surface of the sensor, generally a gold or silver chip. Normally it is used for determining binding constants for generally molecules of biological nature. Where one of the molecules, the antigen is bound to the chip and the other, the target is run across the surface. Binding events change the index of refraction and cause a shift in the maximum absorption angle. These shifts give us a binding curve and can be used to determine the binding constant between the two molecules as the molecules associate and disassociate.

However since the machine detects change in index of refraction anything measureable by such properties can be detected. The question becomes does surface Plasmon resonance have enough

resolution to detect the change in index of refraction of the double layer do to the higher ion concentration.

Applications that use the double layer in the biomedical field:

The Double layer effect matters for determining the rate at which an electrolyte is transferred between an interface of two solusions.[1] In this case the limiting rate depends on rate of transfer between layers created by the two double layers. This rate limiting step can influence how fast a drug can travel since depending on the properties of the double layer.

Another biomedical application where the double layer matters involves electrodes in contact with bodily fluids. This model not only had to account for electric resistance of passing through organic matter but also impedance created by the formation of an electric double layer between the body and the electrode. An Important part of this model is the impedance generated from the double layer that forms around the electrode in a biological solution. [2] In biomedical applications impedance is important. You don't want impedance too high or you might cause damage to the cells while at the same time have impedance high enough to stimulate whatever the target is, generally nerves.

The electric double layer has recently come up in ultrasound. It was found that the deformation of the electric double layer could be measured by a dealloyed gold/silver electrode in liquid solution to sense ultrasonic frequencies.[3] distortion of the double layer will give off potential energy in the form of electric current. Sensitivity can be scaled up by increasing the surface area of the material required. This approach at ultrasound sensors is far less expensive sensor than the piezoelectric sensors used currently.

Knowledge of the double layer is also important with Micro fluidics which deals with small scale fluid transfer mainly electroosmonics. The double layer has greatly different mechanics. The stern layer is generally treated as a stationary layer while the diffuse layer moves freely. Because the diffuse layer has a net charge to it this layer can be moved when a electric field is applied. When dealing with such small scales these layers although small become significant and have to be taken into account. [4]
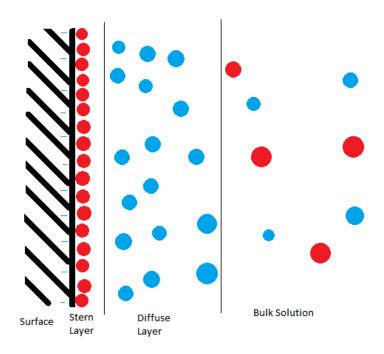
The electric double layer:

Figure 1: the double layer (red ions, blue counter ions)

The double layer consists of two layers of ions of opposite charge found on a surface. The first layer is the stern layer that consists of ions with a charge opposite of the surface potential. Every surface has a charge weather van der walls or ionic. This charge attracts opposite ions to the surface depending on the surface. This attraction forms a thin layer of charged ions bound along the surface.

This however because of electron shielding a second weaker layer of counter ions forms resulting in a net neutral charge. The second layer is called the diffuse layer. this layer forms as a response to the net charge exhibited created by the sturn layer and consists of ions opposite in charge from the sturn layer. These ions unlike the stern layer move with the liquid and are found in much higher concentration than in bulk solution. The experiment is to see in SPR can detect the change in index of refraction caused by NaCl.[5]

Surface Plasmon Resonance (SPR):

Surface Plasmon resonance measures change in index of refraction. A laser is shined through a glass prism that is covered with a thin layer of gold. The laser is internally refracted but the electromagnetic wave from the light propagates a short distance outside of the interface, about a wavelength distance. This electromagnetic wave interacts with the thin layer of gold or silver to excite a Surface Plasmon wave under specific conditions: dielectric constants of opposite signs for the real component of the interface between the chip and the glass[6] and the wave vector of the glass-chip equals the wave vector of the chip-sample. [7]
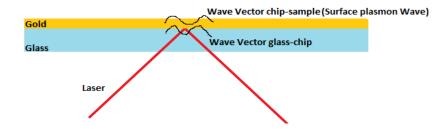

Figure 2: A laser is internally refracted but it's electromagnetic wave exits a short distance. If conditions are right and the wave vector of this electromagnetic wave match the wave vector of the chip-sample interface this results in energy being absorbed to form a surface Plasmon wave.

