

BIOSENSORS BASED ON SURFACE PLASMON RESONANCE PHENOMENON: A THEORETICAL REVIEW

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Abstract. Surface Plasmon's are waves that propagate along the surface on a conductor, and have been studied intensively. The phenomenon has been utilized in studies of the properties of surfaces and thin films devices and, in particular, to recent developments in biosensor devices. Surface Plasmon resonance (SPR) biosensors gained an important place in detection, point- of- care diagnostics, and even as potential therapeutic agents. In this way the SPR biosensors have becomes central tool for characterizing and quantifying biomolecular interaction. Not all the materials can be utilized as surface active media; gold (Au) and silver (Ag) are the best examples of materials which can support surface Plasmon's. The optical properties of metallic nanostructures are particularly important for the field of Nano science and technology. This paper attempts to review the major developments in SPR technology. Main application areas are outlined and examples of applications of SPR sensor technology are presented.

Keywords: surface Plasmon resonance, Plasmon's, biosensors

1. Introduction

Conforming to Zdzislaw Salamon and Gordon Tollin [1], the current interest in the properties of the thin films based on surface plasmon resonance (SPR) phenomenon has increase do to the applications to thin film devices, and in particular, to recent developments in biosensor devices.

Doing for their significant proprieties many biosensors using SPR phenomenon was developed in recent years, for examples electrochemical (amperometric, potentiometric or conductimetric), calorimetric, acoustic, and optical biosensors [2].

Various researchers intensively studied the phenomenon of SPR. Otto [3], Kretschmann and Raether [4] and Swalm that brought understanding and showed the versatility of the technique initially observed the phenomenon.

In the work of P. Anton van der Merwe [5] is explained how the SPR phenomenon occurs. The detection principle relies on SPR, an electron charge density wave phenomenon that arises at the surface of a metallic film when light is reflected at the film under specific conditions. The resonance is a result of energy and momentum being transformed from incident photons into surface plasmons, and is sensitive to the refractive index of the medium on the opposite side of the film from the reflected light.

Concerning the measurements of SPR, according to Kooyomann [6] these could be done using optical systems that control parameters to

which SPR is sensitive, namely incident angle, wavelength of incident light, degree of polarization, and optical materials. The SPR technique is used in development and characterization of ultra- thin films. Generally, a typical SPR experiment requires a dielectric substrate, a prism in most cases, which has been coated with a suitable noble metal at a precise thickness. The combination of the substrate, noble metal, and sample in contact with the metal, allows for generation and support of Surface Plasmon Polaritons (SPPs) that are formed along the metal-dielectric interface. These polaritons are highly damped charge density waves oscillating at optical frequencies, and may be excited if the materials and optical properties of an experimental system are chosen correctly.

2. Theoretical aspects regarding SPR phenomenon

The SPR phenomenon occurs at the surface by shining on a layered system consisting of a transparent medium on one side, a metal film (most often gold or silver) and a dielectric on the other. The physical explication regarding the SPR phenomenon is described below as a review of P. Anton van der Merwe research [5].

When a beam of light passes from material with a high refractive index (e.g. glass) into material with a low refractive index (e.g. water) a part of the incident light is reflected at the interface.

When the angle at which the light strikes the interface (the angle of incidence or θ) is greater than the critical angle value (θ_c), the light is completely reflected (total internal reflection) as shown in figure 1.

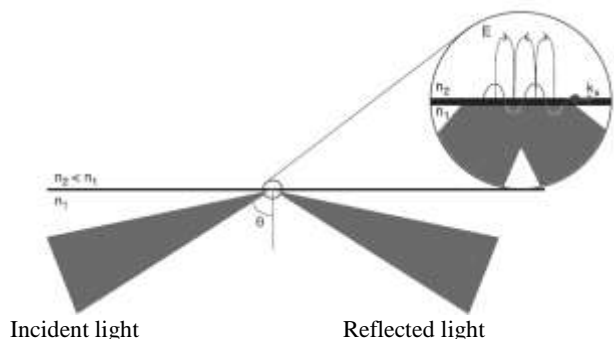


Figure 1. Total reflection of light when the incident beam passes from a material with refractive index n_1 into another material with refractive index $n_2 < n_1$ [5]

Since the wavevector of the plasmon wave is bound to the conductor surface, it is the wavevector of the component of the incident light which is parallel to the conductor surface that can be equal to the wave-vector of the surface plasmons (k_{sp} , k_x in figure 2).

