SURFACE PLASMON RESONANCE FOR GAS DETECTION AND BIOSENSING*

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Abstract

Surface plasmon resonance is a new optical technique in the field of chemical sensing. Under proper conditions the reflectivity of a thin metal film is extremely sensitive to optical variations in the medium on one side of it. This is due to the fact that surface plasmons are sensitive probes of the boundary conditions. The effect can be utilized in many ways. A description of how it can be used for gas detection is given, together with results from exploratory experiments with relevance to biosensing.

Introduction

Physical phenomena that can be used in the development of new sensors are currently being sought. A number of principles have been tried but there are still many that are unexplored. One optical phenomenon, which is promising for chemical sensing, is surface plasmon resonance (SPR) [1]. We have recently shown how this effect can be used for gas detection [2]. In this paper we briefly describe the SPR phenomenon and its application to gas sensing. We thereafter report on some exploratory experiments with relevance to biosensing.

A surface plasmon is a surface charge density wave at a metal surface. It can also be described in terms of a TM wave. The type of plasmon that is interesting in this context is the non-radiative surface plasmon. The dispersion relation for such a plasmon is given by

$$k_{sp} = \frac{\omega}{c}\left(\frac{1}{\epsilon} + \frac{1}{\epsilon_m}\right)^{-1/2} \quad (1)$$

where ϵ_m is the real part of the dielectric constant of the metal at the given frequency and ϵ is the dielectric constant for the dielectric medium outside

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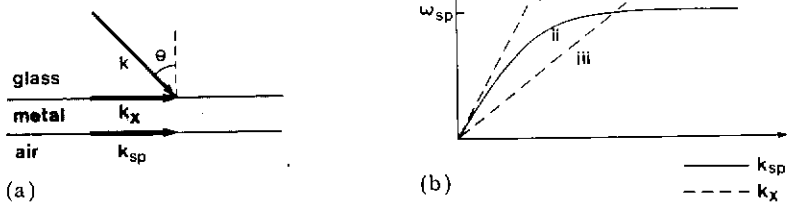


Fig. 1(a) The layer system used for surface plasmon resonance. k_{sp} and k_x are the wave vectors given in eqns. (1) and (2). (b) Dispersion curves for (i) light in air, (ii) the surface plasmons and (iii) light in glass. Surface plasmon resonance occurs at the intercept of curves (ii) and (iii). By varying the angle of incidence, the resonance can be obtained for any frequency below ω_{sp}.

the metal. The frequencies of interest are those where ϵ_m is negative. If light is incident to the surface with an angle θ, then the wave vector of the light parallel to the surface is

$$k_x = \frac{\omega}{c}\sqrt{\epsilon}\sin\theta \qquad (2)$$

It is realized from eqns. (1) and (2) that for a negative ϵ_m the parallel wave vectors for the incident light and the surface plasmon are not equal for any values of ω and θ. However, if the metal is in the form of a thin film on, *e.g.*, a glass substrate, then the dielectric constants on the two sides of the metal are different, *i.e.*, ϵ is larger in eqn. (2) than in eqn. (1). If the film is thin enough, light incident on the glass/metal interface will also affect the metal/air interface. In this case there exists an angle of incidence where eqns. (1) and (2) match each other (Fig. 1). Thus, a surface plasmon resonance will occur.

The surface plasmon resonance is experimentally observed as a very sharp minimum of the light reflectance when the angle of incidence is varied. The resonance angle is very sensitive to variations in the refractive index of the medium just outside the metal film. For example, if the medium outside a silver film is changed from air ($n = 1$) to water ($n = 1.33$), then the resonance angle for HeNe light changes from 43° to 68°. Since the electric field probes the medium within only a few thousands of Ångströms from the metal surface, the resonance is very sensitive to variations in thin films on this surface. By choosing an angle of incidence half way down the reflectance minimum and measuring the intensity of the reflected light at that constant angle, changes in the refractive index of about 10^{-5} are easily detected. This is due to the sharpness of the resonance minimum. The sensitivity is hence very high, even compared with ellipsometry.

Gas detection

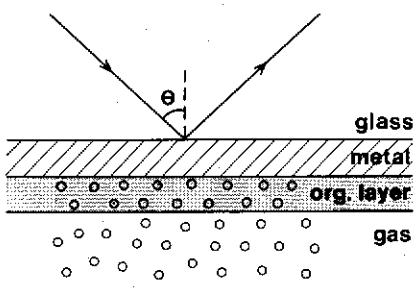
One application of SPR, which we have reported on earlier [2], is gas detection. There are a large number of reversibly gas-absorbing organic materials which are used in gas chromatography. If a thin film of such a material is formed on top of a metal film, the small change in refractive index of the organic material upon gas absorption can be detected by SPR (see Fig. 2(a)). By choosing a fixed angle of incidence half way down the reflectance minimum, very small shifts of the resonance angle are seen as large variations in the reflected light intensity. Figure 2(b) shows the result when a 430 Å thick film of a silicon-glycol copolymer is exposed to different concentrations of the anaesthetic gas halothane.

