Considered in this review are main directions of developing technology and construction of the available sensors based on surface plasmon resonance phenomenon to increase their sensitivity and accuracy of measurements. It has been shown that reducing roughness of the plasmon carrying layer in the sensor as well as application of an additional covering dielectric layer with developed surface enables two-fold increase in the sensitivity due to the twice increased surface of interaction between the sensor and studied substance. The main technical way enabling to diminish surface roughness is thermal annealing, and the best result is usually reached for the annealing temperature 120 °С.

In most cases, as a dielectric layer they use metal oxides Al₂O₃, TiO₂, SiOₓ and ZnO, which allows attaining the detection limit in changes of the studied substance refraction index close to 1 • 10⁻⁹, what is one order better than that in available commercial analytic devices based on SPR phenomenon. Shown in the review are promising directions for development of SPR sensorics such as application of multilayer graphene coatings and polymer layers prepared by polymerization in high-frequency plasma of inert gas. Also, adduced in the paper are statistical data that show the number of publications in scientific journals within this topic. This number underwent an exponential growth and to the end of 2015 was about six thousands, which is indicative of these investigations topicality and stimulates further studying the possibilities to improve and develop new analytical devices based on the SPR principle. In our opinion, further development of these sensors will be directed to increasing the selectivity, wear resistance of the sensitive element surface as well as to developing the methods for regeneration of receptors suitable for multiple using the sensitive elements.

Abstract

In most cases, as a dielectric layer they use metal oxides Al₂O₃, TiO₂, SiOₓ and ZnO, which allows attaining the detection limit in changes of the studied substance refraction index close to 1 • 10⁻⁹, what is one order better than that in available commercial analytic devices based on SPR phenomenon. Shown in the review are promising directions for development of SPR sensorics such as application of multilayer graphene coatings and polymer layers prepared by polymerization in high-frequency plasma of inert gas. Also, adduced in the paper are statistical data that show the number of publications in scientific journals within this topic. This number underwent an exponential growth and to the end of 2015 was about six thousands, which is indicative of these investigations topicality and stimulates further studying the possibilities to improve and develop new analytical devices based on the SPR principle. In our opinion, further development of these sensors will be directed to increasing the selectivity, wear resistance of the sensitive element surface as well as to developing the methods for regeneration of receptors suitable for multiple using the sensitive elements.

Introduction

Modern trends in the development of analytical devices require a reduction in the size of both the equipment as a whole, and sensors in general, by integrating the functions of the laboratory on one chip, which is called Lab-on-a-Chip Technology (LOC technology) [1]. The advantages of this technology are reducing the volume of samples and reagents, increasing the automation of the measurement process and reducing the measurement time. Most of the modern technology of analysis, such as electrochemical, mechanical and optical methods, are represented by equipment with LOC technology. It is known that optical methods possess a high operation speed and enable to reach high accuracy and sensitivity in measurements. One of the promising optical methods for analysis of various compounds and micro-objects as well as processes at the molecular level is the refractometric method based on surface plasmon resonance (SPR) phenomenon. As compared with traditional measuring methods, the SPR-method provides possibility to study processes of molecular interaction in micrometer-thickness layers in the real-time scale; low value of the sample volume required for measurements (less than 10 μL); the method does not require any markers or fluorescent labels for studying the analyte. Optical measurements based on the SPR phenomenon are widely used in chemical and biological analyses that are found on registration of molecular adsorption in different media from gases to liquids and solids (e.g., inorganic solid particles and the organic films of Langmuir-Blodgett type [2-5]. Diagnostic devices that operate using the SPR phenomenon possess high sensitivity to low concentrations of studied substances, which enables to use them as precise analytical tools in laboratory investigations performed in food, chemical and pharmaceutical industry, agriculture, medicine and ecology [6-9].

