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Surface Plasmon Resonance: An Introduction to a Surface Spectroscopy Technique

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Abstract

Surface plasmon resonance (SPR) has become an important optical biosensing technology in the areas of biochemistry, biology, and medical sciences because of its real-time, label-free, and noninvasive nature. The high cost of commercial devices and consumables has prevented SPR from being introduced in the undergraduate laboratory. Here we present an affordable homemade SPR device with all of its components accessible to visualization. This design allows ease of integration with electrochemistry and makes the device suitable for education. We describe a laboratory experiment in which students examine the relationship between the SPR angle and the solution refractive index at the interface and perform a coupled SPR–electrochemistry experiment. Students also study the antibody–antigen binding activity. Most of the experimental work was done as a project by a grade 12 high-school student under proper supervision. We believe that the SPR device and the SPR laboratory will enhance undergraduate chemical education by introducing students to this important modern instrumentation and will help students to learn and understand the molecular interactions occurring at interfaces.

Keywords

Analytical Chemistry; Bioanalytical Chemistry; Biochemistry; Biotechnology; Electrochemistry; Hands-On Learning/Manipulatives; Kinetics; Laboratory Equipment/Apparatus; Laboratory Instruction; Mechanisms of Reactions; Molecular Biology; Quantitative Analysis; Second-Year Undergraduate; Spectroscopy; Surface Science; Upper-Division Undergraduate

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Since its first use in a real-time analysis of a biological system in 1990s (1), surface plasmon resonance (SPR) has become an important optical biosensing technology in the areas of biochemistry, biology, and medical sciences because of its real-time, label-free, and noninvasive nature (2). Commercial SPR devices are prohibitively expensive and require consumable sensor chips that fit certain specifications of size, thickness, and so forth. For example, Biacore (acquired by General Electric Healthcare in 2006) provides several models of SPR-based instruments (cost $120,000–$250,000) that are compatible only with

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Supporting Information Available
Quantitative study of how solar cell voltage changes with light intensity. This material is available via the Internet at http://pubs.acs.org.

expensive Biacore accessories and consumables (electrode chips cost $60–$120/each). The high operational cost prevents introduction of this technique into laboratory classes at the undergraduate level. Additionally, the commercial SPR instrument is not a good teaching tool because all the instrument components are enclosed, thus preventing students from visualizing the details of the SPR instrumentation.

We constructed a low-cost SPR instrument (less than $2000) specifically targeted for undergraduate education (3). The homemade SPR instrument is small (8 × 4 × 2 in.), and all components are accessible to visualization and manipulations. This design allows ease of integration with various electrochemical techniques, such as voltammetry, and also provides a great teaching tool for students to understand modern instrumentation. The ease of use of the homemade SPR instrumentation makes the techniques accessible to undergraduate students.

Here we describe a three-part laboratory for undergraduate laboratory courses that uses this affordable homemade SPR device. The purpose of this experiment is to illustrate the principles of SPR, its integration with electrochemistry, and its application for biosensors. The experimental results can be interpreted quickly and accurately using any spreadsheet software such as Microsoft Excel. The SPR laboratory was successfully introduced in the fall 2009 analytical chemistry laboratory. In addition to analytical chemistry, this SPR laboratory could be incorporated into various undergraduate laboratory courses, such as instrumental analysis, general chemistry, biochemistry, or physical chemistry.

Background

The Kretschmann configuration (4) is used in most SPR applications (Figure 1), where a metal (usually silver or gold) film is placed at the interface of two dielectric media. Medium 1 with higher refractive index (n_1) is a prism and the medium 2 with lower refractive index (n_2) can be the air or the solutions of interest. Theoretically, only the parallel-polarized light is practical in the SPR sensing techniques; however, we do not polarize the laser light in the homemade device to maintain the low cost.

Total Internal Reflection and Evanescent Waves

When the light travels from the higher refractive index medium 1 to the lower refractive index medium 2, the total internal reflection (TIR) can take place within medium 1 as long as the incident angle, θ, is greater than the critical angle, θ_c, where $\sin(\theta_c) = n_2/n_1$. Evanescent waves are formed in the lower refractive index medium 2 under the condition of TIR. The amplitude of this type of standing waves decays exponentially with the distance to the interface of the media 1 and 2. When a nonmagnetic gold film with suitable thickness is placed at the interface, the evanescent wave is enhanced, penetrating the gold film and existing in the medium 2. The magnitude of the parallel wave vector of the evanescent wave, $k_{evan,\|}$, is expressed as

$$k_{evan,\|} = \frac{2\pi}{\lambda} n_1 \sin(\theta) \quad (1)$$

where λ is the wavelength of the incident light, n_1 is the refractive index of the higher refractive index medium 1, and θ is the incident angle.

