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SURFACE PLASMON RESONANCE SPECTROSCOPY: POTENTIALITIES AND LIMITATIONS

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Summary

SURFACE PLASMON RESONANCE SPECTROSCOPY: POTENTIALITIES AND LIMITATIONS

V. I. Chegel, Yu. M. Shirshov

The review deals with (i) investigation of the physical processes of light interaction with multilayer film structures at the “TIR prism surface-medium under investigation” interface and (ii) development of a novel high-sensitivity optoelectronic sensor spectrometer for registration of biological reactions and real-time quantitative determination of medium dielectric characteristics and geometry. The spectrometer is developed on the basis of surface plasmon-polariton resonance in thin gold and silver films.

Keywords: surface plasmon resonance, optoelectronic sensor, biosensor, physical sensor, biomolecule, polarization.

Анотація

СПЕКТРОСКОПІЯ З ВИКОРИСТАННЯМ ПОВЕРХНЕВОГО ПЛАЗМОННОГО РЕЗОНАНСУ: МОЖЛИВОСТІ ТА ОБМЕЖЕННЯ

В. І. Чегель, Ю. М. Ширшов

Представлена оглядова робота присвячена дослідженню фізичних процесів взаємодії світла з багатошаровою плівковою структурою межі поділу: поверхня призми повного внутрішнього відбивання — досліджуване середовище та розробці нового високочутливого оптоелектронного сенсорного спектрометра для реєстрації біологічних реакцій та кількісного визначення діелектричних характеристик та геометрії середовища в реальному режимі часу. В основу розробки було покладене явище поверхневого плазмон-поляритонного резонансу в тонких плівках золота та срібла.

Ключові слова: поверхневий плазмонний резонанс, оптоелектронний сенсор, біосенсор, фізичний сенсор, біомолекула, поляризація.

Аннотация

СПЕКТРОСКОПИЯ С ИСПОЛЬЗОВАНИЕМ ПОВЕРХНОСТНОГО ПЛАЗМОННОГО РЕЗОНАНСА: ВОЗМОЖНОСТИ И ОГРАНИЧЕНИЯ

В. И. Чегель, Ю. М. Ширшов

Представленная обзорная работа посвящена исследованию физических процессов взаимодействия света с многослойной пленочной структурой на границе раздела: поверхность призмы полного внутреннего отражения — исследуемая среда и разработке нового высокочувствительного оптоэлектронного сенсорного спектрометра для регистрации биологических реакций и количественного определения диэлектрических характеристик и геометрии среды в реальном режиме времени. В основу разработки положено явление поверхностного плазмон-поляритонного резонанса в тонких пленках золота и серебра.

Ключевые слова: поверхностный плазмонный резонанс, оптоэлектронный сенсор, биосенсор, физический сенсор, биомолекула, поляризация.

1. Introduction

At present a need for novel “high technologies” becomes of crucial importance. The reason for this is that further progress of mankind has to occur against the background of aggravating contradictions between human beings and environment accompanied by exhaustion of natural resources and appearance of new hazardous deceases. In this situation science is facing an urgent problem, namely, development of new generations of sensors capable of fast and accurate monitoring and estimation of biomolecular interactions of various kinds and physico-chemical interactions between molecules on the whole. However, scientific advances in a single field cannot give a solution to the above problem. A sufficient basis for development of highly sensitive sensor systems can be provided only by combining the achievements of physics of solids and optoelectronics with advances in other disciplines, such as physical chemistry, biochemistry and biophysics which provide recognition of molecules.

The optoelectronic chemical sensors and biosensors are the actual realization of synthesis of advanced technologies. At that surface plasmon-polariton resonance (SPR) in thin metal films, being the main nondestructive instrument for investigation of kinetics of interactions between molecules, is finding increasing application when developing sensors. Some drawback of this technique is relative complexity of interpretation of experimental results obtained with it. The corresponding devices existing at the moment are expensive and particularly special-purpose. Nevertheless the leading research laboratories of the world apply this technique, and the number of publications on it is growing progressively.

At present several firms from different countries produce commercial versions of such biosensors, and the SPR technique is becoming the leading instrument when investigating kinetics of biomolecular interactions. Such firms as Pharmacia Biosensor (Sweden), Affinity Sensors (UK), Xantech (Germany) and DKK Company (Japan) produce commercial laboratory equipment for biological studies with SPR technique known, correspondingly, as “Biacore”, “IASYS”, “IBIS” and “SPR-2”. The main drawbacks of this equipment are that it is expensive, of considerable size and requires highly skilled operating crew. The firm Texas Instruments (USA) produces an integrated version of the SPR-based sensor element “Spreeta”. It is intended mostly for application in environmental monitoring and is not a completed SPR-device. The commercial versions of the SPR-based device “PLASMON-4m” (that has been developed and is produced at the V. Lashkaryov I Institute of Semiconductor Physics of the National Academy of Sciences of Ukraine, Kiev, Ukraine) is presented in the European market (since 1999) by the firm *Analytical Micro-systems* (Germany, www.micro-systems.de) and in the American market (since 2004) by the firm *Biosuplar-USA* (www.biosuplarusa.com).

Optical excitation of surface plasmons using attenuated total reflectance (ATR) technique was demonstrated by Otto [1] and Kretchmann [2]. After the pioneer work [3] by Abeles (1976), SPR was used for characterization of the optical properties of thin films [4]. The real SPR potentialities for investigation of films and processes at metal (semiconductor)–ambience interfaces were determined during the last decade. The principles of SPR application for gas detection and biosensing have been demonstrated for the first time in 1982-1983 by Nylander, Liedberg, Lind and Lundstrom. Since publication of their works [5, 6] the SPR technique is attracting more and more attention. In mid-nineties most of the works dealing with SPR application were made in the field of biosensing and aimed at quantitative estimation of biomolecular interactions. As we know, in Ukraine the works on biosensing with application of the SPR technique have started in 1993-95 [see 7, 8].

In recent years the use of SPR in different lines of studies has expanded considerably. The reason for this was appearance of new fundamental theoretical and experimental investigations [9-11], as well as novel types of laboratory SPR sensors combining several methods (e.g., SPR and electrochemical measurements, SPR and absorption spectroscopy). A considerable contribution has been made into such lines of SPR technique application as investigation of monolayers and self-assembling structures, polymers and composites, nanoparticles, redox-materials, biomolecular structures, etc. [12-20]. The area of SPR technique usage is rather large. First of all, it involves monitoring of kinetics of interaction between biomolecules or molecular complexes, as well as the whole field of studies of thin (up to 200 nm thick) films of various origins.

2. Experimental results and discussion

2.1. Physics of the phenomenon and theoretical foundation of the technique

Theoretical foundation of surface plasmon waves follows from the Maxwell theory and concept of free electrons in metal as a high-density electron liquid. Then the surface plasmon waves are density oscillations at the surface of such liquid (plasma). The physics of surface plasmon waves propagating at the metal (semiconductor)–dielectric interface have been studied intensely. It is believed that the basic properties of such waves are in good agreement with the theoretical concepts [21].

The SPR phenomenon may be described exactly in the physical terms as propagation of free electron oscillations at a metal surface. From the viewpoint of calculations and obtaining parameter values, however, it is more convenient to apply directly the conclusions of the electromagnetic theory of thin films. The dispersion equations for a surface plasmon propagating along an interface between a metal (whose complex dielectric function is $\varepsilon(\omega)$) and ambience (with permittivity ε_a) may be presented as [9]

$$k_{sp} = \frac{\omega}{c}\sqrt{\frac{\varepsilon(\omega)\varepsilon_a}{\varepsilon_a + \varepsilon(\omega)}}, \qquad (1)$$

where k_{sp} is the surface plasmon wave vector and c (ω) is the speed (frequency) of light.

