

SURFACE PLASMON RESONANCE SPECTROSCOPY OF Au/HEXANETHIOL/9-(5'-FERROCENYLPENTANOYLOXY) NONYL DISULFIDE THIN FILMS

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The surface plasmon resonance method was used for determination of 9-(5'-ferrocenylpentanoyloxy) nonyl disulfide (FPONDS) and lipase thin film thicknesses and optical constants. Surface plasmons were excited in the Kretschmann configuration on the thin 50 nm gold film on the BK7 or SF10 glass prism. Sufficient sensitivity of the method for investigation of lipase interaction with a thin FPONDS layer ($d = 2.9$ nm) kinetics was demonstrated. From the experimental plasmon minimum shift to higher angles it could be concluded that lipase is adsorbed on the FPONDS and as a result it causes an increase in the effective layer thickness. The obtained results are important in understanding the interaction of these molecules, determination of their size and distribution on the surface.

Keywords: surface plasmons, biosensor, FPONDS, lipase

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1. Introduction

Surface plasmon resonance (SPR) is a widely recognized analytical technique for measuring the concentrations of various species in chemical and biochemical processes [1, 2], for the detection of ethanol in water [3] as well as the detection of CO and humidity in the air [4]. Light excited SPR has been used in the surface analysis of metals because the resonance conditions are very sensitive to physical properties of the metal surface [5–8]. SPR offers real time *in situ* analysis of dynamic surface events and is capable of defining rates of adsorption and desorption for a wide range of surface interactions. It is a sensitive technique to investigate the biological interactions via sensitive detection of binding of proteins to functionalized surfaces [1, 2]. It has been widely used in biosensing, gas sensing, immunosensing, and electrochemical studies [1, 2, 9–13]. The SPR method does not require fluorescent or other labelling of the analyte, which is a great advantage of the method.

A surface plasmon (SP) is a coupled, localized transverse magnetic (TM) electromagnetic field / charge-density oscillation which may propagate along an interface be-

tween two media possessing opposite sign of real part of dielectric permittivity (such as a metal and dielectric). The SP field reaches maximum at the interface and decays evanescently into both adjacent media. The propagation constant of the SP wave propagating at the interface between a semi-infinite dielectric and metal is given by the following expression [8]:

$$\beta = k \sqrt{\frac{\epsilon_m n_s^2}{\epsilon_m + n_s^2}}, \quad (1)$$

where k denotes the free wave number, ϵ_m is the complex dielectric constant of metal, $\epsilon_m = \epsilon'_m + i\epsilon''_m$, and n_s is the refractive index of dielectric. As may be concluded from Eq. (1), the SP may be supported by the structure providing that $\epsilon'_m < -n_s^2$. At optical wavelengths, this condition is fulfilled for several metals [6], whereas noble metals such as gold and silver are most commonly used.

Optical excitation of the plasmon can be achieved if a p -polarized collimated light beam undergoes the total internal reflection at a glass / metal / dielectric interface (Kretschmann configuration). The effect is observed as a sharp minimum in the reflectance curve when the angle of incidence is varied and condition of resonance (1) is

fulfilled. The resonance condition includes the following parameters: wavelength and angle of incidence, dielectric constants of all adjacent media, and thickness of the metal film. By covering the metal layer with a chemically active thin membrane, a very sensitive sensor, the sensitivity of which depends on the chemistry of the membrane, can be constructed.

It has been recently shown [14] that composite organic multilayers can be utilized as a biosensor platform for the lipase activity assay. These surface constructs include the chemically anchored hexanethiol (HT) self-assembled monolayer, and an overlayer of the physisorbed 9-(5'-ferrocenylpentanoyloxy) nonyl disulfide (FPONDS), a compound that contains two ester bonds, which are the potential targets for the ester bond cleaving enzymes. It was demonstrated that the wild-type lipase from *Thermomyces lanuginosus* (TLL) is capable of cleaving ester bonds in FPONDS molecules, thus triggering the release of the redox species from the surface. These events can be monitored electrochemically [14]. However, the molecular level details of this process such as thickness of the physisorbed FPONDS layer and / or amount of lipase present on the surface remain unclear.