Surface Plasmon

Surface plasmons are quanta of plasma, a surface electromagnetic wave whose propagation is confined to the metal–dielectric interface. The magnitude of the wave vector of the

surface plasmon (k_{SP}) is related to the dielectric constants of both the medium 2 and the gold film. For nonabsorbing media, the dielectric constant equals the square of the refractive index, $\varepsilon = n_2$, where ε is the dielectric constant and n is the refractive index. Therefore, k_{SP} is determined by n_2 and n_g according to

$$k_{SP} = \frac{2\pi}{\lambda}\sqrt{\frac{n_2^2 n_g^2}{n_2^2 + n_g^2}} \tag{2}$$

where n_2 is the refractive index of medium 2 at the vicinity of the interface and n_g is the refractive index of the gold film (5).

Surface Plasmon Resonance

The surface plasmon can be excited by the evanescent wave and this phenomenon is called surface plasmon resonance (SPR). When this happens, the intensity of the reflected light decreases sharply. The decays of the excited surface plasmon include energy conversion to phonons or photons. One requirement for the SPR is that k_{SP} equals to $k_{evan,\parallel}$. Thus, using eqs 1 and 2 gives

$$\theta_{SPR} = \sin^{-1}\left(\frac{1}{n_1}\sqrt{\frac{n_2^2 n_g^2}{n_2^2 + n_g^2}}\right) \tag{3}$$

The angle required for the resonance, θ_{SPR}, is related to n_2 when n_1 and n_g are fixed. Adsorption and desorption on the gold surface (Figure 1) changes the refractive index of media 2 near the metal–dielectric interface and the resonance angle changes accordingly. Therefore, the monitoring of the θ_{SPR} change can be used to analyze the adsorption–desorption or association–dissociation activities that take place on the gold surface.

Methodology

SPR

The homemade SPR device is based on the Kretschmann configuration (4) and contains the following components: light source, prism, gold film, and detector (Figure 1). A testing cell is attached to the gold film and may be filled with different solutions to be studied. When a light beam propagates in the prism and encounters the interface of the gold film and the solution, TIR takes place and the evanescent wave forms as long as the incident angle is greater than the critical angle. Usually, the intensity of the reflected light does not change with the incident angle under the condition of TIR. However, at a specific angle larger than the critical angle, the evanescent wave excites the delocalized electrons or plasmons of the gold film, causing SPR; the intensity of the reflected light decreases sharply at this point. The incident angle at which the minimum reflectivity is observed is called the SPR angle.

When the prism is fixed, the SPR angle changes with the refractive index of solution at the interface, and the latter changes with the mass and density of foreign items attached to the surface of the gold film. Therefore SPR angle changes provide information about mass and density change on the gold surface. Additionally, the SPR is an interfacial phenomenon, an ideal technology to analyze the interaction in the vicinity of the interface, usually within 200 nm from a metal surface (6). A typical reflectivity versus incident angle curve (Figure 2) shows that the SPR angle in the air is 35.21° obtained with the homemade SPR instrument.

SPR technology is used widely as a transducer for affinity-based biosensors. An SPR-based affinity biosensor is composed of a recognition element or ligand immobilized on the metal surface of a SPR transducer. An example of a ligand–analyte pair is antibody–antigen. When the target molecules, species, or the analytes in solution phase bind to the ligand, the solution refractive index at the interface changes, and consequently the SPR takes place at a different angle. This change in SPR angle can be used to obtain information such as the binding amount (how many analytes bind to surface-immobilized ligands), the association and dissociation rate constants, and the apparent association constant by measuring the time relationship of refractive index changes at various analyte concentrations.