Surface plasmon cannot be excited using normal incidence of light from an ambience of smaller density, because the photon wave vector value $k_a = \frac{\omega}{c}\sqrt{\varepsilon_a}$ is not sufficient in this case. A straightforward way to coordinate the values of the above vectors (to provide fulfillment of the law of conservation of the wave vector component k_x parallel to the interface plane) is application of the attenuated total reflection (ATR) technique, for instance, after Kretchmann [2] (Fig.1). In this case a metal film is deposited either immediately onto a prism with high refractive index $\sqrt{\varepsilon_g} > \sqrt{\varepsilon_a}$ or onto a glass plate being in optical contact with the prism via a matching liquid. In the ATR technique light is reflected at angles over the critical one (θ_c). At that total internal reflection of light (with reflection coefficient $|R| \approx 1$) is observed. However, some part of light (an evanescent wave) still penetrates to the outer glass surface and excites a plasmon in metal if the film is sufficiently thin, so that the energy flow reaches the metal-ambience interface.

Accordingly, SPR occurs when $k_x = k_{sp}$. This is achieved by variation of the angle of incidence θ in the range $\theta_c < \theta < 90°$ and manifests itself as a minimum on the $R(\theta)$ curve for intensity of the reflected p-polarized light (from now on it will be refer to as the SPR spectral curve, or simply SPR curve). An abrupt drop of the reflection curve, that is observed at angle scanning, fixes light absorption and appearance of resonance in the near-surface layer of electron plasma. The resonance angle θ_{sp}, that corresponds to the reflection intensity minimum, is determined from the following equation:

$$\sqrt{\varepsilon_g}\,\sin\theta_{sp} \approx \sqrt{\frac{\varepsilon(\omega)\varepsilon_a}{\varepsilon_a+\varepsilon(\omega)}}, \tag{2}$$

where ε_g is permittivity of the prism material.

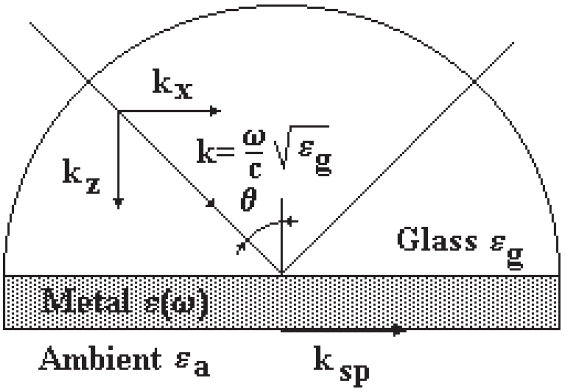

Fig.1. SPR excitation after Kretchmann.

Figure 2 presents the calculated SPR curves for silver (curve 1) and gold (curve 2). Presence of dielectric coating (say, adsorbed biomolecules) on metal surface leads to an increase of the surface plasmon wave vector:

$$k^1_{sp} = k^0_{sp} + \Delta k_{sp}\,. \tag{3}$$

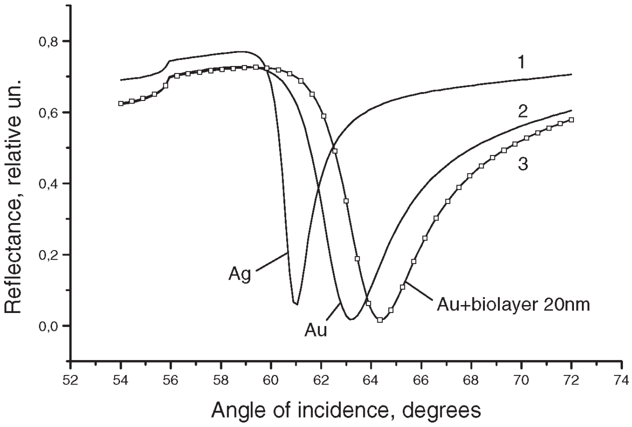

Fig.2. Calculated reflection intensities vs angle of incidence of laser beam curves $R(\theta)$ (SPR curves) for silver and gold layers and for gold with a layer of biomolecules.

According to Eq. (2), this will shift the position of SPR curve minimum (Fig.2, curve 3). By calculating the angular shift $d\theta$ with application of the Fresnel formulae, one can estimate the optical thickness of the dielectric layer. Using the Maxwell equations, it is possible to describe propagation of plane monochromatic linearly polarized electromagnetic field in a multilayer thin-film system.

The experimentally taken SPR curve depends on the optical constants (refractive indices and absorption coefficients) of all the phases with which electromagnetic wave interacts. These are the prism material; metal (say, gold) layer; substance adsorbed on the layer surface; ambience and other phases that may be present in the system studied (depending on the measurement conditions). The SPR curve depends also on the geometric thicknesses of all the layers (in particular, the gold film and absorbed layer). These dependencies may be presented in the following form (using the Fresnel integral reflection coefficient for p-polarized light [22]):

$$R\ (\theta)=\frac{\left(r_{01}+r_{12}e^{-i2\beta_1}\right)+\left(r_{01}r_{12}+e^{-i2\beta_1}\right)r_{23}e^{-i2\beta_2}}{\left(1+r_{01}r_{12}e^{-i2\beta_1}\right)+\left(r_{12}+r_{01}e^{-i2\beta_1}\right)r_{23}e^{-i2\beta_2}}\,. \tag{4}$$

Here r_{01}, r_{12} and r_{23} are the Fresnel reflection coefficients at the corresponding interfaces, and β_1 and β_2 are the phase thicknesses of the layers:

$$\beta = 2\ \ N_j\ \ \pi\frac{d_j}{\lambda}\cos\ \ \varphi_j\,,$$

where $N_j = n_j - ik_j$ is the complex refractive index of the layer considered; φ_j is the angle of incidence inside the j-th layer; l is the light wavelength; d_j is the j-th layer thickness.

Equation (4) enables one to calculate a three-layer structure. In the case of $j > 3$ layers it is convenient to apply the formalism of the matrix of effective optical constants [22]:

$$S = I_{10}L_1I_{12}L_2\ldots I_{(j-1)j}L_j\ldots L_mL_{m(m+1)}, \tag{5}$$

where I_{ab} is the matrix of the interface between the a-th and b-th layers, and L_j is the layer matrix:

$$I_{ab}=\begin{bmatrix} 1 & r_{ab} \\ r_{ab} & 1 \end{bmatrix},\quad L=\begin{bmatrix} e^{i\beta_j} & 0 \\ 0 & e^{-i\beta_j} \end{bmatrix}.$$

Here r_{ab} are the Fresnel reflection coefficients for the corresponding interfaces; β_j is the phase thickness of the j-th layer. The reflection coefficient for a layered structure is determined by the elements of the first column of the matrix of effective optical constants $R = S_{10}/S_{00}$.

Equation (4) describes the SPR phenomenon as function of the angle of incidence θ in the case when monochromatic light is used (i.e., the wavelength λ is constant). However, one can obtain easily the expression for reflectivity as function of λ, assuming the angle of incidence θ to be constant. In practice, both types of spectra (dependence on wavelength or angle of incidence) are used when developing SPR sensors. The calculated shift of SPR angular position vs refractive index and adsorbed layer thickness curves (Fig.3) enable one to determine quickly (though with low accuracy) thickness of the adsorbed molecular layer at the known value of refractive index.

