Chapter
Surface Plasmon Resonance Sensors for Concentration and Reaction Kinetic Detections

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Abstract
Surface plasmon resonance (SPR) is an optical phenomenon that occurs on the metal (normally gold or silver) film surface and the light that excited this phenomenon changes with the refractive index of materials on the metal surface. SPR sensors are constructed based on this phenomenon and are used in fields of biological and chemical analyses, drug screening, environmental monitoring, and so on. Here, we will make an introduction to applications of SPR sensors on reaction kinetic and concentration detections. To make this chapter readily comprehensible, we will divide it into three portions. The first part will be an abbreviated depiction of surface plasmon excitation and constructions of an SPR sensor. Then, we will aim at an introduction to the bimolecular interactions in SPR sensors. At last, we will make a summary on applications of SPR sensors.

Keywords: surface plasmon resonance, concentration, dissociation constant, kinetics, molecular reaction, sensors

1. Introduction
Surface plasmon phenomenon has been observed for over one century [1], and its applications in sensors have over 40 years [2, 3]. Surface plasmon resonance (SPR) sensors constructed based on this phenomenon are sensitive to the binding and unbinding of molecules on the sensor chip surface [4]. By online recording the reaction process, we obtained the SPR sensorogram and applied it to analyte concentration, especially molecular reaction kinetics analyses. These sensors are applied to the detections of the reactions between protein and protein, protein and DNA/RNA, small molecules and proteins/DNA/RNA, proteins/DNA/RNA/ small molecules and cells, and so on [5–8]. The application fields include chemical and biological analyses, food safety and environmental monitoring, and drug screening [9, 10].
Since the automatic sampling system and a series of commercial chips have been developed, the SPR instruments have been becoming operation-oriented design. And this is further expanding the application fields. But it still cannot maintain the molecular reactions occur at an ideal condition. As the reactions are occurred in solutions, the hydrodynamic conditions are a great variable factor [11]. To be better understanding of this technique and obtaining reliable data, we have to possess a good knowledge on the theory of molecular reactions in the SPR system.
2. SPR sensors

2.1 Excitations of surface plasmon

Surface plasmon (SP) phenomenon was first observed as anomalies in the metallic diffraction grating experiment by Wood in 1902 [1]. In 1941, Fano concluded these anomalies on the metallic surface to the excitation of electromagnetic wave [12].

Figure 1 shows the relationship between the incident light angle and the reflected light intensity in the absence and presence of thin gold film [13]. The total reflection occurs at 39 degree in the prism and air system (Figure 1A and B) and a dip (the drop of reflection) is observed around 43 degree in the presence of gold film (Figure 1C and D).

This approximated semicircular prism (Figure 1A and C) makes it possible that the angle of incident light remains unchanged. This significantly simplifies the optical system. As a result, Kretschmann geometry [14], which couples a prism and attenuates the total reflection, is the most common used excitation approach. In another geometry, Otto also used a prism to implement the SP excitation [15], but placed the dielectric layer between the prism and metal layer.

Figure 1.
(A) A polarized light illuminates the interface of air and a prism. (B) The intensity of reflected light corresponding to different incident light angle in panel (A). (C) a polarized light illuminate the interface of gold and prism. The dielectric material on the opposite side of the gold film is air. Panel (D) is the reflected light intensity in panel (C). The dielectric constants of air and the prism are 2.29 and 1, respectively. The thickness of the gold film is 50 nm, and its real and imaginary parts of dielectric constants are $12.3$ and $1.29$. The simulation was finished via the Winspall software.
As the SP wave intensity decays exponential along with and perpendicular to the propagation direction (Figure 1C), the SPR sensor can only be sensitive to the reflective index change next to the metallic surface and the sensitivity decays along with the distance from the metallic surface.

At last, the shape and position (resonance angle) of the dip (Figure 1D) are also affected by the reflective index of the materials next to the metallic film. For instance, the dip is much sharper in an air system than water, thus the sensor is more sensitive in an air solution than water.