SPR Experimental Setup

The design of the homemade SPR device (Figure 3) is composed of a light source, detector, gold film, prism, and sample cell. The light source is a diode polarized laser assembly made by Melles Griot (part # 06DAL103) with output wavelength of 650 nm and output power of 4.0 mW. A silicon solar cell (purchased from a local RadioShack store, cat # 276–124) that generates a voltage of 0.55 V in full sunlight is used as the detector. The test on the logarithmic response of the solar cell to the light intensity is included in the supporting information. The solar cell is fixed on a turntable, where the gold film, prism, sample cell, and angular displacement transducer (Trans-Tek, Ellington, CT) are also placed. The gold films are prepared by sputter coating a 50 nm-thick gold film on a 22 × 22 mm microscope cover glass (Electron Microscopy Sciences, Hatfield, PA, cat # 72200-10). To ensure firm contact, a drop of immersion oil with the similar reflective index of the prism (Fluka, cat # 56822) is applied between the cover glass and the prism. Next to the gold film and prism is the homemade sample cell. The cell has an opening on the top for adding the sample solutions and a larger opening on the side where the sample solution contacted the gold surface. An O-ring is used for this larger opening to seal the sample cell system and prevent leaking.

To measure the SPR angle, the change in the intensity of the reflected light with the angle of incidence is monitored. The angle of incidence is changed by rotating the turntable connected to an angular displacement transducer (Figure 3). The intensity of the reflected light is measured by a silicon solar cell. Both the angular displacement transducer and silicon solar cell transduce the optical signal to the electric signals and to a computer through an analog–digital signal converter (Measurement Computing, Norton, MA, cat # USB-1608FS). A program created by LabVIEW (National Instruments, Austin, TX) is used for data acquisition and plotting both the reflectivity– time curve and the reflectivity–incident curve in a real-time fashion. In addition to LabVIEW, other software programs including the one bundled with the analog–digital signal converter are capable of collecting the data or the voltages generated by the angular displacement transducer and by the solar cell.

Chemicals

Anti-rabbit IgG Fab fragment was purchased from Jackson ImmunoResearch Laboratories, Inc. (cat # 111-007-003). PBS buffer (phosphate buffered saline) was purchased from GIBCO (cat # 20012, 1X, pH 7.2). Poly(vinyl ferrocene) or PVF was purchased from Polysciences, Inc. (cat # 09746). Tetrabutylammonium perchlorate or TBAP was purchased from GFS Chemicals (cat # 394). All other chemicals were purchased from Sigma-Aldrich, including ethanol (187380), acetone (179973), dichloromethane (D65100), protein A (P6031), and rabbit IgG (I5006).

Hazards

Direct eye exposure to the laser beam should be avoided. Dichloromethane is a carcinogen, is toxic by inhalation, and causes irritation and burning pain on prolonged contact. Acetone and ethanol are flammable. Acetone is irritating to the skin and eyes. Tetrabutylammonium perchlorate causes irritation and may be harmful if swallowed. It is a strong oxidizer. The toxicological properties of PVF are not known, but it is not expected to be hazardous. The normal precautions should be taken in handling.

Results and Discussion

Part 1

Students validate that the SPR angle, θ_{SPR}, has a linear relationship with the solution refractive index (RI) at the interface. The SPR angles in various media with refractive indices ranging from 1 to 1.5 were measured. Accurate values of RI were obtained from the Merck Index (7). A correlation between θ_{SPR} and RI was obtained (Figure 4). The slope of the fitted line has a value of 87.43, which means that every change of 0.1 in RI will cause a θ change of 8.743°. Thus, every 0.1° change in θ_{SPR} is equivalent to about a 0.0011 change in RI. Similar results have been reported by other laboratories (8).

Part 2

Students explore the fixed-angle SPR analysis, which is adopted by most commercially available SPR instruments because of its simplicity and ease of manipulation. The fixed-angle analysis is based on the linear relationship of SPR angle change and the change of reflected light intensity at certain conditions. Instead of monitoring SPR angle, which cannot be done in a real-time fashion, the reflected light intensity change is monitored while maintaining a constant incidence light angle. This real-time monitoring is important for kinetic studies. Additionally, students explore the hyphenated SPR–electrochemistry technique in which the SPR gold electrode is also the working electrode of an electrochemistry system.

A fixed-angle SPR analysis is used to monitor the process of conductive polymer deposition in which poly(vinyl ferrocene) (PVF) is oxidized in tetrabutylammonium perchlorate (TBAP) electrolyte according to the following equation:

$$\mathrm{PVF} + \mathrm{ClO_4^-} - \mathrm{e^-} \rightarrow \mathrm{PVF^+(ClO_4^-)(s)} \quad (4)$$

Both PVF and TBAP are soluble in dichloromethane. When a positive potential applied to the electrode, PVF at the electrode interface is oxidized to be poly(vinyl ferrocenium) (PVF^+), which is not soluble in dichloromethane. Therefore, PVF^+ is deposited on electrode surface. The perchlorate ions (ClO_4^-) are incorporated into PVF^+ matrix to balance the excess of positive charge.