The objective of this work is to investigate the multilayer system composed of HT, FPONDS, and TLL with the SPR spectroscopy. Specifically, our aim was to determine thicknesses and optical constants of composite organic layers on the polycrystalline gold surface, which are used in TLL lipase enzymatic activity assay.

2. Experimental set-up

2.1. Samples

The procedure of sample fabrication was the following. We used two types of prisms as a substrate for Au film evaporation. The first one was a 45° BK7 glass prism that was used for experiments at the air / solid interface. First, we precisely determined the refraction index of the prism n_p from the total internal reflectance angle measurement at the glass / air interface. It was found to be 1.508 for BK7 and 1.722 for SF10 glass prism at $\lambda = 632.8$ nm. Then, we thermally evaporated ~50 nm Au film, the thickness of which was measured using profilometric technique.

Another type of the solid support for gold films was a high refraction index 60° SF10 glass prism. After measurement of the prism refraction index it was thermally coated with ~50 nm Au film as mentioned above. This

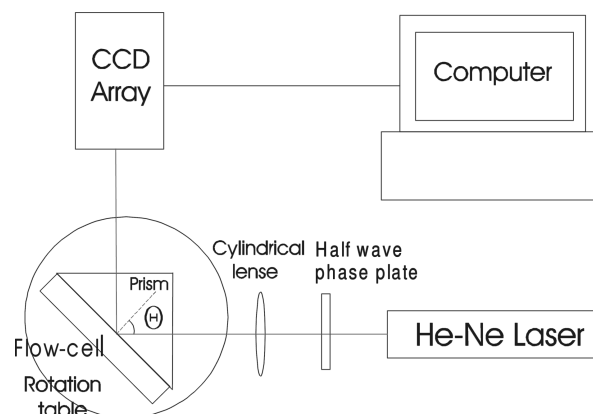


Fig. 1. Experimental set-up.

prism was used for experiments at the water / solid interface.

The hexanethiol (HT) self-assembled monolayer was chemically formed on the evaporated gold film by incubating the sample prism hypotenuse in 0.1 mM solution of hexanethiol in 96.3% (volume) ethanol [14] for at least 12 hours. Then, the FPONDS overlayer was formed by dripping and drying a small droplet of FPONDS solution in ethanol. The concentration of FPONDS and the precise volume of the droplet were chosen in such a way that the resulting FPONDS overlayer thickness would be equal to ~1 monomolecular layer.

2.2. Optical sketch

Optical sketch of SP experiments is shown in Fig. 1. Radiation of the 3mW He–Ne laser ($\lambda = 632.8$ nm) was passed through the half wave phase plate to obtain *p*- or *s*-polarized wave, pinhole, $f = 2.5$ cm cylindrical lens, and was totally reflected at the interface between the glass prism and a gold layer. The lens formed an angular spread of the beam of about 4 degrees, which covered the SPR angle in air. The measured reflected intensity in the vicinity of the SPR angle showed deep minimum which was detected with a 12 bit CCD camera SONY ILX526A. It allowed a simultaneous measurement of a complete SPR angular range and made it possible to escape uncertainties caused by the laser power fluctuations.

3. Results and discussion

The experimental results are summarized in Figs. 2–4. First, we obtained optical constants and thickness of the gold film, which depend on the solid substrate and evaporation process technology. After the best fit procedure of curve 1 (Fig. 2) using noncommercial “Thin