Similar as Wood’s observation, the metallic diffraction grating is also applied to accomplish the SP excitation (Figure 2). This method is based on the diffraction of light and is named as grating coupling [16].

The third method is the waveguide coupling method [4]. As is shown in Figure 3, a metal film is striped on the wave guiding layer to achieve the SP excitation, and a superstrate is placed on the other side of the metal film for analytic applications.

2.2 Constructions of an SPR sensor

The successful excitations of SP via convenient methods introduce a various applications of this optics. Surface plasmon was first used to characterize thin films in 1978 [2]. In 1982, Nylander and Liedberg applied it to gas detection and biosensors [17]. Nowadays, a series of operation-oriented SPR instruments have been developed. As a result, the main work for the construction of an SPR sensor is choosing an appropriate sensor chip and establishing a reproducible procedure.

Figure 2.
An incident light with different wavelength illuminate the metallic grating and the narrow dark band is caused by the surface plasmon phenomenon.

Figure 3.
Waveguide coupling method for surface plasmon excitation.
Nonetheless, a brief introduction on the structure of the SPR sensor is helpful on the construction of an adequate chip. Figure 4 shows a typical SPR sensor, it consists of the portions for surface plasmon (SP) excitation, solutions delivery, signal detection and conversion, and data processing [18].

The solution delivery portion is applied for the transportation of solutions, which contain the analyte solutions and running buffers. It combines the SP excitation portion on the SPR sensor chip. In a typical Kretschmann geometry, the SP excitation accessories are placed next to the glass side of the sensor chip, and the hydrophobic fluidic channels, which have a width down to hundreds of micrometers, are placed next to the metallic film. The receptors pre-immobilized on the metallic side are reversed for the analytes in the solution transported by the fluidic channels. The reaction between the analytes and receptors causes the reflective index change next to the sensor chip surface (see Section 2.2.3). Furthermore, it causes the resonance angle shift. This shift is online collected by the detector and output as SPR signal. At last, we gather the SPR signal for data processing.

2.2.1 The dilution in the sample delivery

The dilution in the solution delivery process is a hindrance for obtaining reliable data. Figure 5 shows the concentration distribution of an analyte and its corresponding SPR sensorgram in a system without any molecular reactions or physical adsorptions. This concentration change can be confused with the concentration change caused by the mass transport limitation, and miss leading the data processing procedure.

Figure 4. The configuration of an SPR sensor.

Figure 5. (A) The dilution in a laminar flow tube during sample delivery. The arrow indicates the flow through direction. (B) The SPR sensorgram of the solution shown in panel (A).
To solve this problem, the microfluidic systems are applied. Also, the bi-direct flow system is efficient on cutting off the diluted samples [19, 20]. In reality, this problem can be alleviated by decreasing the sample delivery distance and increasing the sample delivery rate.

2.2.2 Constructions of SPR sensor chips

SPR sensor chip is the most critical accessory in the whole instrument for the construction of a sensor [21]. It contains a glass substrate and a thin metallic film. For simplify, this glass slide normally has the same reflective index as the prism in the instrument. To simplify the storage condition of the sensor chips and supply a longer shelf life, we usually choose gold film as a substrate. However, silver film supplies a more sensitive experimental condition.

Bare gold chip, containing glass slide and gold film, is the most common used sensor chip and is basic for the constructions of other chips. As this chip is susceptible contaminated and is not so biocompatible, the surface modifications are essential. Besides, these modifications also satisfy the condition for the receptors immobilization (see Section 2.2.3).

Figure 6 shows the most common applied modification case, the self-assemble method based on the interactions of gold and thiol- or disulfide groups [22]. The uniform of the alkyl chains on the chip surface is maintained by the van der Waals interactive force. The active groups on the other side of the alkyl chains are essential for the immobilization of receptors.