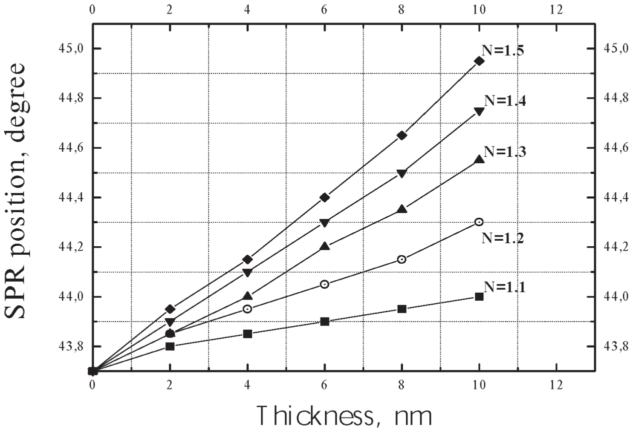

Fig.3. Calculated SPR angle positions θ_{sp} as function of refractive index and thickness of the adsorbed molecular layer. Optical parameters of the SPR sensor: $n_{Cr} = 2.1 + 2.37i$, $d_{Cr} = 5$ nm, $n_{Au} = 0.15 + 3.6i$, $d_{Au} = 45$ nm; $\lambda = 670$ nm. Ambience: air ($n = 1.0$).

Traditionally, the adsorption coatings and monomolecular layers on liquid and solid surfaces are characterized by the effective thickness *d* and effective refractive index *n*. When dealing with close-packed monomolecular layers on a surface where one can determine clearly presence of two boundaries, upper and lower, such approximation may be valid at oriented adsorption of, say, immunoglobulins Ig [23]. But for irregular adsorption of protein molecules that form a rough surface, the notion of effective thickness of a submonomolecular layer ceases to have its meaning (Fig.4). In this case one should use other approaches to description of light reflection from a plane with a system of separate molecules on the surface. In [24-25], for instance, the expansion of field in terms of the derivatives of delta function or the Green's function formalism are used instead of the traditional boundary conditions on both sides of a molecular layer. According to [24], a molecular layer is described by the following parameters: surface concentration N_s and molecular polarizability tensor $A_м$. In this case the expression for the coefficient of reflection of *p*-polarized wave is [25]

$$R_p = \overline{R}_{0p} + a\frac{(\beta\alpha_{\|}g_{xx} - \alpha_{\perp}f_{zz})(1+\overline{R}_{0p}^2) - 2(\beta\alpha_{\|}g_{xx} + \alpha_{\perp}f_{zz})\overline{R}_{0p}}{(1+\alpha_{\|}\alpha g_{xx})(\beta+\alpha_{\perp}\alpha f_{zz}) - a(\beta\alpha_{\|}g_{xx} - \alpha_{\perp}f_{zz})\overline{R}_{0p}}. \tag{6}$$

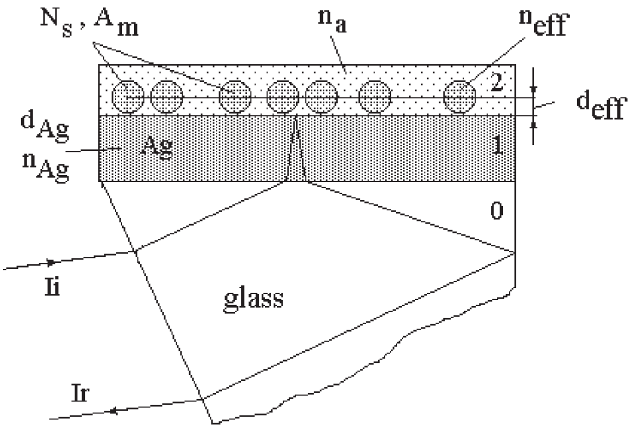

Fig.4. Ray paths in the system studied. 0 — glass prism, 1 — silver film ($d = 50$ nm), 2 — ambience from which molecular sorption occurs. The measured quantity is reflection $R = I_i/I_r$.

Here R_p is the Fresnel integral reflection coefficient for *p*-polarization; R_{0p} is the Fresnel reflection coefficient for *p*-polarization without a molecular layer; $a = 2\pi N_s$; $a_{\|}$ ($a_{\perp}$) is the component of polarizability tensor parallel (normal) to the surface; $\beta = 1 + 4\pi A_m/n_3^2$; n_3 is the complex refractive index of the ambience; g_{xx} and f_{xx} are some combinations of the wave vector.

The integral reflection coefficient *R* of the system considered may be presented also according to Eq. (5) in which all matrices are determined by the optical constants and material thickness except the matrix that describes the adsorbed molecular layer (this matrix is determined by Eq. (6)). When dealing with the layers formed by protein molecules of globulin type which are transparent for visible light and whose shape is close to spherical, one can replace the molecular polarizability tensor in Eq. (6) by the scalar quantity $A_м$. It should be noted also that for a volume phase polarizability may be calculated independently, according to the Lorentz-Lorenz equation:

$$A_m = \frac{3M}{4\pi N_a\rho}\frac{\varepsilon-1}{\varepsilon+2} = \frac{3M}{4\pi\ N_a\rho}\frac{n^2-1}{n^2+2}, \tag{7}$$

where *n* is the refractive index of the adsorbate in the condensed phase; *M* (ρ) is the adsorbate molecular weight (density); N_a is the Avogadro number.

Equation (6) involves a number of parameters. Each of them affects the form and angular position of the $R(\theta)$ curve. Simulation (Fig.5) demonstrates interrelation between θ_{sp} and N_s for different values of $A_м$. These dependencies turn to be linear up to monolayer coating. Obviously one cannot determine both N_s and $A_м$ from the only measured parameter $\Delta\theta_{sp}$. However, inaccuracy in N_s determination appears to be small (about 10-15%), since the refractive indices (and consequently the polarizabilities) of protein molecules formed by a small number of amino acids lie usually between 1.45 and 1.48 [26]. Therefore the nomograms similar to Fig.5 give, in principle, solution to the problem of (not very accurate) determination of N_s from SPR measurements.

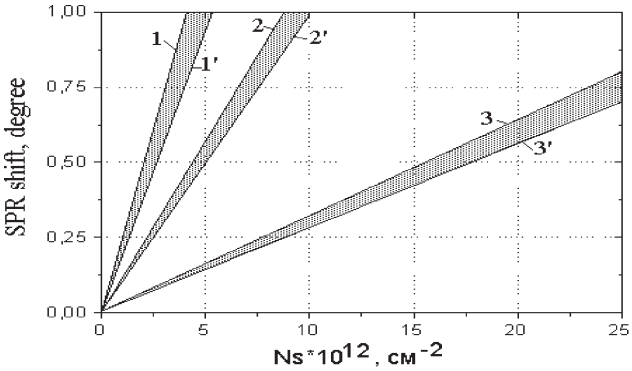

Fig.5. Shift of the SPR angular position $\Delta\theta_{min}$ vs surface molecular concentration N_s curves. 1 — IgG, 2 — BSA, 3 — Mb. 1, 2, 3 (1′, 2′, 3′) — minimal (maximal) polarization.