The magnitude of the surface parallel wave vector, k_x , can be expressed according to as:

$$k_x = \frac{2\pi}{\lambda} \cdot n_1 \cdot \sin \theta \quad (1)$$

The wavevector of the plasmon wave, k_{sp} , depends on the refractive indices of the conductor - n_c (being a constant complex number) and the sample medium - n_2 :

$$k_{sp} = \frac{2\pi}{\lambda} \cdot \sqrt{\frac{n_c^2 \cdot n_2^2}{n_c^2 + n_2^2}} \quad (2)$$

In both expressions, λ is the wavelength value for the light in vacuum. Thus, an increased refractive index of the sample, n_2 , penetrated by the plasmon enhanced evanescent field increases the wavevector of the plasmon wave. The wavevector of the light k_x can be tuned to equate the plasmon wavevector by varying either the angle of incidence θ , or the wavelength of the light, figure 2.

If the surface of the glass is coated with a thin film of a noble metal, this reflection is not total; some of the light is “lost” into the metallic film. There exists a second angle greatest and at which the intensity of reflected light reaches a minimum or “dip”. This angle is called SPR angle (θ_{spr}) – figure 2, and it is a consequence of oscillation of mobile electrons or plasma at the surface of the metal film.

These oscillating plasma waves are called *Surface Plasmon's (SPs)*. When the wavevector of the incident light matches the wavelength of the surface Plasmon's, the electrons “resonate”, hence the term SPR. Surface Plasmon's can be excited by shining on a layered system consisting of a transparent medium on one side, a metal film (most often gold or silver) and a dielectric on the other. When the light is incident at an angle bigger than the critical angle of total internal reflection, an evanescent wave is produced and penetrates into the adjacent medium.

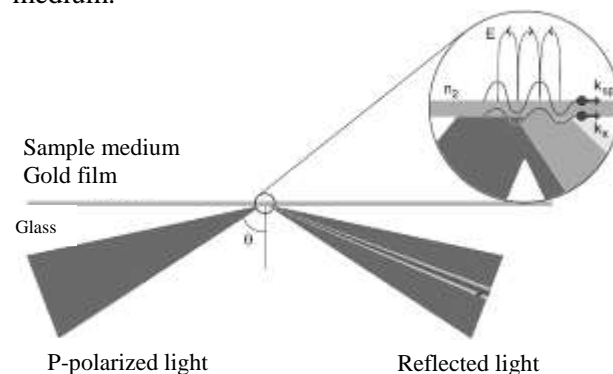


Figure 2. Partial reflection of light when the surface is coated with a metallic thin film [5]

The maximum coupling between the evanescent wave and the surface Plasmon takes place when their phase velocities coincide at which point the surface Plasmon's is excited at resonance. Thus, SPR occurs at a characteristic angle of incidence. This angle depends on the thickness as well as the dielectric of the layers of the adjacent media. Since the permittivity depend on the frequency of the exciting laser light, the resonance angle does too. The most convenient geometry for the development of a sensor is the Kretschmann-Raether configuration which consists on a glass prism, a metal film and the adjacent medium that is to be probed (figure 3) [5].