Equations:[7]

λ= wave length in vacuum,   θ= angle perpendicular to the glass, K= wavelength vector

$K_{glass\text{-}chip} = K_{chip\text{-}sample}$

$K_{glass\text{-}chip} = (2*\pi/\lambda)*n_{glass}*\sin(\theta)$

$K_{chip\text{-}sample} = (2*\pi/\lambda)*(n_{chip}{}^2*n_{sample}{}^2/(n_{chip}{}^2+n_{sample}))^{.5}$

When both the wave vectors match energy is absorbed from the light by the Surface Plasmon effect. Because of conservation of energy we can determine the resonance angle by the angle of incidence at which we achieve maximum adsorption. By knowing the angle and wavelength that the surface Plasmon wave occurs at you can figure out the index of refraction of the sample. Most interments vary angle and have a set wavelength.
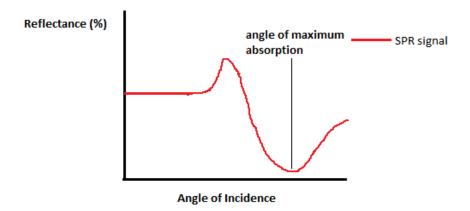
Figure 3: This is a graph showing the Reflectivity verses angle of a gold chip. The angle of maximum absorption is the angle at which both wave vectors mach allowing us to determine the index of refraction of the sample.

Each sample has a index of refraction that determines the angle at which the maximum energy is absorbed due to excitation of a Surface Plasmon wave. If the fluid/gas changes or if stuff starts to bind surface of the gold chip it will change index of refraction and thus the angle

of maximum absorption. By measuring the change of the angle at which the maximum energy is absorbed we can get a binding curve that will tell us how fast does association and dissociation occurs. Different concentrations of the same type of sample will give proportional peak hights.[7]Also It was surprising to learn that not all binding curves look like first order association/dissociation.[8]
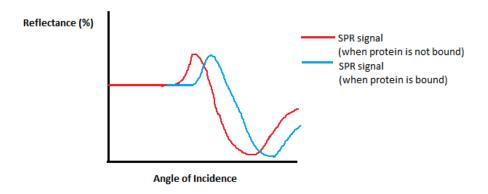

Figure 4: When the index of refraction of the sample changes the maximum absorption angle will shift.

The SPR channel has deionized water constantly flowing. The sample is filled into the loading chamber and then that sample is injected for a calculated amount of time. this creates a plug, which deforms over time to look more like a bullet.
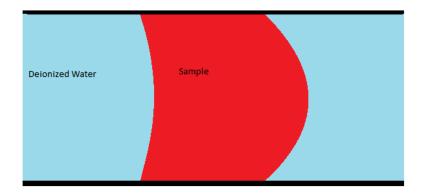
Figure 5: The injected sample will deform as it travels.

When the plug flows over the gold chip the sample binds to the chip changing the index of refraction and when the plug leaves the sample disassociates from the chip returning the index of refraction back to that of water
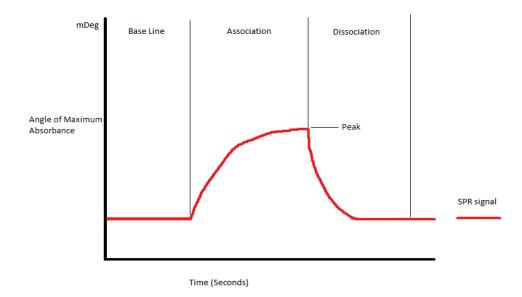