The SPR sensor consists of a sensitive layer, which provides a selective response to a definite analyte through some chemical reaction and a physical transducer that converts the respective chemical signal into the electric or optical one. The latter can be further used for qualitative or quantitative determination of...
SPR phenomenon was observed as a sharp drop in the intensity of light reflected from the interface at some definite (resonance) angle of incidence. It is related with the fact that, at the angles corresponding to total internal reflection, conduction electrons of the thin film are excited by laser radiation. SPR occurs in a thin metal film with the negative dielectric permeability (high electrical conduction), which is located on a transparent dielectric substrate. In designing analytical devices, it is important that the resonance angle value depends on the concentration of analyzed substance contacting with the sensitive element. As a sensitive layer, the most often used are metals with high electrical conductivity and chemical passivity.

The most widely used method for excitation of surface plasmons is realized via a coupling prism. In this case, to provide total internal reflection, the following condition should be satisfied: the refraction index of studied substance has to be lower than that of the coupling prism, i.e., \( nt < np \). There are two optical setups to realize this method of excitation, namely: Kretschmann geometry and Otto one. In the Kretschmann geometry, under the condition of total internal reflection, the prism with a high refraction index \( n_t \) is coupled with a metal-dielectric waveguide that consists of a film possessing the dielectric permittivity \( \varepsilon_m \) and thickness \( d \) as well as semi-infinite dielectric with the refraction index \( n_p \), the role of which is played by the studied substance (Fig. 1a). This scheme is more often used, because it is easier-to-produce in practice. The principle of operation of SPR-devices lies in determination of changes in the analyte refraction index (RI) by observing the shift of the analyte reflection curve \( R(\theta) \) minimum. The preferential majority of SPR-devices are designed using the Kretschmann geometry (Fig. 1a) that consists of a laser (1), prism for total internal reflection (TIR) (2), sensitive element (3) and photodetector (4) [10].

The most widely spread sources for exciting surface plasmons are lasers. The reflection characteristic \( R(\theta) \) is the dependence of the intensity of laser light on the angle of its incidence onto the surface of sensitive element (SE) within the range of angles higher than the TIR angle at the boundary SE – analyte (Fig. 1b). The analyte RI value is related with the value of the minimum inherent to the reflection curve \( \Delta \theta \) via the following parameters of device optical scheme elements: laser light wavelength, refraction indexes of SE, TIR prism and analyte. If the SE metal layer is sufficiently thin (< 200 nm), then a considerable part of electromagnetic wave decaying in metal can reach the opposite surface of this layer [11]. Then SPR becomes sensitive to properties of the medium contacting with metal. A position of the minimum in the reflection curve depends on electric polarization (dielectric permittivity) of this medium. When properties of the metal layer or the refraction index of medium being above this layer are changed, the reflection minimum is essentially shifted.

Therefore, original measurements of reflection characteristics under SPR conditions were only considered as a very sensitive method for studying the optical properties and states of metal surfaces [12]. In what follows, it became used as the most exact method of refractometry for determining the refraction indexes of liquids and gases.

**Figure 1**: Optical scheme of the SPR-device based on the Kretschmann geometry (a) and angular dependences for the intensity of light reflected from the boundary SE-analyte before (–) and after (---) changes in the analyte RI by the value \( \Delta n \) (b) [11].

**Technology and materials of sensors**

When creating the analytical devices, it is important to analyze the influence of SE material and technology for its making on the shape of reflection characteristics \( R(\theta) \) as well as accuracy in determination of its minimum position and sensitivity of sensor caused also by a relative shift of the minimum \( R(\theta) \).