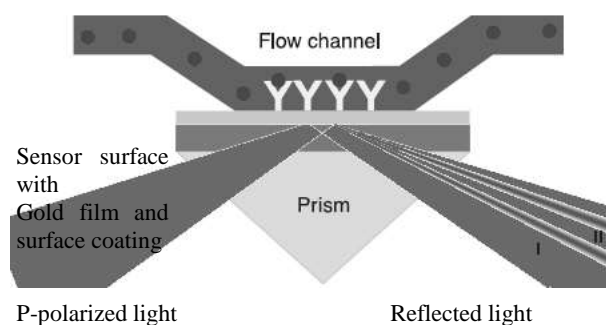


Figure 3. Kretschmann-Raether configuration [5]

3. Surface Plasmon Properties

Speaking about the SPR phenomenon, and related to SPs, a number of specific properties are associated that are particularly relevant to sensor applications: (1) the field enhancement, (2) the phase jump of the reflected field upon SP excitation and (3) the SP coherence length.

Field enhancement. A calculation of the electric field transmission coefficient based on Fresnel's equations for the interface reveals that the electric field at the low index side of the metal can be much larger than that at the other side of the metal layer. Kooyman [6] showed as well the fact that the intensity enhancement is depicted as a function of the angle of incidence of incoming light for a number of different thicknesses of a gold layer. It is found that very close to the SPR angle the intensity can be enhanced by a factor of more than 30. This circumstance accounts for much of the remarkable sensitivity that the SPR condition has for a changing dielectric environment.

Phase jump. A reflection event at an interface is generally accompanied by a phase jump of the reflected field. This is illustrated in figure 4a for a prism–gold–water system. For comparison, the “conventional” SPR dip is shown in figure 4b for the same layer system. Around the SPR dip the phase of the reflected electric field undergoes a relatively large change. The significance of this phenomenon for sensing purposes is more clear when plot the reflectance and phase changes as a function of incident angle for a certain change in dielectric constant of the water. This is depicted in figures 4c and 4d (Kooyman assume that both the change in reflection coefficient ΔR and the phase change $\Delta\phi$ are proportional to $\Delta\varepsilon$). From figure 4c he estimated that $\Delta R/\Delta\varepsilon \approx 30$, whereas from figure 4d he found that $\Delta\phi/\Delta\varepsilon \approx 250$.

Experimentally, a minimum $\Delta R \approx 10^{-3}$ can be measured, whereas a minimum $\Delta\phi \approx 10^{-3}$ is feasible, using interferometric techniques. The conclusion is that on the basis of reflectance measurements a minimum $\Delta\varepsilon \approx 4 \times 10^{-5}$ can be detected, whereas a phase measurement provides a sensitivity of $\Delta\varepsilon \approx 4 \times 10^{-6}$.

In view of the very approximate character of this calculation, the absolute values found are of limited validity; however, the finding that a phase measurement provides an order of magnitude better sensitivity is a hard conclusion and, indeed, this was demonstrated by Nikitin [7, 8].

The only drawback of this approach seems to be the much more complicated experimental setup.

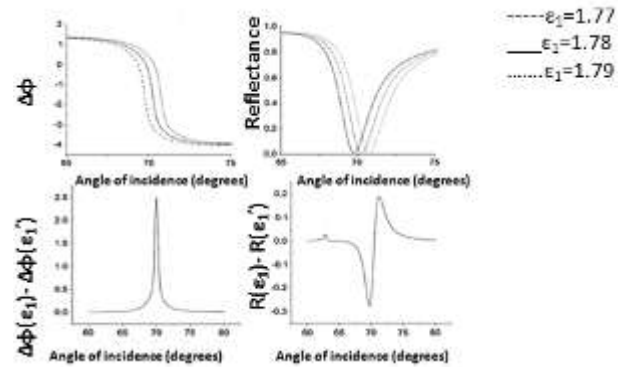


Figure 4. Comparison of the angle-dependent phase changes (a, c) and reflectance changes (b, d) for variation of the dielectric constant at the low-index side of the metal layer.