Figure 6: Typical binding, unbinding curve. As the sample is absorbed/unabsorbed onto the surface the index of refraction will change causing the angle of maximum refraction to change.
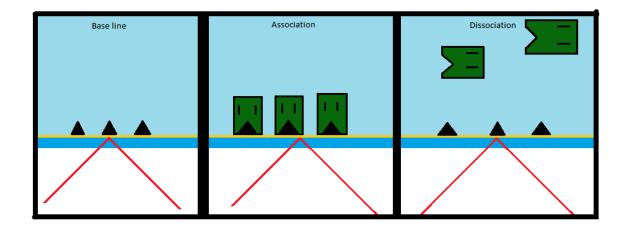
Figure 7: Example of what physically occurring in figure 6. The triangles represent binding sites (often antibodies) and the blocks represent the sample being analyzed.

The most common use of SPR is to bind protein to the surface of the gold chip and then add substrate. This gives them the association and disassociation constants of the substrate to the protein bound on chip. While silver offers a sharper maximum absorbance angle peak gold is more chemically stable. In fact research has been done to use a thin gold layer to protect the silver layer forming a two layer silver gold chip.[9]

Method

To see if we even get a SPR signal salt solutions of .1 M, .01 M, .001 M, .0001 M, .00001 M were made using salt packets. While these packets had plenty of additives we want to see if we get a signal before buying reagent grade salt. (results not shown)

After seeing that the salt packets do in fact show a binding curve we bought reagent grade NaCl from fisher Fisher Scientific. Using serial dilutions with deionized water salt solutions

of .1 M, .01 M, .001 M, .0001M, and .00001 M NaCl are made. Each solution concentration was

ran through the SPR machine randomly.

Results:

Below are the signals of the five different NaCl solutions used in this experiment concentrations varying

from .1 to .00001 molar. What is being measured is maximum absorption angle over time. The base line

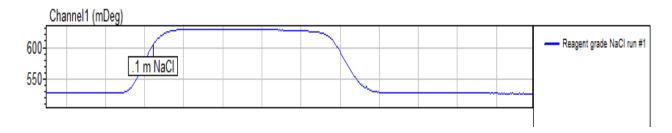is determined by the solvent in this case deionized water.

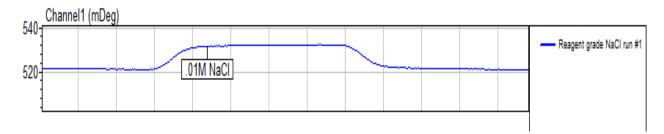**Figure 8: SPR signal at .1m NaCl. Angle of maximum adsorption mDeg vs. time**

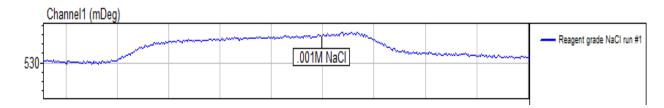**Figure 9: SPR signal at .01 NaCl. Angle of maximum adsorption mDeg vs. time**

Figure 10: SPR signal at .001 NaCl. Angle of maximum adsorption mDeg vs. time
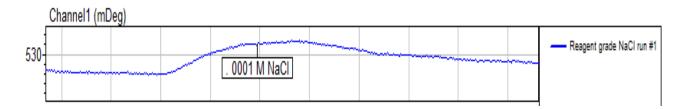
Figure 11: SPR signal at .0001 NaCl. Angle of maximum adsorption mDeg vs. time
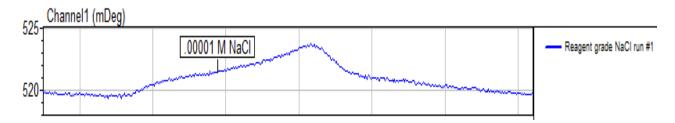

Figure 12: SPR signal at .00001 NaCl. Angle of maximum adsorption mDeg vs. time

It is interesting to note that the general shape of the binding curve changes depending on the concentration of the NaCl. The first two curves at .1 and .01 molar seem to have s shaped association/disassociation. While the .001 molar signal looks like a traditional binding curve. The last seems odd it has a point of inflection in its association but has what looks like a normal logarithmic disassociation.

Table 1: calculated diffuse layer concentration and observed signal peak for bulk sample concentration.