2.2. Experimental procedure

Detection of biomolecules and molecular complexes by a sensor operating on the basis of SPR, with quantitative determination of mass of the substance to be analyzed (that is present at the sensitive surface of the sensing instrument), involves the following operations:

– the sensitive surface of a metal (gold, silver) layer of the sensor is brought into contact with an ambience (say, air or water) whose refractive index is known;

– the angular dependence of the intensity of reflected light is measured over the range of angles of incidence $\theta_{min} < \theta < \theta_{max}$ (here θ_{min} and θ_{max} are the minimal and maximal angles, respectively; they are chosen in such a way that the above range includes the plasmon resonance angle). The acceptable results (i.e., those that provide sufficient certainty) were obtained when the angular dependence was taken with an interval of 20′;

– the optical parameters (the refractive index *k* and conducting film thickness *d*, as well as the refractive index n_1 and thickness d_1 of an additional layer (if any) at the conducting film surface) of the layered structure in the absence of the substance to be analyzed are determined from the results of measurements using nomograms or the special procedure of fitting the experimental and theoretical SPR curves (see subsection 2.4). Usually the parameters *k* and *d* of the conducting film are known;

– the conducting film surface is brought into contact with a sample of the substance to be analyzed in such a way that the latter could be adsorbed or in some other way be bound to the surface;

– the angular dependence of the intensity of reflected light is measured over the range of angles of incidence $\theta_{min} < \theta < \theta_{max}$ (the minimal and maximal angles are chosen from the same considerations as in the above case);

– the refractive index of the adsorbed substance n_x is set. It may be chosen in different ways, so that the condition $n_x > n_0$ (where n_0 is the refractive index of the ambience) is fulfilled. The optimal n_x value is that close to the refractive index of the substance to be analyzed. For instance, if the substance to be analyzed is protein molecules, and their study is performed in a water solution, then one may take $n_x = 1.45$;

– the thickness d_x of the layer of the substance to be analyzed is determined from the results of measurements using the special procedure of fitting the experimental and theoretical SPR curves (see subsection 2.4) (or nomograms) and the set refractive index;

– knowing the optical parameters *n* and *d* of the layer studied, one can determine the mass of the adsorbed substance using the following equation obtained from the expression for molecular refraction of the substance analyzed [26]:

$$\Gamma = d(n - n_0)/(dn/dc), \qquad (8)$$

where Γ [g/cm^2] is the mass of the adsorbed substance; *d* (*n*) is the thickness (refractive index) of the layer studied; n_0 is the refractive index of the ambience. Typical value of *dn*/*dc* (i.e., the ratio between the refractive index and solution concentration increments) for different proteins is 0.188 cm^3/g [27]. It should be noted that the only true physical quantity that is invariant under variation of n and d values and characterizes the actual amount of adsorbed substance is Γ value, while n_x and d_x are not physically meaningful at the above measurement procedure.

If the optical characteristics of a multilayer sensor structure vary with time, then the angular position of SPR varies accordingly. To register kinetics of variation of the angular position of SPR, one should perform measurements of both the total SPR curve and the angular position of SPR at set intervals. In such way one can register kinetics of additional mass (say, biomolecules) inflow to the area of damping electric field of surface plasmon, as well as kinetics of mass outflow from that area. Then the kinetic curves $\theta_{sp}(t)$ (SPR sensograms) can be analyzed using special-purpose computer software to obtain kinetic constants of association, dissociation, etc.

2.3. Sensor sensitivity and construction characteristics

In the SPR technique two main information-carrying parameters are (i) the intensity of reflected laser radiation vs angle of incidence curve $R(\theta)$ (SPR curve) and (ii) SPR angular position θ_{sp}. Contrary to the existing commercial devices that determine the SPR angular position only, our device (Fig.6) makes it possible to determine both parameters. To provide more accurate and convenient determination of parameters, we use precise calibration of a scale in angle, retroreflection and variation of the angle of incidence of laser beam, thus making it possible to perform measurements in a gas, as well as liquid, ambience. Application of precise graduation of a scale in angle enables one to obtain information on the optical parameters and layer thicknesses directly from the form of the SPR curve. A considerable (18°) angle range of the device makes it possible to deal with thick films, as well as improves accuracy of calculations at mathematical analysis of the experimental SPR curves.

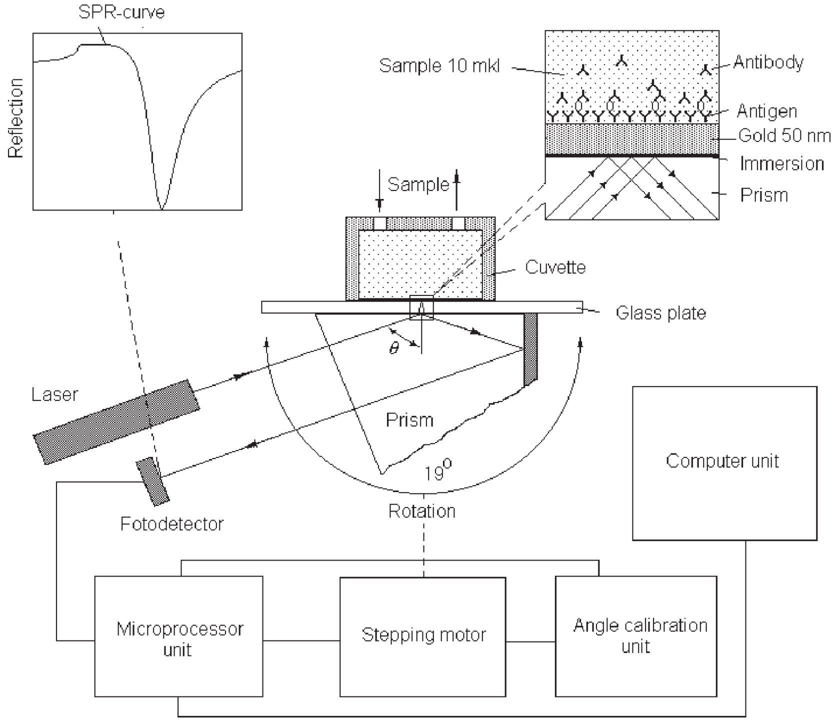

Fig.6. Simplified diagram of the sensor PLASMON-4m.

The simulation performed by us enabled to refine the optimal (from the viewpoint of device sensitivity) values of layer thicknesses and refractive coefficients, accounting with allowance made for possible modification of the metal layer with dielectric. At that the device sensitivity was considered as a comparative parameter characterizing θ_{sp} shift at adsorption of biomolecules with certain characteristics onto the metal surface. The results of simulation show that the effect of the parameters *d, n* and *k* of the metal (Au, Ag) layer (where plasmon oscillations occur) and adhesion sublayer (Cr, Mn) on sensor sensitivity within the range defined for SPR is much below that of a dielectric layer. To illustrate, deposition of a dielectric layer (about 40 nm thick, with refractive index $n = 1.45$ and absorption coefficient $k = 0$) onto the silver layer can result in considerable growth of sensitivity due to increase of the tangential component of the phonon wave vector. Indeed, at dielectric deposition onto the metal layer θ_{sp} shifts toward bigger angles where the tangential component of the phonon wave vector increases. To retain the condition of equality of the wave vectors, any increment of the plasmon wave vector has to be accompanied by the same increment of the photon wave vector, $\Delta k_x = \Delta k_{sp}$. When θ_{sp} is close to $\pi/2$, this can be achieved by bigger increment $\Delta\theta_{min}$ (Fig.7). As the dielectric absorption coefficient grows, the effect of enhancement is disappearing gradually (Fig.8).