A gold layer is used, SPs are excited at $\lambda=700$ nm. (c) and (d) depict the differential phase and reflectance, respectively, for a change in the medium's dielectric constant of 0.01 [13]

SP coherence length. Generally, the metal's dielectric constant ε_2 is complex and this circumstance results in a complex propagation constant $k_x = k_x'' + j \cdot k_x'''$, where k_x'' and $j \cdot k_x'''$ are real and imaginary parts, respectively. For a surface plasmon, travelling along the interface with wavevector k_x , this implies that the field intensity decays with a characteristic distance $1/2k_x'''$. For gold and silver, the standard metals in sensor applications the imaginary part of the dielectric constant increases with decreasing wavelength and the SP propagation length decreases accordingly.

This is illustrated in figure 5: here a layer system was prepared where a 30 nm SiO_2 strip was deposited on a 50 nm silver layer. For a series of wavelengths the angle of incidence was chosen such that SPs were excited in the area outside the strip and for each wavelength the whole area was illuminated with a collimated light beam under a constant angle of incidence.

Because of the contrast in dielectric constant between the strip and its surroundings (air), the SP resonance condition is not fulfilled in the area below the strip and we see the decaying SP (increasing reflectance) at the left edge of the strip. Beyond the right edge of the strip, the SPR condition is again fulfilled and the SP resonance builds up. The figure nicely demonstrates that with decreasing wavelength the SP propagation length becomes shorter: the blurring on the left side of the strip becomes less prominent for shorter wavelengths. It turns out that in the wavelength range 500 nm the propagation length varies between 10 and 40 nm.

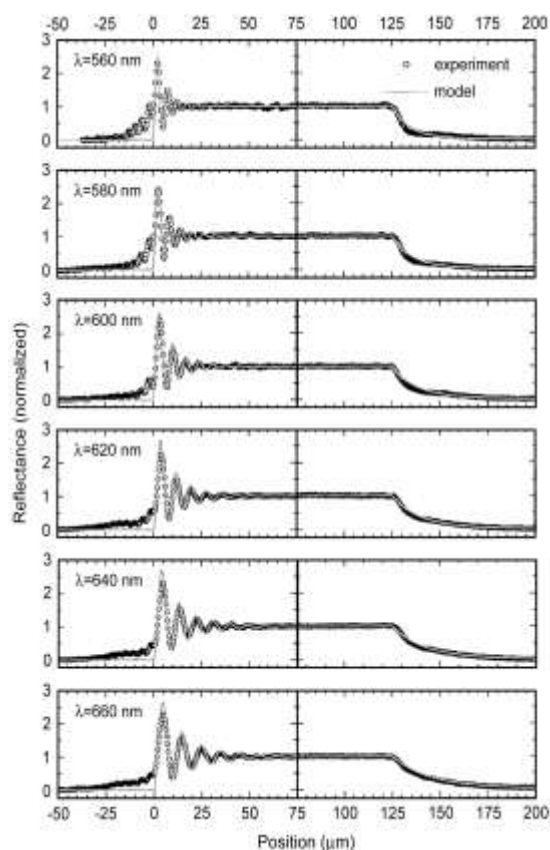


Figure 5. SPR response to a dielectric step at several wavelengths. For each wavelength the light angle of incidence is set such that outside the strip (extending from 0 to 125 μm) the interrogating k_x is resonant with the surface plasmon wavevector. The surroundings of the strip has dielectric constant $\epsilon_3 = 1$ [13]

4. SPR biosensors

Yuanyuang et al. [9] related that biosensors have been first reported in 1962 [10] and are generally defined as sensors that consist of biological recognition elements, often called bioreceptors, or transducers conform as Vo- Dinh et al. [11]. The biosensors have two basic principles, which are very different from conventional chemical sensors: (1) the sensing elements are biological structures, such as cells, enzymes, or nucleic acids; (2) the sensors are used to measure biological processes or physical changes. Owing to the constant desire for novel devices that offer higher sensitivity, greater analyse discrimination, and lower operating costs, intensive research efforts have been done to improve the sensing and transducing performance.

According to Jain With investigations, the recent advances in nanotechnology, nanomaterials have received great interests in the field of biosensors due to their exquisite sensitivity in chemical and biological sensing [12].