**Influence of technology of sensor element**

An important factor is the influence of surface relief inherent to the metal layer on light absorption, since just the surface is characterized by availability of a strong electric field. Therefore, surface roughness of metal layer defines an essential effect on propagation of surface plasmons, which, as a result of energy dissipation, leads to early decay of plasmons and reduction of their phase velocity [13-16]. In this case, the shape of the dispersion curve is changed, and the resonance frequency of surface plasmons is shifted. If the wavelength of incident light is fixed, with growing the SE surface roughness the minimum position of the reflection characteristics is shifted to the side of higher angles, the reflection amplitude in the resonance minimum \( R_{\min} \) increases, the reflection characteristic is widen, and, as a consequence, the error of determining the minimum position grows. The principal factors influencing the structure and properties of metallic layers made by thermal evaporation in vacuum are the speed of deposition and temperature of substrate [17,18]. In the case of gold metallic layer of SE, the deposition speed 4 to 5 nm/s provides a layer with a maximal density as well as reproducible optical parameters and smooth homogeneous surface. At the same time, low speeds of deposition results in fine-dispersed, rough and friable structure of deposited layers, while high deposition speeds lead to the coarse-grained structure of surface [19]. To reduce the influence of substrate relief on the surface roughness of deposited metal layers, the substrate surface is prepared using traditional optical technology that is usually used when making optical parts.
Influence of sensitive element material

In practice, gold and silver are preferentially used as SE material. Copper and aluminum are not practically used: copper – through its high oxidation capability, and aluminum – through its very high value of the imaginary part of dielectric permittivity $\varepsilon''$, which essentially widens the reflection characteristic. Analyzed in the works [31-33] is the problem of optimal choosing the metal and exciting light wavelength from the viewpoint of reaching the maximal sensitivity and chemical inertness of SE operation surface. It is known that usage of silver layers enables to obtain rather narrow minimum in the $R(0)$ characteristic as compared with that of other metals. However, the gold operation surface of SE is more stable and chemically inert. Therefore, just the gold layer is most widely used as carrier of surface plasmons. The most spread way to increase the surface sensitivity is provided by using a porous dielectric layer covering PCL [34].

The authors noted in this work that the obtained structure is heterogeneous and changes its adsorbing, optical properties and geometrical profile, which is related with features of PLD method. This fact makes it practically impossible to reach the necessary reproducibility and stability in the results of measuring the refractometry index by using this structure, i.e., it can be used only in qualitative analysis. As shown in, por-Al$_2$O$_3$ films prepared using PLD can be applied for creation of sensitive elements in optical sensors of humidity and solvent vapors [40]. In, it was shown that the films por-SiO$_2$ prepared by using thermal deposition in vacuum can be used for construction of highly sensitive optical sensors for liquid and gas-like substances [41]. In this work, the growth of sensor response reached at least the six-fold level due to application of an additional layer (AL). In, they studied SPR performances of porous gold films prepared using PLD, but their sensor properties were not investigated [42].

In, the sensor sensitivity was enhanced due to application of an adhesive layer from zinc oxide (ZnO) between PCL and TIR prism [43]. This layer was prepared from metal zinc by irradiation with the high-frequency electromagnetic field (13.56 MHz, 200 W) and heating up to 200 °C. The layers of gold and chromium were prepared using electron-beam evaporation. There was compared the sensitivity of the offered sensor with that of a standard sensor with the adhesive layer from zinc oxide (ZnO) between PCL and TIR prism, and the same sensor with the adhesive layer from zinc oxide (ZnO) between PCL and TIR prism, but the adhesive layer was performed by using self-assembled monolayer (SAM) of 1-dodecanthiol [44]. The authors reported 1.6-fold increase in the sensitivity (~6•10$^{-6}$ RIU), when analyzing liquid media, and 9.4 (~1•10$^{-6}$ RIU), when investigating gases. In, the authors used the waveguide optic-fiber method for SPR excitation [45,46]. To enhance the sensitivity, the authors applied the optic fiber with variable geometry (in the range of PCL the optic fiber was narrowed, which increased the density of electromagnetic energy), and an
additional dielectric layer. In, PCL was aluminum layer (8 nm), and the dielectric layer was titanium dioxide TiO₂ (60 nm) for the range of wavelengths exciting surface plasmons 750 – 850 nm [45]. The sensitivity of this sensor for liquid substances was 4000 nm/RIU (≈2.5 × 10⁻⁷ RIU for the scanning step by wavelengths 0.001 nm). In, PCL was aluminum layer (8 nm), and the dielectric layer was indium nitride InN (30 nm) [46]. In this case, the sensitivity for liquid substances reached 10800 nm/RIU (≈1 × 10⁻⁸ RIU for the same scanning step by wavelengths).