The dextran chip, containing carboxyl groups on the alkyl chain, is applied to construct a series of chips for certain applications. These commercial sensor chips include nitrilotriacetic acid chips (histidine tag capture), streptavidin chips (biotin capture), and lipid capture chips. The chips rely on DNA hybridization or the interactions of streptavidin and biotin are also produced. Other commercial chips contain click chemistry chips, cell capturing chips, and hydrophobic chips [23].

2.2.3 Molecular reactions on the sensor chip

The molecular reactions on the SPR sensor chip are different from the reactions in solutions. As is shown in Figure 7, receptors (B) are immobilized on the sensor chip surface and analytes (A) are transported to the fluidic channel on the top of the sensor chip to accomplish the reactions. The immobilization of A causes the change
of reflective index next to the sensor chip surface and further leads to the dip shifting from position 1 to 2 (bottom right in Figure 7). This change is recorded by a camera (the detector) and transfers to the SPR signal [24]. By gathering the signal collected at a specific time interval, we obtained the SPR sensorgram and applied it for data processing.

3. Bimolecular interactions

The molecular reactions in reality solutions can be affected by a series of factors, like temperature, concentration, mass transport, and so on. To obtain a better signal, the temperature is normally remained at 25°C in the SPR system. But the temperature control usually allows a higher temperature interval to satisfy different reactions.

As the receptors (B) are pre-immobilized on the chip surface, whose total amount is inalterable, the molecular reaction is the receptors’ consumption process. For the analytes (A), they are being transporting to react with B during the binding process. In an ideal condition, the analyte concentration can be maintained the same during the binding process. For the bimolecular reaction between B and A, it can be regard as a pseudo first-order reaction under this circumstance. It is the most common reaction in the SPR system and is a basic reaction for multiple molecules reactions (one B and more than one A).

3.1 Pseudo first-order reaction

Eq. (1) is the bimolecular reaction between B and A in the SPR system at an ideal condition.

\[
B + A \xrightarrow{k_a \ \ \ k_d} AB
\]

where \(k_a\) and \(k_d\) are the association and dissociation rate constants.

If the binding rate of A is higher than its unbinding rate, the SPR signal increases. In reverse, the signal decreases. Figure 8 are the SPR sensorgrams showing analytes (A) pass through the fluidic channel in the presence and absence of
receptors (B) on the sensor chip surface. Figure 8A shows the concentration change of A without any reactions, and Figure 8B is the binding and unbinding of A on the sensor chip surface. By combining the sensorogram with a mathematical approximate model, we obtain the kinetic constants of the reaction.

In an ideal condition, the concentration of analytes, [A] or C, maintains the same during the reaction. The pre-immobilized receptors can be divided into two portions, free receptors on the sensor chip surface (B) and receptors have already been combined with analytes (AB). The formation rate of AB can be expressed as \[25, 26\].

\[
\frac{d[AB]}{dt} = k_a[A][B] - k_d[AB] \tag{2}
\]

where [B] is the concentration of free receptors and [AB] is the concentration of AB. As [AB] is proportional to the SPR signal \( R \) and the total amount of B is proportional to the highest SPR signal \( R_{\text{max}} \), Eq. (2) can be further expressed as

\[
\frac{dR}{dt} = k_aC(R_{\text{max}} - R) - k_dR \tag{3}
\]

After the integral, Eq. (3) can be expressed as

\[
R = \frac{k_aCR_{\text{max}}}{k_aC + k_d} \left[ 1 - e^{-(k_aC + k_d)t} \right] \tag{4}
\]

At the end of the binding process, [A] or C becomes to zero. Analytes begins to unbind from the sensor chip surface. Eq. (2) can be expressed as

\[
\frac{d[AB]}{dt} = -k_d[AB] \tag{5}
\]

or

\[
\frac{dR}{dt} = -k_dR \tag{6}
\]

After the integral, Eq. (6) can be expressed as

\[
R = R_0e^{-k_dt} \tag{7}
\]

where \( R_0 \) is the SPR signal at the point [A] becomes to zero.
By making a good match between the experimental and simulated sensorgrams, the reaction rate constant \(k_a\) and \(k_d\) can be obtained. Furthermore, the association and dissociation constants can be obtained via Eq. (8).

\[
K_A = \frac{1}{K_D} = \frac{k_a}{k_d}
\]  

(8)

where \(K_A\) is the association constant and \(K_D\) is the dissociation constant.