Homola [13] has also studied SPR sensors. According to this, SPR sensors are thin-film refractometers that measure changes in the refractive index occurring at the surface of a metal film supporting a surface Plasmon. A surface Plasmon excited by a light wave propagates along the metal film, and its evanescent field probes the medium (sample) in contact with the metal film. A change in the refractive index of the dielectric gives rise to a change in the propagation constant of the surface Plasmon, which through the coupling condition alters the characteristics of the light wave coupled to the surface Plasmon (e.g., coupling angle, coupling wavelength, intensity, phase) [13].

Clerc showed that based on which characteristic of the light wave modulated by a surface Plasmon is measured; SPR sensors are classified as sensors with angular, wavelength, intensity, or phase modulation [14].

In SPR sensors with angular modulation, a monochromatic light wave is used to excite a surface Plasmon. The strength of coupling between the incident wave and the surface Plasmon is observed at multiple angles of incidence, typically by employing a convergent light beam. The excitation of surface Plasmon's is observed as a dip in the angular spectrum of reflected light. The angle of incidence yielding the strongest coupling is measured and used as a sensor output [15].

In SPR sensors with wavelength modulation, a surface Plasmon is excited by a collimated light wave containing multiple wavelengths, typically a beam of polychromatic light. The excitation of surface Plasmon's is observed as a dip in the wavelength spectrum of reflected light.

SPR sensors with intensity modulation are based on measuring the strength of the coupling between the light wave and the surface Plasmon at a single angle of incidence and wavelength, and the intensity of light wave serves as a sensor output [16]. In SPR sensors with phase modulation the shift in phase of the light wave coupled to the surface Plasmon is measured at a single angle of incidence and wavelength of the light wave and used as a sensor output [17].

To increase the sensitivity of intensity-modulated SPR sensors, Lechuga's group proposed an approach based on combination of the magneto-optic activity of magnetic materials and a surface Plasmon resonance in a special multilayer structure [18]. They demonstrated an improvement in sensitivity by a factor of three compared to a conventional intensity-modulated SPR sensor and a

refractive index resolution of 5×10^{-6} RIU.

A typical example of a high-throughput SPR sensor is the SPR imaging [19, 20]. In a typical SPR imaging configuration, a beam of monochromatic light passes through a prism coupler and is made incident on a thin metal film at an angle of incidence close to the coupling angle. The intensity of reflected light depends on the strength of the coupling between the incident light and the surface Plasmon and therefore can be correlated with the distribution of the refractive index at the surface of the metal film [19, 20].

Corn's group has researched SPR imaging for over a decade. In their earlier works, they employed a HeNe laser as a source of illumination [20].

However, a highly coherent light source generated images with parasitic interference patterns that were disturbing SPR measurements. In 1997 they improved their SPR imaging instrument by introducing an incoherent light source and a Near-IR (NIR) narrow band-pass filter [21].

Using this approach, they detected hybridization of short (18-base) oligonucleotides at concentrations as low as 10 nM [22] (this was estimated to correspond to a refractive index resolution in the 10^{-5} RIU range). The use of a white light source and a bandpass filter was also advocated by Yager's group [23] that demonstrated that their SPR imaging instrument operating at a wavelength of 853 nm can provide a refractive index resolution of 3×10^{-5} RIU [24].

In 2005 Corn's group reported SPR imaging with a special multilayer structure supporting long-range surface Plasmon's; however, the use of long-range surface Plasmon's led only to minor sensitivity improvements of 20% (experiment) and 40% (theory) compared to the conventional SPR imaging [25].

A dual-wavelength SPR imaging system was reported by Zybin et al. [26]. In their SPR sensor, they used two sequentially switched-on laser diodes, and the intensities of the reflected light at the two different wavelengths were measured and the sensor output was defined as the difference of these two signals. A refractive index resolution of 2×10^{-6} RIU was achieved when the signal was averaged over a large beam diameter (6 mm^2).