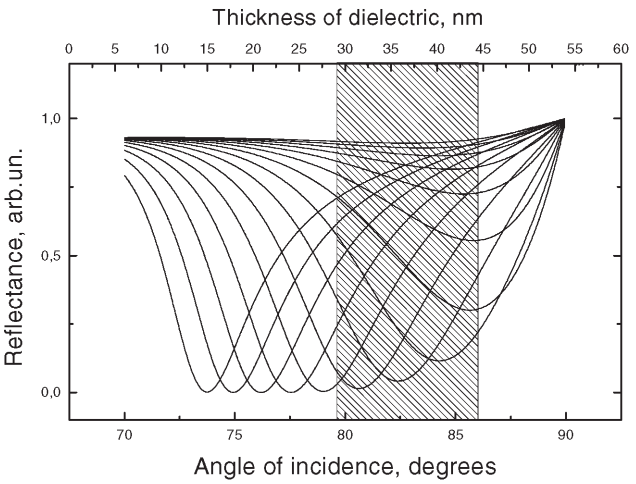

Fig.7. Calculated SPR curves for the structure “metal film–nonabsorbing dielectric film” ($n = 1.5 + 0i$) at stepwise (5 nm) dielectric thickness increasing. Shaded area — region of $\Delta\theta_{min}$ increase.

Correct determination of the θ_{sp} position is of importance for the device sensitivity. Interpolation of the area of SPR curve minimum makes it possible to reduce the measuring channel errors and determine θ_{sp} with rather high accuracy, on condition that the adequate mathematical analysis procedure has been chosen. Simulation shows that optimal interpolation for a symmetric (in the area of minimum) SPR curve is that using a polynomial of degree three (Fig.9). When performing interpolation, the calculated SPR curve was used. It corresponded to the standard experimental conditions in a water ambience for a gold film without dielectric coating and with a chromium adhesion sublayer. The optical parameters of the layers were as follows: the gold film thickness $d_{Au} = 45$ nm, $n_{Au} = 0.15$ and $k_{Au} = 3.6$; the chromium layer thickness $d_{Cr} = 5$ nm, $n_{Cr} = 2.1$, $k_{Cr} = 2.37$.

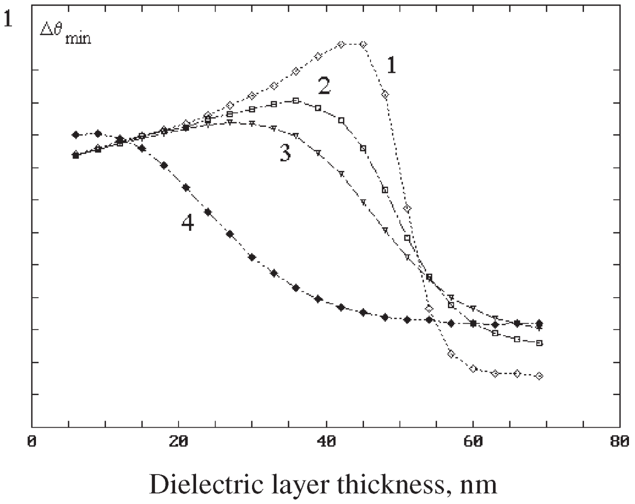

Fig.8. Calculated shifts of SPR curve minimum ($\Delta\theta_{min}$, relative units) vs dielectric layer thickness curves ($n = 1.45$) for different *k* values at registration of biomolecular layer with refractive index $n = 1.46$ and thickness $d = 2$ nm. 1 — $k = 0.0i$, 2 — $k = 0.02i$, 3 — $k = 0.04i$, 4 — $k = 0.2i$.

Shown in Fig.10 are the comparative experimental kinetic characteristics of the angle position of SPR minimum when the measured SPR curves are interpolated with polynomials of the degrees two and three. It is obvious that in the latter case the deviation amplitude for the SPR angular position is less by several times (25″ and 8″, respectively). This makes it possible (in theory) to detect variation of the refractive index of the ambience under study by 0.00005. In practice the developed device detects variation of refractive index at a level of 0.0001. This result, however, is true only if at least one of the following two conditions holds: (i) one performs mathematical processing of only a restricted curve part in the area of minimum, or (ii) the SPR curve in the area of minimum is of the form close to symmetric. In actual practice the above conditions may not hold, for example, when measuring thick polymer films or films with high absorption coefficient (in this case the resonance curve loses its extremum and ceases to be symmetric because its right shoulder tilts).

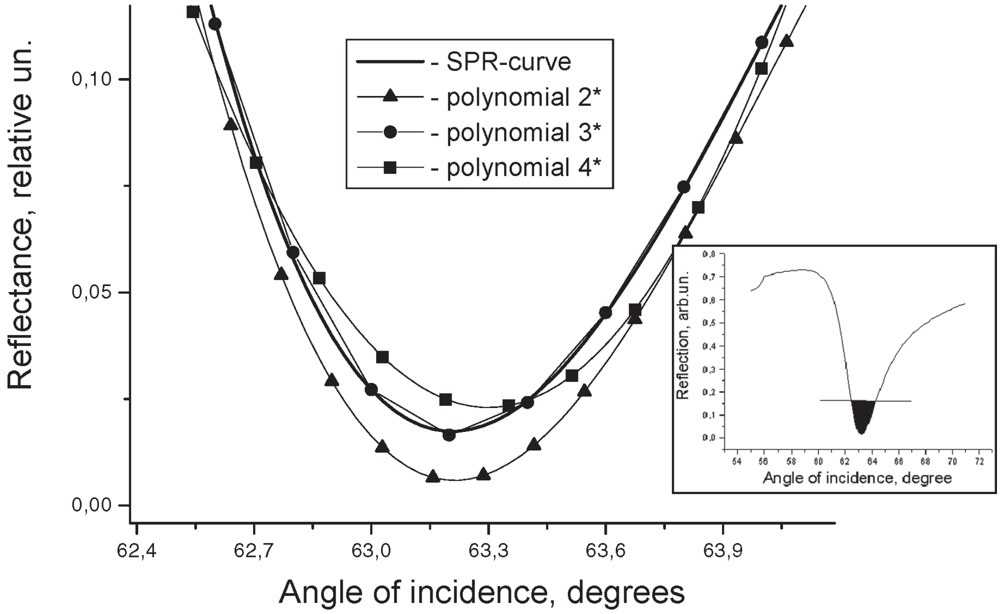

Fig.9. Interpolation of the minimum of calculated SPR curve with polynomials of the 2nd, 3rd and 4th degrees. Inset: dark area — interpolated part of SPR curve.

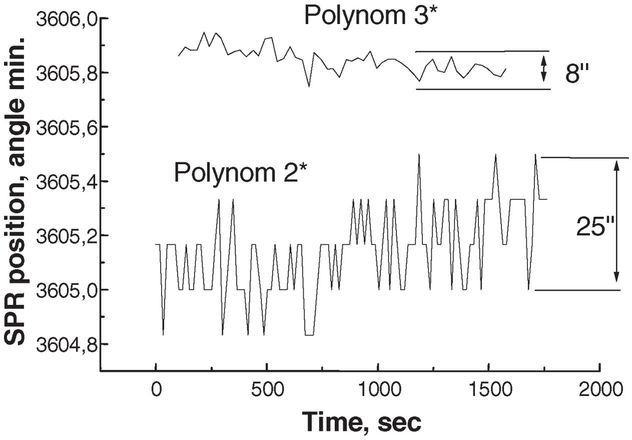

Fig.10. Comparative experimental kinetic characteristics of the angle position of SPR curve minimum obtained using polynomials of the 2nd and 3rd degrees for processing of SPR curve minimum position.

It should be noted that considerable noise amplitude at reflection intensity measurement may affect the result of polynomial interpolation. In such case one should process larger portion of the SPR curve, when optimal interpolation is achieved by application of polynomials of higher (or lower) degrees.