Sensitivity, stability and speed of response are very good for this system. Gas concentrations from a few ppm up to several per cent can be measured with good reproducibility. The responses are very fast and completely reversible. This particular organic material is sensitive to halogenated hydrocarbons such as halothane, trichloroethene, carbon tetrachloride etc. However, it is also sensitive to variations of the humidity.

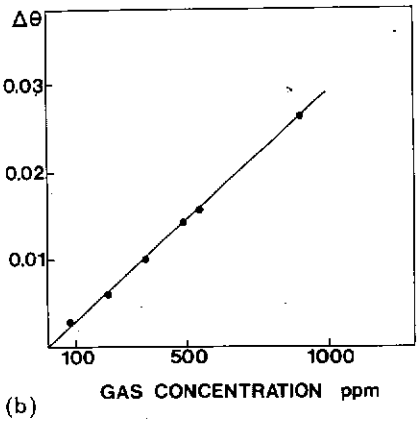
A number of other materials have also been studied. For example, the substance OV-225, which is a cyanopropylphenyl, is very sensitive to methanol and toluene but less sensitive to halogenated hydrocarbons, while squalane is very sensitive to octane and toluene.

In the experiment shown in Fig. 2(b) SPR was used in parallel with a quartz microbalance [2, 3]. We conclude that the two techniques are generally comparable with respect to sensitivity, stability, speed of response and selectivity. However, in certain applications one of them may have some advantages over the other.

By means of a near-visible laser diode a very compact SPR gas detector can be made. Since the divergence of such lasers is large, the metal film



(a)



(b)

Fig. 2(a) Schematic view of the layer system used in the gas sensing application. (b) Measured response to halothane gas exposure in the ppm range.

should be made as a very narrow strip. This can be done by means of photolithography.

Antibody adsorption

Surface plasmons can also be excited at a metal/water interface. From eqns. (1) and (2) it is found that for a silver film deposited on to glass, the resonance will occur when the angle of incidence is 68 degrees. In these experiments we have used the glass slide as one wall of a 2 ml cavity through which water solutions could flow. Figure 3 shows the reflectance *versus* angle of incidence for a number of situations. Starting from the left, the curves are explained as follows. First, we show surface plasmon resonance when the cell is empty, *i.e.*, for a silver/air interface. Thereafter the cell was filled with water and the second curve from the left (marked 'clean' in the Figure) was measured. The fact that this curve is broader than the first curve is expected from theory [1]. The curves were measured on a number of silver films with excellent reproducibility. The water was then exchanged for a water solution of the antibody human γ-globulin (IgG). The antibodies are known to adsorb as a single monolayer on the surface. As seen in Fig. 3 (third curve from the left) this results in a shift of the resonance angle of about 0.6 degrees. Using a refractive index of 1.47, which is reasonable for protein, we have calculated the effective thickness of the IgG layer to be 50 Å thick. This is reasonable for a protein of this weight (160 000 a.u.). The absorbed layer is very stable. An increase of the IgG concentration in the cell did not affect the result. Nor did the curve change when the cell was rinsed with distilled water. The IgG layer is also stable in air and can therefore also be analysed in it.

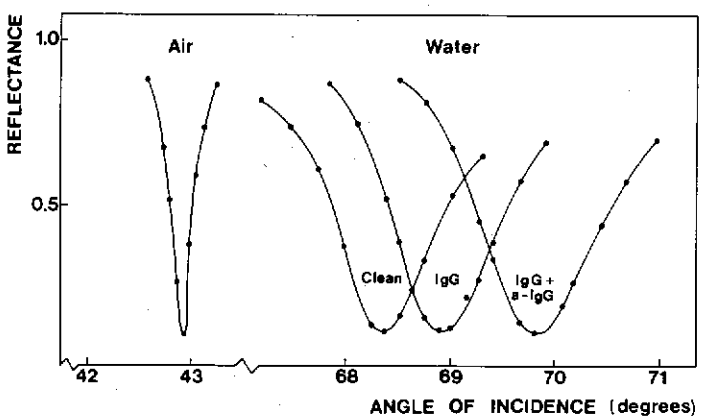
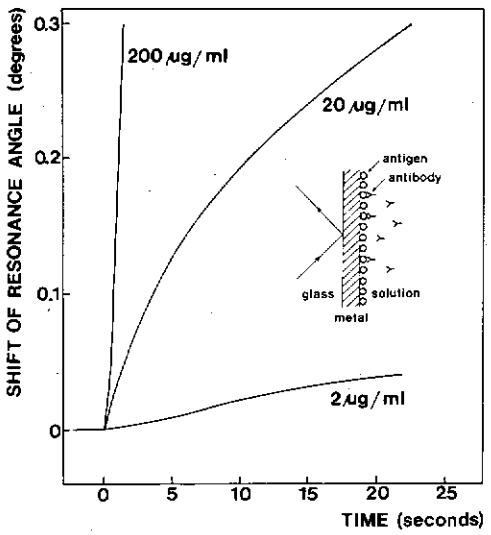