In, the authors used nanoporous aluminum oxide prepared using anodization with a periodic structure of pores (nanocaps) and step 60 nm [47]. In this case, the layer of porous anodized aluminum was covered with gold nanoparticles, which promoted adhesion of biomolecules to the sensor surface. The sensitivity of this sensor for interaction “antigene – antibody” was 1 fg/mL, which corresponds to the change of refraction index in the medium over the sensor ≈1 × 10⁻⁸ RIU.

When the studied substance interacts with PCL, there can occur such processes as physical adsorption of substance and surface oxidation. These processes cause degradation of the sensitive element as well as to distortions of measurement results as a consequence of changing the physical characteristics both of analyte and PCL. To enhance the sensitivity and selectivity of SPR sensors, they use additional organic polymer layers of calixarenes, polymethylmetacrylate, alkanthiols, complex oxides etc [48-50].

To reduce the influence of temperature on results of SPR measurements, they use local or complex thermal stabilization [51]. Besides, keeping the set temperature of the studied object is necessary, for instance, when analyzing DNA mutations by using the method of polymerase chain reaction. The deficiency of available technical solutions is related with a definite distance between the range of heating (cooling) and the range of sensitivity in the SPR device, where the chemical or biological reaction takes place. As the range of SPR sensitivity is close to the half wavelength of laser radiation exciting plasmons (0.2…0.5 µm), and the distance between the heat source and the surface of the sensitive element exceeds 100 µm, there is a discrepancy between the set and real temperature. Besides, the temporal delay of the heat flux front in conditions of high SPR-device sensitivity leads to considerable temperature errors in measurement results. This problem can be solved by joining the functions of PCL and heater in one sensitive element. As shown in, the sensitive element based on the ITO layer can combine functions of PCL and heater [52].

Evolution of devices based on surface plasmon resonance

In recent two decades, high demands from biological research and pharmaceutical industries have led to successful commercial implementations of SPR based sensors. Commercial SPR units are now available from a number of companies including Biacore, AutoLab, Biosensing Instrument, ICx Nomadics (manufacturer of SPREETA previously under Texas Instruments) and Hofmann Sensorsysteme etc. Most of current commercial SPR sensors are based on controlling the position of reflectivity dip in angular or wavelength spectra (angular and spectral interrogation), or the intensity under a fixed angle of incidence and wavelength (intensity interrogation) [53,54]. Such amplitude-sensitive interrogations are capable of detecting 1 pg/mm² of biomaterial accumulating at the biosensor surface [55]. This sensitivity is sufficient for studies of many interactions involving relatively large molecules such as, e.g., antibody-antigen, protein-DNA, DNA-DNA etc [56]. However, the sensitivity still needs to be greatly improved for detection of low molecular weight analytes (typically less than 500 Da) such as drugs, vitamins etc., as well as lower copy number analytes such as e.g., antigens, viruses, which are deadly or pathogenic even in ultra-low quantities [57,58]. The main problem of current amplitude-sensitive SPR technology consists in the existence of a physical limit of detection (LOD). This limit is defined by the level of noises in measurements and normally is estimated as 10⁻⁶…10⁻⁵ Refractive Index Units (RIU) for various sensor implementations with angular, spectral or intensity interrogations [59]. In 2014, the firm Biacore reported the LOD result close to the value 10 RIU, in 2016 – to the value 3 × 10⁻⁵ RIU (Biacore T200), and the firm Reichert reported of its achievement in the sensitivity 1 × 10⁻⁷ RIU.

Our analysis of literature data for the latter 25 years enabled to ascertain the tendency of development of SPR-devices with regard to enhancing their sensitivity and lowering their detection limit. Starting from 1990, the detection limit for the analyte concentration in buffer was lowered by 160 times from 8 ng/ml down to 50 pg/ml (Fig. 2a) [60]. Also increased is the number of publications (Fig. 2b). With account of the growth in the number of publications devoted to improvement and application of SPR-devices, one can draw a conclusion that this direction of scientific researches is topical and promising.