The SPR angle with a gold film in dichloromethane solution containing 2.5 mM PVF and 0.1 M TBAP was measured. Then the turntable angle was fixed at that SPR angle, θ_{SPR}, which was 67.95°. A 6 cm-long gold wire was placed in the sample cell as the counter electrode. A conductive metal wire was connected to the gold film for connecting to a potential power supply (Figure 5).

The potential of the gold wire relative to a saturated calomel electrode (SCE) was measured to be −0.30 V. Usually the oxidation potential for PVF is higher than 0.7 V relative to SCE. Therefore, PVF can be oxidized when the potential applied is higher than 1.0 V relative to

the gold wire. A AA size battery (1.5 V) provides more than enough driving force for the PVF oxidation.

As the turntable was fixed at the SPR angle, θ_{SPR}, the reflected light intensity was minimized. When a 1.5 V AA battery was connected to the gold wire and the gold film (Figure 5), the positive potential at gold film allowed the PVF in solution to be oxidized to PVF^+ (the ferrocenium form of PVF), which was not soluble in dichloromethane and precipitated on the gold film surface. The mass change on gold film surface changed the SPR angle and θ_{SPR} was no longer the angle where the minimum reflection took place; therefore, the reflected light intensity[1] increased at the angle of θ_{SPR} (Figure 6).

Part 3

The binding between a protein and an antibody was studied, that is, protein A binding with rabbit IgG and rabbit IgG binding with anti-rabbit IgG Fab. After the gold film and the sample cell were mounted (Figure 3), 0.5 mL of PBS buffer and 0.2 mL of protein A solution (0.5 mg/mL in PBS) were added to the sample cell. After 30 min, the immobilization of protein A onto the gold film surface was completed. Then, 0.2 mL rabbit IgG solution (0.2 mg/mL in PBS) was added to the sample cell. The Fc portion of rabbit IgG can bind to protein A and results in a refractive index change at the gold surface and subsequently to an SPR angle change. The SPR angle was measured after 15 min to allow the binding to be completed. Finally, 0.2 mL Fab solution (0.2 mg/mL in PBS) was added to the sample cell. Addition of monoclonal anti-rabbit IgG Fab fragment into the system can further change the SPR angle because the Fab bound both the heavy chain and the light chain of rabbit IgG. The SPR angle was again measured after 15 min.

As shown in Figure 7A, the binding of rabbit IgG to protein A caused the SPR angle to increase by 2.1°. Further addition of anti-rabbit IgG Fab caused the SPR angle to increase by another 1.2°. The SPR angle change was linearly proportionate to the mass change on the surface within a range; therefore, the mass ratio of rabbit IgG to the anti-rabbit IgG Fab was roughly 2.1:1.2 or 1.75. If we assume the molar ratio of rabbit IgG to anti-rabbit IgG Fab fell between 1:1 and 1:2 (Figure 7B), we would predict the mass ratio of rabbit IgG (MW ~ 66 kD) to anti-rabbit IgG Fab (MW ~24 kD) was in the range of 1.37 and 2.75.

Conclusions

We described a surface spectroscopy technique for the undergraduate chemistry curriculum. The SPR instrument and laboratory developed here enhanced the undergraduate chemical education by introducing students to this important class of modern instrumentation and also through the improved understanding of molecular-interfacial processes. This homemade SPR device was easily and successfully operated in the analytical laboratory. The SPR experiment allowed students to become familiar with the concept of SPR, learn more about biological interactions between proteins and ligands, and view firsthand how SPR works and see the potential of the additional capabilities of hyphenated SPR–electrochemistry technique. By setting up a low-cost homemade SPR device, we endeavored to make this important technique more accessible to students at the undergraduate or high-school level for research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

[1]The test on the logarithmic response of the solar cell to the light intensity is included in the supporting material.

Acknowledgments

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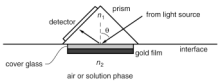

Figure 1.
Schematic diagram of an SPR setup.

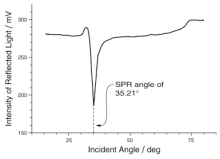

Figure 2.
Reflectivity as a function of incident angle for gold film in the air.