At the condition, Eq. (4) is used for concentration detections, a higher amount of receptors have to be pre-immobilized to obtain a higher SPR signal. It also leads to a huge consumption of the analytes. At the point that the amount of analytes bound with receptors is higher than the analytes transported to the sensor chip surface, the concentration of the analyte next to the sensor chip surface changed.

By combining Eqs. (4) and (8), we obtain Eq. (9)

\[
R = \frac{R_{\text{max}}}{1 + \frac{K}{C}} \left[ 1 - e^{-(k_aC + k_d)t} \right]
\]  

(9)

where \(R_{\text{max}}\) and \(K_D\) are constant for a given reaction. This decreased analyte concentration \((C)\) directly leads to the numerical increase of \(k_a\). But the value of \(k_a\) should be the same for a given reaction at a certain temperature and pressure. Thus, the equations above cannot fit any more.

By introducing the mass transport coefficient into the reactions at a mass transfer limited condition \([27]\), we obtain Eq. 10.

\[
A \xrightarrow{k_m} A + B \xrightarrow{k_d} AB
\]  

(10)

where \(k_m\) is the mass transport coefficient. For the laminar flow system in the SPR fluidic channel, \(k_m\) can be expressed as

\[
k_m = 0.98(D/h)^{2/3}(f/bx)^{1/3}
\]  

(11)

where \(D\) is the diffusion coefficient, \(h\) and \(b\) are the height and width of fluidic channel, \(f\) is the volumetric flow rate, \(x\) is the distance from the receptor immobilization site.

Still, Eqs. (4) and (7) can be expressed as the following at the mass transport limited condition.

\[
R = \frac{k'_aCR_{\text{max}}}{k'_aC + k'_d} \left[ 1 - e^{-(k'_aC + k'_d)t} \right]
\]  

(12)

and

\[
R = R_0e^{-k'_dt}
\]  

(13)

where \(k'_a\) and \(k'_d\) are the association and dissociation rate constants at the mass transport limited condition and they can be expressed as

\[
k'_a = \frac{k_ak_m}{k_a[B] + k_m}
\]  

(14)
and

\[ k'_{d} = \frac{k_{d}k_{m}}{k_{a}[B] + k_{m}} \]  

(15)

Although the association and dissociation rate constants are affected by the mass transport, \( K_{A} \) and \( K_{D} \) can still be calculated via \( k'_{a} \) and \( k'_{d} \).

\[ K_{A} = \frac{1}{K_{D}} = \frac{k_{d}}{k_{a}} \]  

(16)

Eq. (12) can be used for concentration detections at mass transport limited condition. Two methods are available, one is built on the equilibrium value \( R_{eq} \) and the other one is based on the curve at the beginning of the binding.

\[ R_{eq} = \frac{k_{a}CR_{max}}{k_{d}C + k_{d}} = \frac{k'_{a}CR_{max}}{k'_{d}C + k'_{d}} = \frac{CR_{max}}{C + K_{D}} \]  

(17)

or

\[ \frac{1}{R_{eq}} = \frac{K_{D}}{R_{max}} \cdot \frac{1}{C} + \frac{1}{R_{max}} \]  

(18)

**Figure 9** are the SPR sensorgrams showing the bimolecular reactions at an ideal (solid line) and a mass transport limited (long dash line) condition. The mass transport limitation significantly prolongs the time to reach the equilibrium. As a result, the method based on \( R_{eq} \) is reliable, but not time consuming.