Fig. 3. Resonance curves for a 600 Å silver film in air and in water solutions. Upon adsorption of an IgG layer, the curve shifts to a larger angle of incidence. Additional adsorption of a-IgG gives rise to a further shift.

If a solution of antihuman γ-globulin (a-IgG) is injected into the cell, antibodies will bind specifically to IgG. This results in a monolayer of a-IgG on top of the IgG layer. This layer gives rise to a further shift (about 0.9°) of the resonance angle as seen in Fig. 3 (right curve). The antigen–antibody binding is strong, as revealed by the fact that the shift does not reverse when the cell is rinsed with pure water. The antigen–antibody reaction is specific in the sense that unrelated antibodies do not bind to the antigen layer. The antibodies used in the described experiments were a-human-IgG. In a few experiments we measured the uptake of a-rabbit-IgG by the human IgG layer. In these cases a small and very slow shift of the SPR angle was observed. This implies that a-rabbit-IgG can to some extent bind to human IgG.

Since a-IgG binding to IgG is not reversible on solid surfaces, there is no concentration-dependent equilibrium on the surface. The time needed to form a layer of antibodies is, however, dependent on the concentration of antibodies in the solution. If the angle of incidence is kept constant at the left side of the resonance during the adsorption, the intensity of the reflected light increases linearly with the shift of the resonance for at least 0.2 of a degree. This is enough to give the initial time derivative of the shift which is proportional to the concentration of antibodies in the injected sample solution. Figure 4 shows the result for three different concentrations of a-IgG in the solution. In these exploratory experiments the sample injection was made manually, resulting in some variations of the initial derivative. However, the maximum time derivative correlates with antibody concentration. Figure 5 shows the results from a series of measurements. The slope of



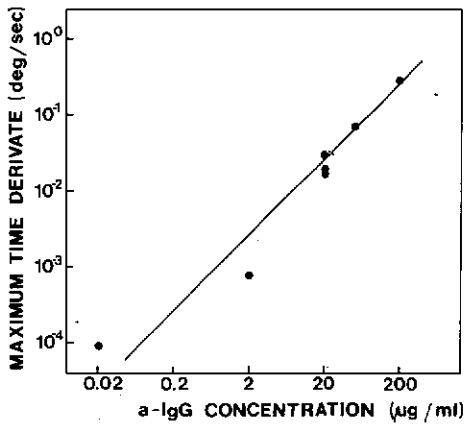


Fig. 4. Shift in resonance angle *versus* time for three different a-IgG concentrations. The shift is measured as an increase of the reflected light intensity. The insert illustrates the antibody adsorption.

Fig. 5. Maximum derivative $d\theta/dt$ *versus* a-IgG concentration from one measurement series.

the line in the logarithmic diagram reveals the expected proportionality with antibody concentration. These measurements can be automated using a differentiating amplifier and a peak holding display.

In one experiment antihuman serum albumin (a-HSA) was adsorbed on to a gold film coated with human serum albumin (HSA). In that case part of the shift was reversible. This probably means that a second layer of a-HSA is weakly bound to the first layer of a-HSA. We feel that this type of experiments can reveal new information of interest to immunology.

Discussion

The simplicity and sensitivity of the surface plasmon resonance technique are promising. In the case of gas detection, a very simple equipment already shows excellent performance. By taking advantage of the optical effects upon specific protein binding, SPR has potential applications in the field of biosensing. We have demonstrated selective antibody reactions with a sensitivity that appears to be higher than in any other known physical method. It should also be noted that due to its sensitivity, SPR can give the result within a few seconds. The technique can be extended to measurements of other biologically relevant parameters such as enzyme activity and hormone–receptor interactions. By using hydrogels and Langmuir–Blodgett films we hope to be able to construct a number of different types of SPR bioselective sensors.

In the described experiments the metal films were made of silver. Gold can also be used for this purpose and has been shown to be superior since it is more stable in buffer solutions.

One problem in the case of antibody binding and enzyme activity is that the effects are irreversible. The prepared metal films must therefore be replaced between each measurement. These can, however, be prepared reproducibly and in large quantities. It should also be possible to prepare surfaces which reversibly bind certain types of organic molecules of biological interest.

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