2.4. Mathematical processing of SPR curves

Both shape and angular position of the experimentally measured $R(\theta)$ curve depend on the optical constants (n_i and k_i) of all the phases with which electromagnetic wave interacts. These are the materials of the: prism; conducting layer; substance to be analyzed that is adsorbed on the surface of that layer; ambience and other phases that, depending on the measurement conditions, may belong to the system studied. In addition, the shape and angular position of the SPR curve depend on the effective geometric thicknesses of all the layers in the system, in particular, the conducting film and adsorbed layer to be studied. Therefore, when having an experimental SPR curve, one can, in principle, calculate the unknown parameters of the system studied (say, the refractive index and thickness of the adsorbed layer) by applying the trial-and-error method, so that the theoretical SPR curve (calculated using these parameters) best fit the experimental one (i.e., by solving the inverse problem using the so-called matching technique) [28].

The effect of the refraction index (n), absorption coefficient (k) and layer thickness (d) values on the characteristics of the SPR spectrum is contained in full in Eqs. (4) and (5). In the simplest case (a metal layer without an additional dielectric layer) the number of parameters of the measured SPR curve (position, width and depth of the resonance curve) is equal to that of the unknown film parameters (n, k and d); thus they can be calculated (in principle) [26]. Simulation shows that in most cases a specific form of the angular reflection spectrum corresponds to a specific set of parameters of the SPR multilayer structure.

The calculated angular dependencies of reflection for the system “chromium adhesion sublayer-gold-the layer (dielectric) studied” are presented in Fig.11. The SPR curves show that there exists considerable independent influence of the optical parameters of a layer system on the angular spectrum parameters. This is particularly true for a system in which the parameters of the metal layer (which is the carrier of plasmon oscillations) remain constant, and the only variable parameters are those of the layer under study. A comprehensive analysis of simulation shows that in this case the angular position of SPR depends mainly on the layer thickness *d* and refractive index *n*, while the depth f the minimum and curve width depend on the absorption coefficient *k* to a greater extent. It should be noted, however, that for any resonance curve there exist no less than two solutions of Eqs. (4) and (5),with different values of *n, d* and *k*.

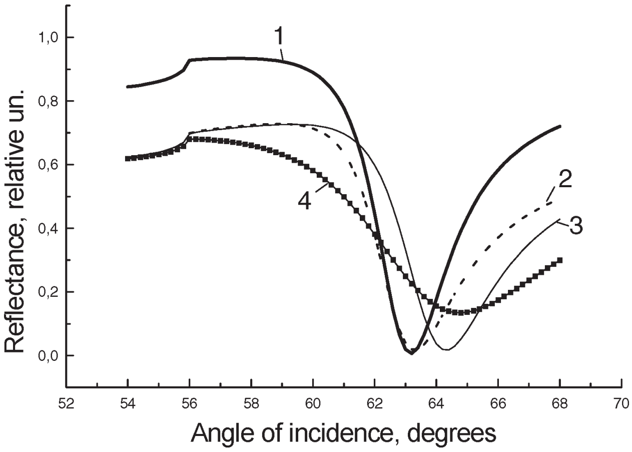

Fig.11. Calculated SPR curves for gold layer (1) and gold with: chromium sublayer (2), nonabsorbing dielectric layer (3) and absorbing dielectric (4).

Obviously such procedure does not provide obtaining the exact parameter values all at once, because their number is big. Nevertheless the procedure of comparing the experimental and theoretical SPR curves is applied rather often, with usage of the appropriate approaches and comprehensive experiment. Thus, when applying the above technique, the parameter determined at the previous stage is introduced into the comparison procedure at every next stage. Such reduction of the number of parameters that are processed concurrently results in improvement of the accuracy. Application of additional procedures (say, atomic force microscopy (AFM) or profilometry) that give exact value of one of the parameters (in this case, this is layer thickness) also increases reliability of the result obtained.

Interfaces and internal junction layers deserve particular attention, because ignoring their effect may lead to considerable final error [29]. AFM provides additional opportunity to determine the effective refraction values for the surface layers by determining the profile of the surface studied. These effective refraction values are required for construction of a more reliable mathematical model of a multilayer structure (Fig.12). The effective refractive index of a layer can be calculated according to the Lorentz-Lorenz equation [11]:

$$\frac{n_{\mathrm{eff}}^2-1}{n_{\mathrm{eff}}^2+2}=\frac{n_{\mathrm{exp}}^2-1}{n_{\mathrm{exp}}^2+2}V+\frac{n_{\mathrm{amb}}^2-1}{n_{\mathrm{amb}}^2+2}(1-V). \qquad (9)$$

Here n_{eff} is the complex effective refractive index; n_{exp} is the refractive index of a gold film, dielectric or some other object that makes surface rough; n_{amb} is the ambience refractive index. *V* is the filling factor that is calculated using the information on surface relief obtained with AFM.

Having a set of experimental data, one can determine approximately the parameter values for the layer analyzed. The procedure of direct fitting of values, even performed using unspecialized software, requires much time (at that at least one of the main parameters, say, the refractive index *n* or layer thickness *d*, has to be known) and is routine *per se*. This problem becomes much simpler when applying the methods of minimization of a function of several variables. The optimal solution is found using the objective function. In the course of optimization procedure one should find such values of parameters determining the problem considered at which the objective function has a minimum. The objective function may be presented as

$$G=\frac{\sum_{i}^{N}\left[R^{e}(\theta_i,n,d)-R^{t}(\theta_i,n,d)\right]^2}{N}, \qquad (10)$$

where *N* is the number of measurements. The function is a generalized difference between the experimental values of the $R^e(\theta)$ curve and the parameters $R^t(\theta)$ of the theory leaning upon the mathematical model of the SPR system. In our case $G = f(n, k, d.....n_i, k_i, d_i)$ is a function of several variables. If, however, the model used is adequate to the actual object, then it is possible, in principle, to recognize more than three parameters of a multilayer

SPR structure. The problem is complicated because, as mentioned above, the solutions of Eqs. (4) and (5) are not uniquely defined. At that the number of minimums of the objective function may increase if an inadequate physical model of the structure analyzed is chosen.

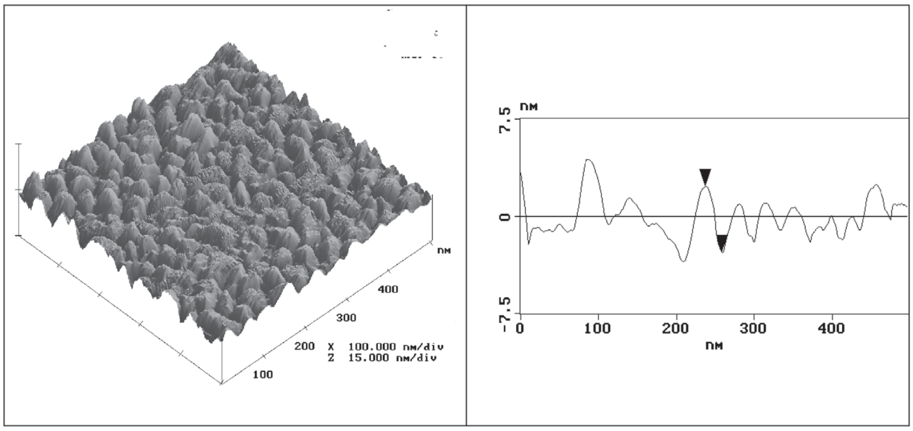

Fig.12. AFM pattern (left) and AFM section (right) of surface layer of gold film used in SPR sensor structure.