[NaCl] molar	Calculated diffuse layer [NaCl]	Peak change from base line mDeg
.1	2.3327E+24	109.764
.01	2.3327E+23	11.309
.001	2.3327E+22	3.546
.0001	2.3327E+21	3.775
.00001	2.3327E+20	4.435

Surface potential of gold 1.5^{10}, temp 298 K^{10}

The peak change was compared to the molar concentration and to the calculated concentration of the diffuse layer, to see if any correlation exists. The SPR peaks should be proportional to the bulk concentration if we are looking at a binding curve created by NaCl.[6] Since the concentration is proportional to the bulk concentration the diffuse layer concentration should also be proportional to the SPR peak since It is based on bulk concentration.

Comparing Signal to Bulk [NaCl]

y = 0.0009x - 0.0027
R² = 0.9991

Bulk [NaCl] Molar (y-axis): 1.20E-01, 1.00E-01, 8.00E-02, 6.00E-02, 4.00E-02, 2.00E-02, 0.00E+00

Signal change from baseline mDeg (x-axis): 0, 50, 100, 150

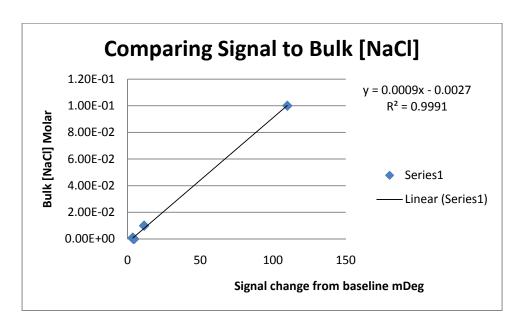◆ Series1
— Linear (Series1)

Figure 13: Looking to see if peek high is proportional to Bulk concentration of NaCl. If this signal is NaCl then it should be proportional and it is.
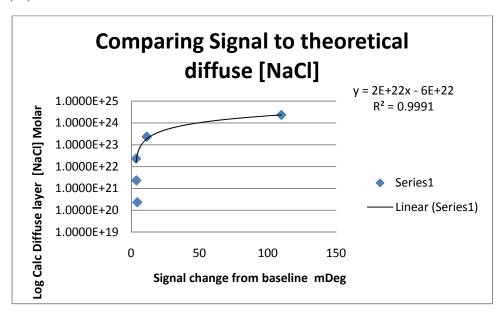
Comparing Signal to theoretical diffuse [NaCl]

y = 2E+22x - 6E+22
R² = 0.9991

Log Calc Diffuse layer [NaCl] Molar (y-axis): 1.0000E+25, 1.0000E+24, 1.0000E+23, 1.0000E+22, 1.0000E+21, 1.0000E+20, 1.0000E+19

Signal change from baseline mDeg (x-axis): 0, 50, 100, 150

◆ Series1
— Linear (Series1)

Figure 14: comparing peak height vs. theoretical diffuse layer concentrations of NaCl

Conclusion:

For all five samples we appear to have gotten binding curves in the SPR signal. Interestingly enough the binding curves seem to change in general shape depending on range of NaCl concentration, but more sampling needs to be done to say for certain. At .1, .01, and .001 molar NaCl sample's binding curves association and disassociation look like an s curve; could they be saturation curves? However the

.0001 molar NaCl samples look closer to a traditional binding curve. The curve at .00001 Molar is a bit odd it has a point of inflection in it's association but has a normal logarithmic disassociation. In addition it seems that the peak height is proportional to the sample concentration and theoretical diffuse layer concentration verifying that we are indeed looking at the SPR signals of salt.

For future experiments it would be interesting to take duplicate SPR readings over shorter intervals to see how the signal's general shape changes over different concentrations of NaCl if it does. Also it would be interesting on establishing a theoretical model to compare to the readings; this would require knowing index of refraction at different NaCl concentrations. Some factors that might be a concern is the shape of the plug since it will deform into more of a bullet shape as it travels and if it has enough time to completely associate. This might change the signal.

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