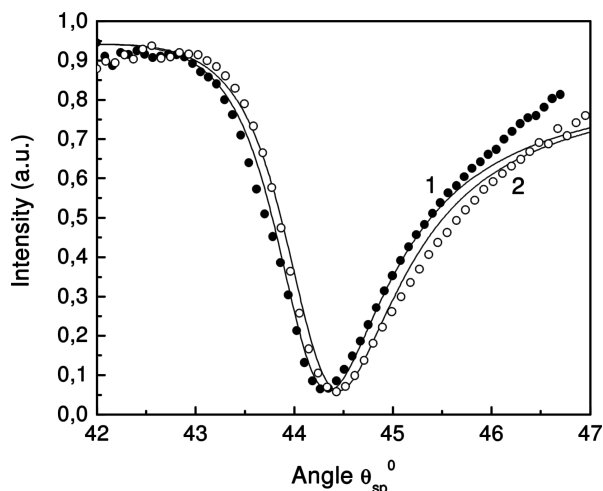


Fig. 2. Experimental (points) and a best fit (lines) curves of surface plasmons in a Kretschmann configuration at the interfaces: 1 – BK7 glass / Au / air; physical constants for Au: $\epsilon' = -0.138$, $\epsilon'' = 3.06$; 2 – BK7 glass / Au / hexanethiol / air (hexanethiol thickness $d = 0.8$ nm), $n = 1.4$.

Films” software a complex refractive index and thickness of Au film deposited on the BK7 glass prism were obtained: $\epsilon' = -0.138$, $\epsilon'' = 3.06$, and $d = 52.7$ nm. The estimated thickness was the same as that measured by the profilometric technique. Similar calculations for the gold film evaporated on the SF10 prism gave $\epsilon' = -0.097$, $\epsilon'' = 3.5$, and $d = 45.2$ nm. The formation of the HT monolayer on the Au film was followed by shift of the SPR curve towards higher angles with some increase in the depth of the surface plasmon curve (curve 2 in Fig. 2). From the best-fit using earlier determined optical constants of the gold film we obtained these physical constants for the hexanethiol monolayer: $n = 1.4$, $d_{HT} = 0.8$ nm. This value is close to that expected from the fully extended methylene (trans configuration) chain in an n -alkane molecule ($\sim 0.85, \dots, 0.95$ nm).

The FPONDS layer physisorbed on the HT self-assembled monolayer significantly altered SP spectra (Fig. 4). The shift of the SP curve towards higher reflection angles is obvious. After determination of optical constants and thicknesses of multilayers at the air interface, the SPR cell was filled with aqueous buffer solution containing 0.01 M NaH_2PO_4 and 0.1 M NaClO_4 buffered at pH 7.0 with NaOH. The refractive index $n = 1.333$ was assigned to aqueous media. Due to SP field redistribution at the interface when air was replaced with water the SP curves shifted to considerably higher angles (Fig. 4). Fitting this curve to the model yielded the refractive index and thickness of FPONDS ($n = 1.45$, $d = 2.9$ nm).

Finally, the lipase injection (~ 200 lipase activity units per 1 ml, for details see Ref. [14]) into the

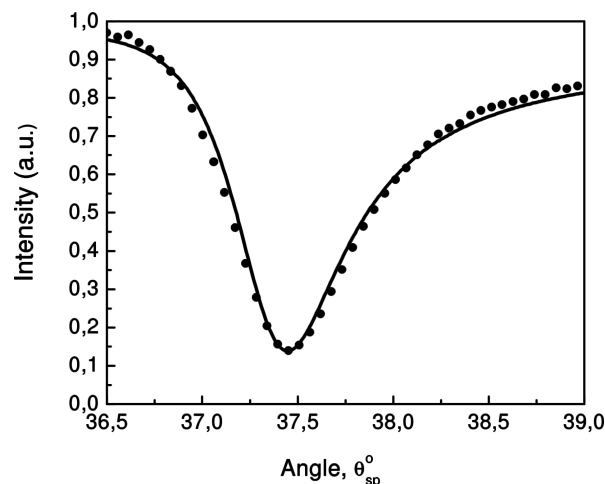


Fig. 3. Experimental (points) and best fit (lines) curves of surface plasmons in Kretschmann configuration at SF10 glass / Au / air (gold thickness = 45.2 nm) interface. Physical constants of gold: $\epsilon' = -0.097$, $\epsilon'' = 3.5$.