Campbell's group reported an SPR imaging system with a controllable angle of incidence [27, 28]. This feature allows SPR images to be acquired sequentially at different angles of incidence and selection of the optimum angle of incidence for the SPR measurements. With a HeNe laser as a source

of light, their sensor was able to measure simultaneously in 120 sensing channels with a refractive index resolution of 2×10^{-5} RIU. Recently, they claimed an improvement in sensor resolution down to 5×10^{-6} RIU [29].

Recently, Piliarik et al. investigated SPR imaging with an elliptically polarized light [30] and concluded that a change in the polarization of light induced by the coupling of light to a surface Plasmon can be exploited to significantly improve the sensitivity and operating range of SPR imaging sensors. In addition, this approach, as illustrated in figure 6, provides high-contrast SPR images (with a low background), which are well suited for automated image analysis.

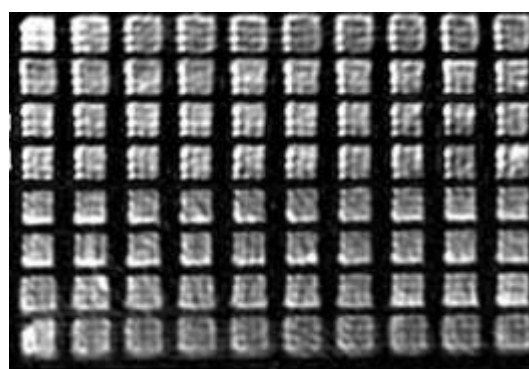


Figure 6. Typical image obtained with an SPR imaging sensor with a polarization control. Bright rectangles correspond to areas of an SPR chip ($300 \times 300 \text{ mm}$) covered with a monolayer of albumin molecules formed on the surface of gold by microspotting [13]

Homola's group developed a SPR imaging approach based on polarization contrast and excitation of surface plasmons on spatially patterned multilayers [31]. In this configuration, a prism coupler with an SPR chip containing a spatially patterned multilayer structure was placed between two crossed polarizers. The output polarizer blocked all of the light reflected from the (inactive) areas outside the sensing areas, generating high-contrast images. The output polarizer blocked all of the light reflected from the (inactive) areas outside the sensing areas, generating high-contrast images. Two types of SPR multilayers with opposite sensitivities to refractive index were employed, and the output signal was defined as a ratio of the intensities generated from the two neighbouring multilayers. This sensor was shown to be able to detect refractive index changes down to 2×10^{-6} RIU and to detect short oligonucleotides (23-mers) at concentrations as low as 100 pM [32].

5. Applications of SPR Sensors for Detection of Chemical and Biological Species

SPR biosensors have been applied in numerous important fields including medical diagnostics, environmental monitoring, and food safety and security. Various formats for the detection of chemical and biological analytes have been applied in SPR sensors [33, 34].

The format of detection is chosen on the basis of the size of target analyte molecules, binding characteristics of available biomolecular recognition element, range of concentrations of analyte to be measured, and sample matrix [34].

The most frequently used detection formats include (a) direct detection, (b) sandwich detection format, (c) competitive detection format, and (d) inhibition detection format (figure 7).

In the direct detection mode (figure 7A), the biorecognition element (e.g., antibody) is immobilized on the SPR sensor surface. Analyte in solution binds to the antibody, producing a refractive index change detected by the SPR sensor. Direct detection is usually preferred in applications, where direct binding of analyte of concentrations of interest produces a sufficient response. The specificity and LOD can be improved by using the sandwich detection format (figure 7B), in which the sensor surface with captured analyte is incubated with a second antibody. Smaller analytes (molecular weight < 5000) often do not generate a sufficient change in the refractive index and therefore are measured using either competitive or inhibition detection format.

Figure 7C shows an example of the competitive detection format, in which the sensing surface is coated with an antibody interacting with the analyte; when a conjugated analyte is added to the sample, the analyte and its conjugated analogue compete for a limited number of binding sites on the surface [13]. The binding response is inversely proportional to the analyte concentration. In the inhibition detection format (figure 7D) a fixed concentration of an antibody with affinity to analyte is mixed with a sample containing an unknown concentration of analyte. Then, the mixture is injected in the flow cell of the SPR sensor and passed over a sensor surface to which analyte or its analogue is immobilized. Noncomplexed antibodies are measured as they bind to the analyte molecules immobilized on the sensor surface. The binding response is inversely proportional to the concentration of analyte.