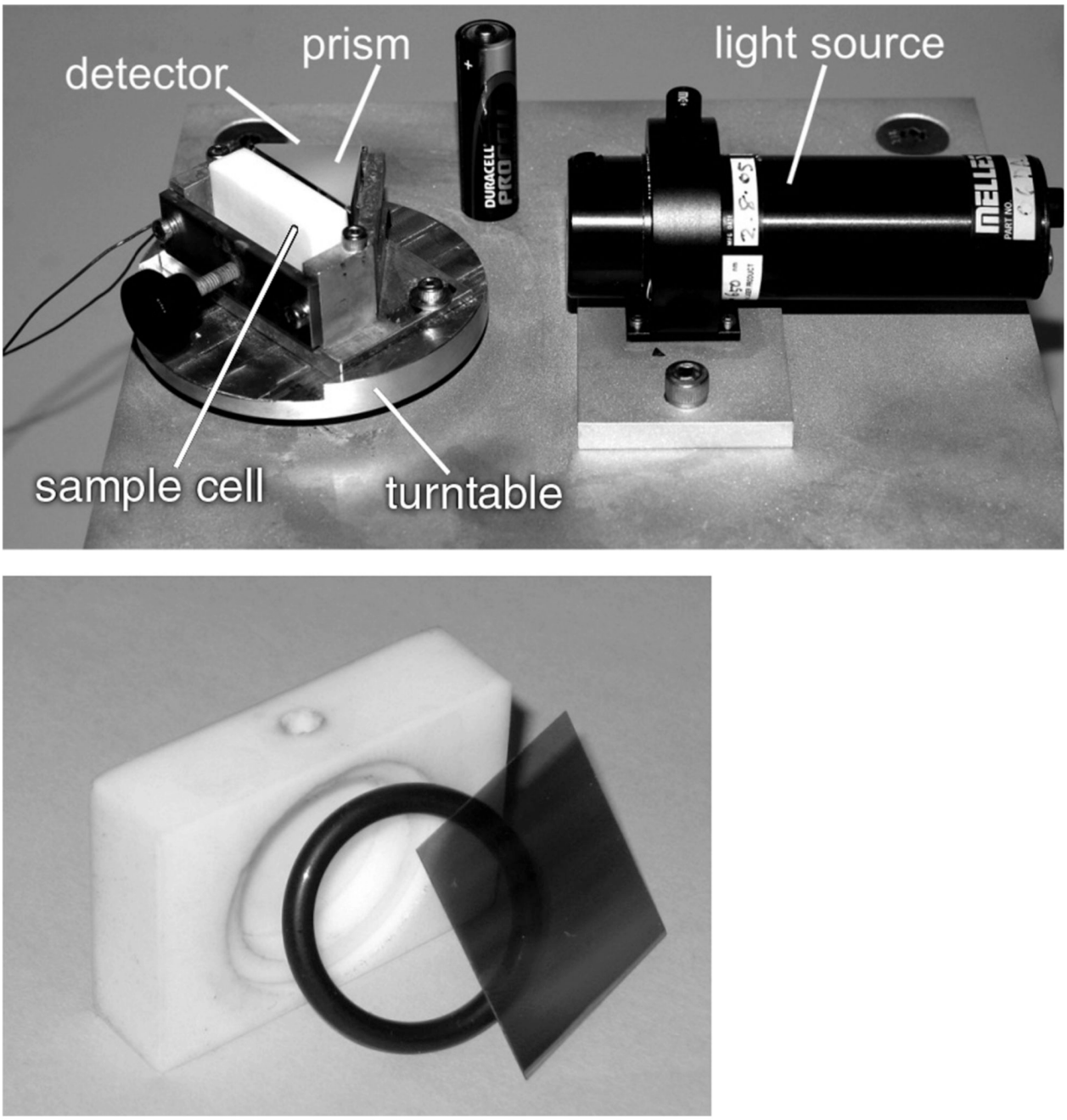

Figure 3.

(Top) The homemade SPR device. One AA-size battery is present to show the size of the setup. The turntable and the laser source are fixed on an 8 × 8 in. table, which is about 6 in. tall. The angular displacement transducer (not shown) is installed beneath the small table. (Bottom) An expanded view of the sample cell, a sealing O-ring, and the cover glass. The hole on the top of the sample cell is used for introducing samples and inserting electrodes. One side of the cover glass is coated with gold and the same side is placed facing the sample cell. The other side is contacting the prism with a drop of immersion oil.