A program with a special-purpose algorithm has been developed for solving such problem in the domain of existence *G* where the objective function minimum is sought. The principal distinction between the proposed algorithm and those existing is determination of the sequence of enumeration of parameters by the Monte Carlo method (random function) at all the stages where enumeration of parameters is made, as well as possibility to test if the minimum is true. The latter possibility is realized in the following way: After the algorithm operation is finished, the program stores the determined value of the objective function and returns to the start of the algorithm operation. The random function of the algorithm makes recurrence of the previous combination of starting parameters impossible and gives rise to a new course of calculations. As a result, if the objective function has several minimums, then another minimum will be fund necessarily. Recurrence of the above procedure and comparing the values of the objective function result in determination of the true minimum. As against other algorithms, the proposed one in most cases excludes ambiguity in situations when the level lines of the objective function have kinks.

2.5. Modification of sensor sensitive surface

A damping electric field of a surface plasmon with a wave vector k_{sp} penetrates at a certain depth d_z into an ambience contacting with metal surface. The *z*-component of the field in a medium is

$$E_z = E_z(0)\exp[-k_z z], \qquad (11)$$

where k_z may be expressed as

$$k_z^2 = k_x^2 - \frac{\omega^2}{c^2}\varepsilon_a . \qquad (12)$$

Here k_x is the tangential component of the photon wave vector; $c(\omega)$ is the speed (circular frequency) of light; $\varepsilon(\omega)$ is the complex dielectric function of metal; ε_a is the ambience permittivity. At $k_x = k_{sp}$ the *z*-dependence of the *z*-component of the damping wave is approximately as follows [30]:

$$E_z(z) = E_z(0)\exp\left[-\frac{\omega}{c}\left(\frac{-\varepsilon_a^2}{\varepsilon'(\omega)+\varepsilon_a}\right)^{1/2} z\right] = E_z(0)\exp[-z/\delta_z] \qquad (13)$$

If one substitutes into Eq. (12) the permittivities of gold, $\varepsilon'(\omega) = -13.5$, and water, $\varepsilon_a = 1.77$ (at a wavelength $\lambda = 670$ nm of the laser radiation used), then one obtains for typical depth of the damping plasmon field penetration into a water ambience over a metal $d_z \approx 200$ nm.

To provide efficient use of the space where an evanescent wave of a surface plasmon (that is sensitive to changes occurring at the sensor surface) propagates and increase of sensor sensitivity, the biomolecules to be studied are often bound not to a flat surface but to flexible polymer chains pre-immobilized at that surface. Such an approach makes it possible to immobilize at a sensor substrate the required amount of molecules that is equivalent to several close-packed layers, with retaining their ability to interact with other biomolecules. The most widespread of such polymer matrix structures is that (from now on matrix) formed according to the method used by Biacore AB (Sweden) — the leading manufacturer of SPR biospectrometers [31-32]. This method seems too complicated to be applied by the ordinary users of the SPR technique in their everyday practice. It involves formation on gold surface of an omega-mercapto-hexadecanol monolayer, its activation with epichlorohydrin, covalent binding of dextran to “the activated surface” and transformation of immobilized dextran into carboxymethyl-dextran whose carboxyl groups can be used for immobilization of the protein to be studied.

It is known, however, that the carboxymethyl-dextran matrix is not optimal for proteins with low pI, because immobilization in a matrix of proteins whose charge is of the same sign as that of matrix requires considerable additional modification of a matrix [32]. That is why a demand arose for development of a simpler and more versatile technique for modification of gold surface to immobilize proteins and other biological objects on it. The most representative objects studied with the SPR technique are proteins. A necessary condition for application of the SPR technique is immobilization of one of the interacting biomolecules on a metal (usually gold) film exposed to the solution analyzed. In many cases immobilization via immediate adsorption onto gold leads to uncontrolled changes in protein spatial structure, thus resulting in loss of its biological activity [33-34]. So, as a rule, gold surface has to be isolated from direct contact with proteins studied.

It is known that the SH groups (contrary to most of other functional groups) interact with gold [35-36]. That is why just SH-containing compounds are used for formation of a binding layer on gold surface. The aliphatic thiols with rather long (*n* > = 10) unbranched chains are most often used for this purpose. Such compounds (or their derivatives with various functional groups at the methylene chain end) spontaneously form at gold surface rather stable ordered monolayers. In them a sulfur atom is bound to gold, and close-packed aliphatic chains make the same angle with the surface. The monolayer is stabilized by numerous van der Waals interactions between chains [37].

We chose aldehyde derivatives of polysaccharides (obtained with periodate oxidation) to form a polymer matrix. These derivatives can be immobilized easily on a surface that carry hydrazide groups, and biomolecules containing amino groups can be attached to the immobilized derivatives of polysaccharides by using the aldehyde groups of polysaccharides. Negative charge of carboxymethyl-dextran becomes its advantage when immobilization is made at pH below the isoelectric point of the protein studied, because electrostatic attraction of protein molecules to the matrix favors protein accumulation in a volume adjacent to the matrix. However, this matrix is of little use in the case of proteins with low isoelectric point, and reduction of the carboxyl group content means drop of the number of active groups via which immobilization proceeds. Immobilization in a matrix formed from periodate-oxidized polysaccharide proceeds via uncharged aldehyde groups. If, however, negative matrix charge is desirable, one can obtain it using periodate-oxidized dextran-sulfate instead of periodate-oxidized dextran. An additional advantage of this approach is that the cost of reagents used is lower and they are readily available.

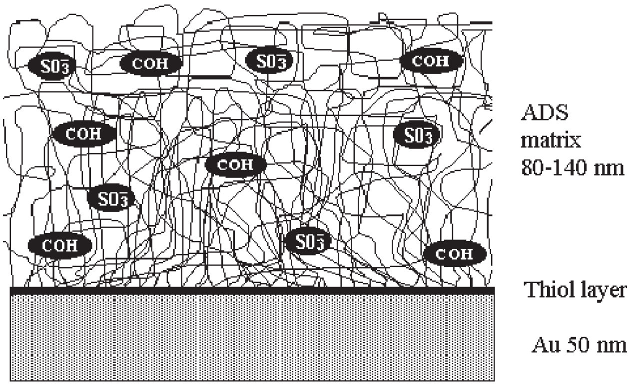

Fig.13. Schematic of aldehyde-dextran-sulfate (ADS) matrix structure.

The proposed aldehyde-dextran-sulfate matrix [38-39] (Fig.13.) has, contrary to the previous one, two functional groups, aldehyde and sulfate. The first of them (which is electrically neutral) forms covalent binding with protein (the carboxyl group in the “Biacore” matrix did). The function of the second group is to provide electrostatic retraction of biomolecules. The results of SPR testing of the developed matrix evidence that adsorption activity for biomolecules in the pI range from 4.1 up to 10 is sufficiently high. The advantage of this matrix is a possibility to vary number of sulfate groups in it, or exclude them completely. A neutral electric charge of the matrix enables one to deal with those proteins which are characterized by low pI, practically at any pH of solution. Testing showed that process of protein immobilization becomes slower in this case; however, the amount of immobilized protein may be even bigger than in the case when sulfate groups are present.

2.6. Electrochemical investigations with application of SPR

A capability of the SPR technique to estimate *in situ* the optical constants and thicknesses of thin film in the real-time mode seems to be very attractive when studying various electrochemical processes, in particular electropolymerization. To realize the above functions, we developed a modification of the SPR device, as well as a procedure of concurrent electrochemical and SPR (ESPR) investigations. A special-purposed electrochemical cell of the device makes it possible to study different films having redox properties, as well as realize monomer electropolymerization directly at gold surface and trace *in situ* thickness of the polymer film being obtained. At that the gold layer is used as a main electrode, platinum wire serves as an auxiliary electrode, and silver wire (treated in NaCl solution) serves as a reference electrode.