According to Homola's research, the application of SPR sensors are in different types of

domains, as follows: Food Quality and Safety Analysis (Pathogens, Toxins, Veterinary Drugs, Vitamins, Hormones, etc.), Medical Diagnostics (Cancer Markers, Antibodies against Viral Pathogens, Drugs and Drug-Induced Antibodies, Allergy Markers, etc.), Environmental Monitoring (Pesticides, Heavy Metals, Aromatic Hydrocarbons, Phenols, etc.).

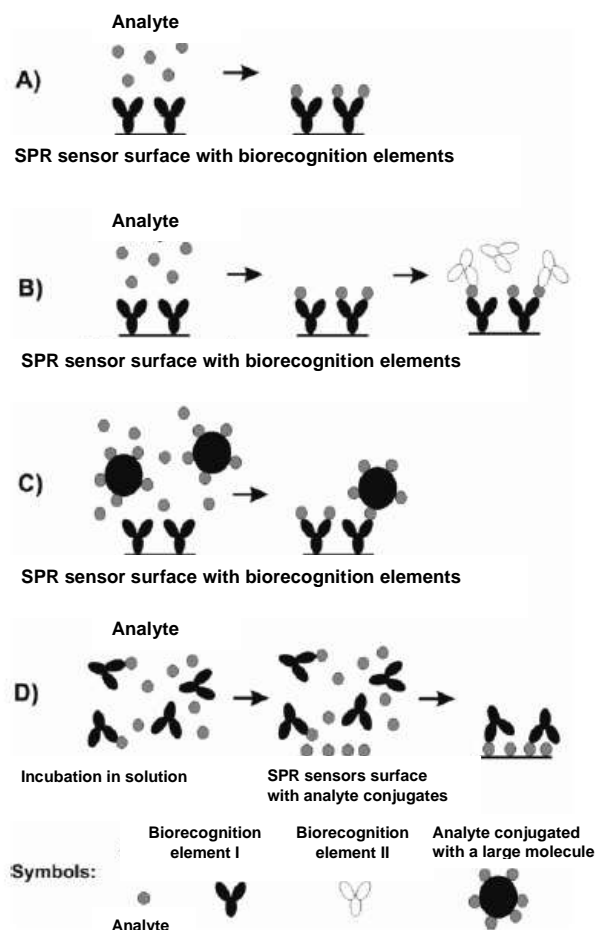


Figure 7. Main detection formats used in SPR biosensors: (A) direct detection; (B) sandwich detection format; (C) competitive detection format; (D) inhibition detection format [13]

6. Future objectives

In present, it is of great interest to encapsulate the TiO₂ nanostructures with highly dispersed noble metal nanoparticles such as Ag, Au and Pt, because the nanoscale noble metals are usually classic high-performance heterogeneous catalysts.

Based on this state-of-art study, the research group will try to investigate the properties of these kinds of materials, under thin-film forms, and it will try to find correlations between these properties and biological sensor applications.

6. Conclusions

In the last years, many biosensors were demonstrated. The most interest research field is for biosensors based on detection of a variety of chemical and biological analytes. Most of these biosensors are based on prism coupling and angular or wavelength spectroscopy of surface Plasmon's.

Commercial SPR systems have played an important role in the development of detection applications due to their increasing spread and the availability of special SPR platforms and kits dedicated to specific applications. SPR biosensor is a result of a multitude of factors (performance of optical platform, characteristics of the employed biorecognition element, suitability and degree of optimization of the immobilization method, detection format, and methodology), and thus low performance of one part of the biosensor (e.g., optical platform) can be compensated for by high performance of another component (e.g., biorecognition elements).

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