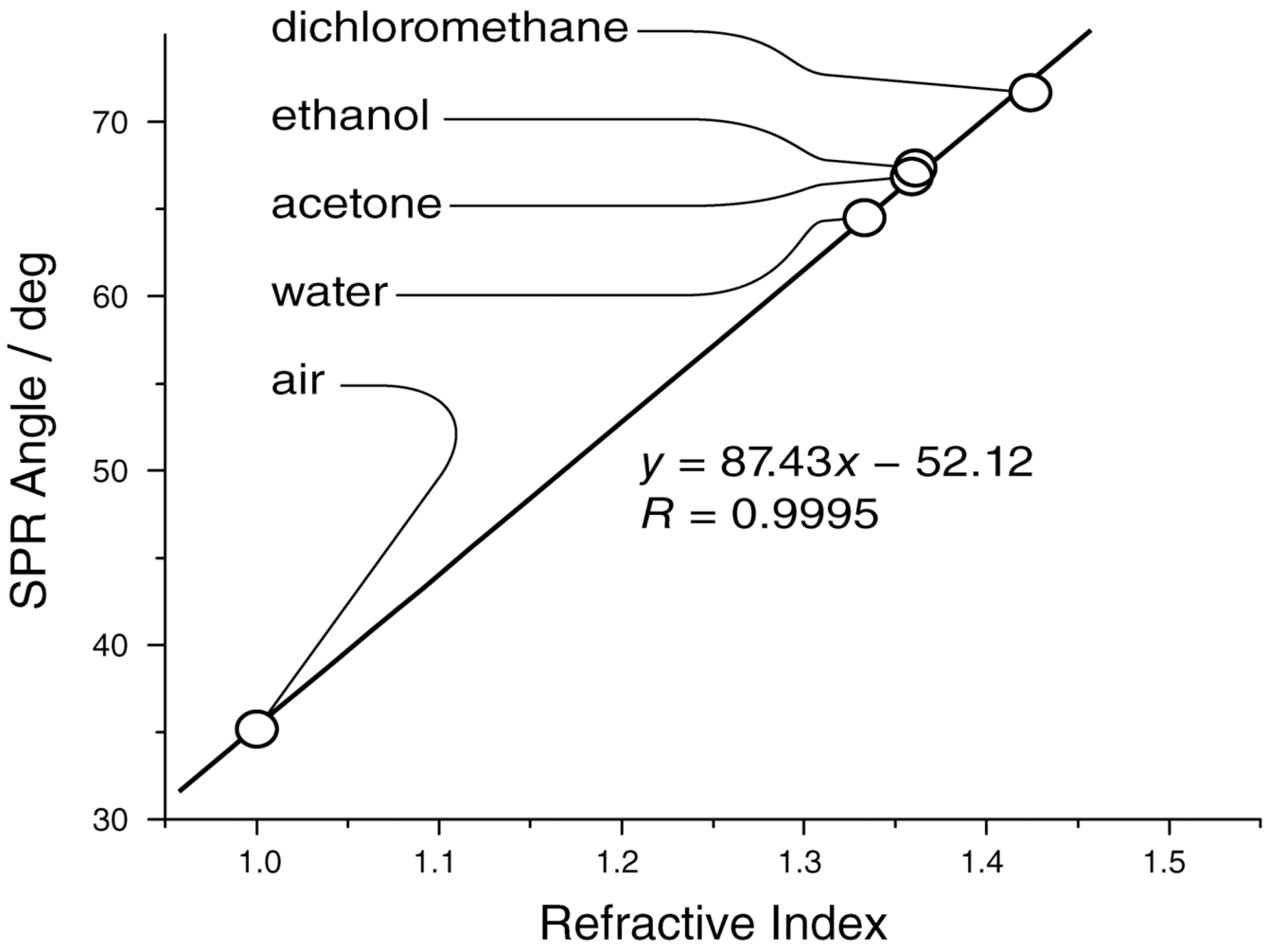

Figure 4.
Correlation between SPR angle and the refractive index.

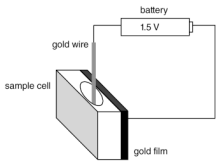

Figure 5.
Setup for the potential applied to gold film.

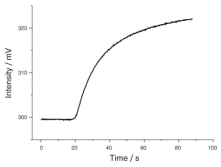

Figure 6.
Reflected light intensity changes with PVF deposition on gold surface. (Response of the silicon solar cell is logarithmic[1]; therefore, kinetics parameters cannot be obtained directly from this curve.)

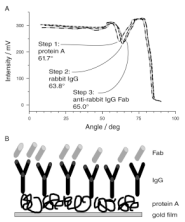

Figure 7.
(A) SPR increases with more molecules attached on the gold surface. (B) Schematic representation of layers formed on gold film surface.