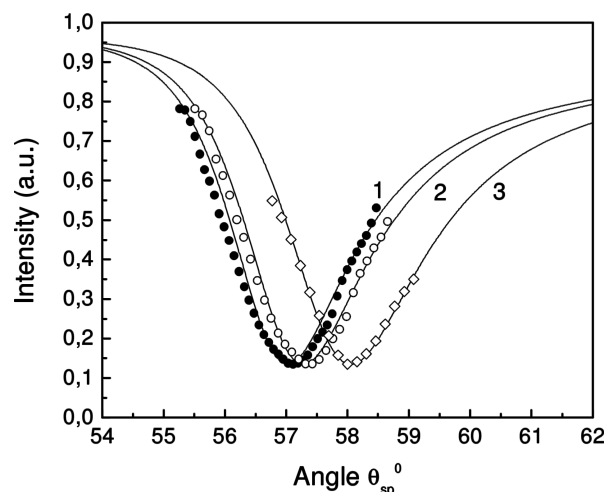


Fig. 4. Experimental (points) and a best fit (lines) curves of surface plasmons at aqueous media (0.01 M NaH_2PO_4 + 0.1 M NaClO_4 , pH 7.0). 1 – SF10 glass / Au / HT / water solution (gold layer thickness is $d = 45.2$ nm, HT self-assembled monolayer $d = 0.8$ nm), 2 – with physisorbed FPONDS film ($d = 2.9$ nm), and 3 – after the lipase injection into the solution ($d = 8.8$ nm) formation.

buffer rendered further transformations of the SPR curve (Fig. 4, curve 3). These transformations occurring several minutes after the lipase injection indicate the interaction between the enzyme and FPONDS surface. The details of this interaction are quite complicated and should be further investigated. However, the shifted SP curve allowed us to estimate the organic material density increase, which was caused by formation of the lipase layer. The refractive index and thickness $n_{\text{lipase}} = 1.45$ and $d_{\text{lipase}} = 8.8$ nm are comparable to the molecular size of the lipase. Thus, the experiment indicates a considerable increase of protein amount at the

interface. It is quite possible that within several minutes after the lipase injection almost all FPONDS surface is covered with a monolayer of enzyme. It has been shown earlier [14] that the lipase injection is causing partial removal of FPONDS. Consequently, a quite complicated non-uniform layer could be formed. In this case, the calculated refraction index ($n = 1.45$) of lipase should be considered as an effective refraction index. For determination of both n and d_{lipase} a more detailed analysis involving other techniques is required.

In conclusion, lipase from *Thermomyces lanuginosus* interaction with the FPONDS layer was directly observed using SPR spectroscopy. The refractive indices and thicknesses of the composite organic layers were estimated. These parameters are important in obtaining the molecular level information about the enzymatic processes that take place on solid surfaces.

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PLONŲ AUKSO/HEKSANTIOLIO/FPONDS SLUOKSNIŲ TYRIMAS PAVIRŠINIŲ PLAZMONŲ REZONANSO SPEKTROSKOPIJOS METODU

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Santrauka

Paviršinių plazmonų rezonanso metodas buvo naudojamas plonų FPONDS (9-(5'-ferocenilpentanoiloksi) nonil disulfidas) ir lipazės sluoksnių storiams ir optinėms konstantoms nustatyti. PPR buvo žadinami, BK7 ir SF10 prizmes padengiant apie 50 nm aukso sluoksniu (Kretschmann konfigūracija). Eksperimentiškai pade-

monstruotas pakankamas metodo jautris, tiriant lipazės sąveikos su FPONDS sluoksniu ($d = 2,9$ nm) kinetiką. Iš PPR kreivių poslinkio į didesnių kampų pusę galima daryti išvadą, kad lipazė sorbuojasi ant FPONDS ir dėl to padidėja efektinis sluoksnio storis. Šie tyrimai svarbūs, siekiant išsiaiškinti šių molekulių sąveiką, dydžius ir jų išsidėstymą paviršiuje.