Novelty of the technique discussed arises some questions concerning interpretation of results of ESPR investigation. This is pertinent to the necessity (usual for SPR measurements) for segregation of two main parameters- the layer thickness d and refractive index n, as well as to the effect of external potential applied to the metal on both the angular position of resonance minimum θ_{sp} and form of SPR spectrum. An opportunity to segregate the n and d values from the SPR measurements has been studied rather well; it was considered in subsection 2.4. The effect of external potential on the angular position of the minimum and form of the SPR curve is less well understood. Application of external potential to a metal layer (that serves as a carrier of plasmon oscillations) is related usually to variation of the free electron concentration in the metal near-surface layer and formation of metal oxide film at anodic decomposition of water [40] and to the effect of electrolyte ions [41-42]. However, if a potential of up to 1 V is applied to the gold placed into electrolyte, then the effect of ambience on SPR can be detected explicitly only when the electrolytic medium has clearly pronounced redox properties, i.e., it can be in two states — reduced and oxidized. The acidic or basic electrolytes that are traditionally used in practice of electrochemical experiment do not possess such properties. In this case shift of the angular position of the resonance minimum and variation of the form of SPR spectrum (SPR response) depend mainly on formation of oxide film on gold or on dissolving of such film.

The situation becomes different if the gold electrode is coated with a polymer film (especially if that film displays its redox properties). Even at application of low voltages under the above conditions a θ_{sp} shift is considerable (up to several degrees) and depends, first of all, on the film thickness, its structure and level of redox properties. It is known that optical parameters of such substances may change due to variation of the electrode layer thickness in the course of electrochemical reaction [43-44]. This fact leads to additional difficulties in estimation of properties of such substances. SPR provides further opportunities in investigation of these structures, because this technique is sensitive to both the concentration of redox-molecules in the electrolyte solution and the adsorbed molecular layer thickness. The cyclic voltammetry, for its part, enables one to estimate gradient of redox-particle concentration near the main electrode surface. A combination of these two techniques becomes a powerful tool when studying the properties of macromolecular structures with pronounced redox-properties.

An example of such combination may be the investigation of the gold-polyaniline structure made with the developed ESPR device-sensor [45]. This polymer is a redox-substance and can be obtained *in situ* by electropolymerization under the conditions of SPR experiment. In such a way a polyaniline film was obtained. Its thickness was calculated by comparing the experimental and theoretical SPR curves. Then the film was studied concurrently with cyclic voltammetry, chronoamperometry and SPR. It is known that polyaniline may be in two states — oxidized $(An^{2+})_n$ and reduced $(An)_n$. Transition of polyaniline from one state to another at gradual variation of potential from–0.2 V up to 1.3 V and at abrupt potential jump from 0 V up to 0.4 V (Fig.14) was studied by processing of the recorded SPR curves, cyclic voltammograms and chronoamperograms. It was found that variations of the form of SPR curve and angular position of the minimum of θ_{sp} of the sensor under action of external electric potential can be observed mainly in the following three cases: 1) change of the free electron concentration in the gold near-surface layer resulting from application of external electric potential to the main electrode; 2) formation and change of characteristics of the double electric layer at the gold-electrolyte interface resulting, say, from variation of pH of the solution serving as electrolyte; 3) change of the ambience dielectric characteristics in the zone of evanescent plasmon wave effect.

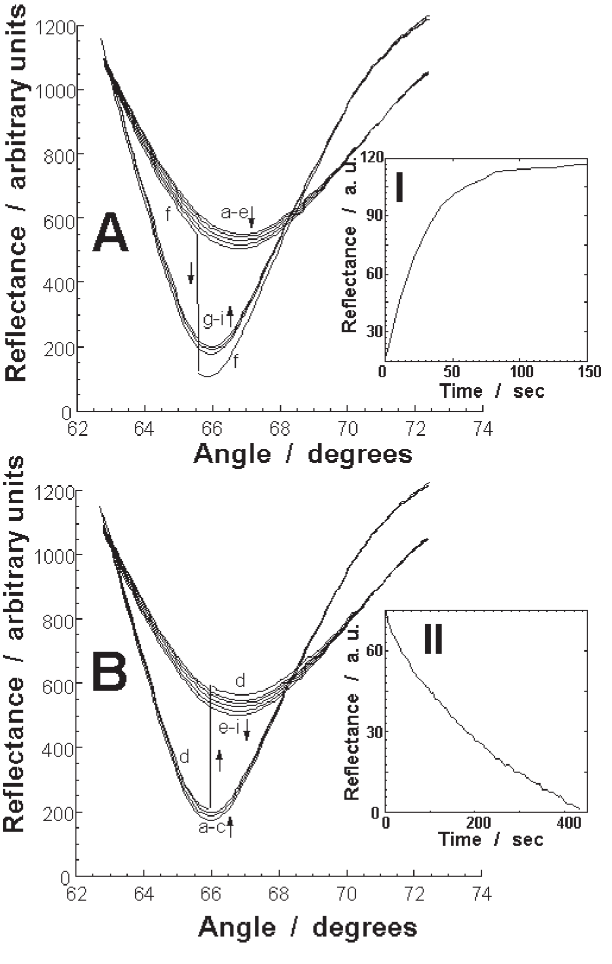

Fig.14. SPR curves for uncoated gold (*I*, *x*) and gold with a polyaniline film (*I*, *y*). Variation of the form of SPR curve (A, B) and kinetic curves of shifts of minimum (II — shrinking, III — swelling) at polyaniline transformation from oxidized to reduced form.

The investigation enabled to calculate the refractive indices at the reduced (1.4 + 0.02i) and oxidized (1.39 + 0.46i) states, as well as the kinetic constants for processes of polyaniline film contraction (biexponential kinetics with the constants 9×10^{-3} s^{-1} and 5×10^{-4} s^{-1}) and swelling (3×10^{-4} s^{-1}). This is of particular importance if one takes into account that application of similar structures as man-made muscles and controlled relief seems to be promising.

3. Conclusions

The developed SPR device-sensor [46] has important advantages over the existing devices. These are an opportunity for wide range variation of the angle of incidence of laser beam; the retroreflection principle of reflection; an automatic calibration system that enables one to obtain quantitative characteristics of permittivities and film thicknesses for a wide range of materials using mathematical analysis of the angular spectrum $R(\theta)$ and kinetic curves $\theta_{sp}(t)$. The processes of excitation of surface polariton-plasmon resonance in the SPR sensors after Kretchmann are described more adequately in the framework of the model of an isotropic multilayer system on the basis of the matrix of effective optical constants and junction layer thicknesses which takes into account presence of junction layers and surface geometric imperfection. This model makes it possible to calculate the main sensor parameters and optimize construction of the sensitive part of sensor. The proposed procedure for mathematical analysis of the form of SPR curve enables one to determine no less than three parameters (the effective refractive indices and absorption coefficients, as well as layer thicknesses) of the structure studied. It is shown that polarizability and concentration of biomolecules in the zone of damping wave of surface plasmon also can be determined from the results of SPR parameter measurements.

The developed novel aldehyde-dextran-sulfate matrix structure on the basis of dextran essentially increases SPR sensor response at protein immobilization in the matrix volume as compared to immobilization directly on metal surface. The developed procedure of electrochemical measurements with application of SPR opens up fresh opportunities for appearance of the novel line of investigations — the ESPR technique.

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