The method based on the curve at the beginning of the binding is attractive, as it is calibration free and high efficient. Although the calibration curve seems to be
ubiquitous and reliable for most of the concentration calculations, the standard samples normally cannot be obtained for a novel biomarker. This significantly hinders the development of the corresponding detection methods.

At a total mass transport limited condition, the binding rate of analytes is decided by the mass transport rate.

\[
\frac{dR}{dt} = k_m \frac{C_0 - C}{C_0} \cdot MW \cdot 10^9 \quad (19)
\]

where \( C_0 \) is the analyte concentration in the bulk solution, and \( MW \) is the molecular weight of the analyte. At the beginning of the binding process, \( C \) is zero. Thus, this equation can be expressed as

\[
\frac{dR}{dt} = k_m C_0 \cdot MW \cdot 10^9 \quad (20)
\]

where the value of \( dR/dt \) approximately is equal to the slope of the curve at the beginning of the bimolecular interaction.

3.2 Other reactions

Bimolecular reaction is the basic reaction in some multistep reaction. For instance, the Eq. (21) can be expressed as Eq. (22), which contains two bimolecular reactions.

\[
2A + B \xrightarrow{k_a} A_2B \quad (21)
\]

\[
A + B \xrightarrow{k_{a1}} AB + A \xrightarrow{k_{a2}} A_2B \quad (22)
\]

4. Applications

The SPR sensors are available for both concentration and reaction kinetic detections [28]. Their application fields include biological and chemical analyses, drug screening, environmental and food safety monitoring [29].

The concentrations of the biomarkers are essential information for the early diagnose of disease [30]. The reaction kinetics is a crucial parameter for drug screening [31], biological molecules interactions, and chemical industry. The association/dissociation constant is helpful on estimating the strength of the bond [32, 33]. The SPR sensor equipped with different chips affords a friendly condition for the biological molecular reactions. And the chips modified with specific materials (fibronectin, polylysine, and so on) can satisfy the condition for cell culturing [34]. This enlarges the applications of the SPR sensors on biomarker concentration detections, reaction kinetics of molecules, and reaction kinetics between cell membrane and molecules [8].

Natural compounds are usually more biocompatible than synthetic ones, thus they are more suitable drug candidates. But natural compound normally has a low concentration and mixes with other chemicals. The low detection limit and the convenient kinetic detection method make SPR sensors to be widely used in small molecule drug screenings [35]. Furthermore, the high throughput [36, 37] and
mapping technique allow a quick grasp of drugs from natural compounds [22, 23], and even reprocessing of approved drugs.

The contaminants in the environment and food are harmful to our healthy [38, 39]. The SPR sensors not only can detect the concentration of a certain contaminant, but also can make a rough screening of the unknown chemical and biological contaminants. For possible contaminants, the SPR sensor can grasp the molecules or cells for further analyses.

5. Summary

The binding and unbinding of analytes on the SPR sensor chip surface induces the refractive index change next to the thin metallic film. Surface plasmon resonance sensors are sensitive to this refractive index change and transfer this change to the shift of the resonance angle. By a real time recording of the angle change, we obtained the SPR signal for data processing.

The bimolecular reaction is a basic reaction on the sensor chip. By making a good match of the simulated and experimental SPR sensorgrams, we obtained the kinetic constants. Furthermore, we also use the SPR sensors on concentration detections. The concentration detection method based on the $R_{eq}$ value is sensitive but time consuming. The novel calibration free method based on the curve at the beginning of the binding process is time saving. Besides, it efficiently avoids the demand of standard samples. Although its sensitivity is not high, it is still a promising method.

Right now, SPR sensors have been applied in the field of biological and chemical analyses, drug screening, environmental and food safety monitoring. As its integration with new technologies, the application fields of SPR sensors will be further enlarged.

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Conflict of interest

The authors declare no conflict of interest.
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