![Figure 2](image_url): Advancement in the analyte detection limit (a), and growth of the quantity of publications in the field of SPR sensors (b) for the last 25 years [60].

Being based on the above analysis, it can be expected that the detection limit will reach the range 3…10 pg/ml for the period 2020-2025 years. In future, decreasing the detection limit will be limited by availability of thermal noises and noises caused by friction of liquid flow in a cell, as well as technological possibilities in preparation of analytes. In most cases, enhancement of the sensitivity
is related with increasing the electromagnetic field at the surface of a sensitive element. The detection limit value can be considerably reduced by using the systems with a high value of the ratio signal-to-noise, namely: interferometry, ellipsometry or polarimetry [61-63]. Leading firms producing SPR-devices as well as scientific community improve both technology and design of SPR-devices to increase their sensitivity and accuracy in measurements.

Experimental researches of applied aspects in construction of biosensors based on SPR phenomenon at the V. Lashkaryov Institute of Semiconductor Physics of the National Academy of Sciences of Ukraine (ISP NASU) were initiated in the early 90's. Constructively completed model of the SPR refractometer (Plasmon-3), which is suitable for use in biochemical and biophysical laboratories, was developed at the end of the nineties (1998). Highly qualified physicists, programmers and designers were involved in this project, which in the future formed the core of the research and development group, which continued the further development of SPR refractometry and its applications in scientific researches. The biosensor "Plasmon-5" was first serially capable one-channel biosensor based on surface plasmon resonance, which was developed at the ISP NASU. Later it was created double-channel refractometer "Plasmon-6" (2004). The device was created during the work of the model series “Plasmon", which were successfully used in many laboratories in Ukraine and abroad. Today, developed in ISP NASU have been the dual-channel refractometers "Plasmon-6", "Plasmon-7", "Plasmon-71", "Plasmon-9" and eight-channel device "Plasmon-8". One of the designed models “Plasmon-71” allows one to measure the refraction index over a wide range from 1 to 1.5 RIU (refractive index unit) with the detection limit ±5×10⁻⁶ RIU and accuracy ±2×10⁻⁷ RIU [64]. All devices possess the supplementary electronic channel for recording data from external equipment (like potentiostat in electrochemical studies) synchronously with the SPR data. Accessories also include thermostabilized flow-cell (up to 95 °C), syringe and peristaltic pump. The “Plasmon” series are versatile measuring devices designed for application in various industries and fields of researches, running with PCs and having complex and versatile software. The developed software allows maximum use of the capabilities of devices when working in laboratory conditions. Versions of the device can be capable of long-term autonomous operation, which provides the ability to work in the field conditions. Devices may have an autonomous power supply and specialized built-in software aimed at solving specific tasks. The most significant advantage of “Plasmon" devices are small dimensions, low weight (less than 3 kg), block-modular design, which makes it easy to update the device for various applications and possibility to investigate gas-like media.

Features trends in SPR technique

Overwhelming majority of sensitive elements is located on a glass substrate, but it seems more promising to use substrates made of polymers [65]. To lower mutual influence of the sensor surface and analyte, as well as being aimed at extension of the sensor exploitation term, the sensitive element is additionally covered with an inert protective layer that can also play the role of citop in the sensors with long-range surface plasmons [66-68]. For surface immobilization of biomolecules onto the sensing metal layer, self-assembled monolayers (SAMs) of alkanethiols or disulfides have been widely used [69]. The formed S-Au linkage is stable in air and water, but it decomposes under UV irradiation and at temperatures above 70 °C. Moreover, although dense monolayers assemble quickly, well-ordered monolayers can take days to form [70]. Therefore, the existing approaches are time-consuming and produce layers with low stability at elevated temperature and under UV irradiation. One of the most suitable candidates to substitute SAMs for bio sensor surface modification is the plasma polymerization of ultrathin functional films (thickness of 5…20 nm). These thin layers should not degrade the SPR formation on the one hand and enable efficient grafting of COOH, NH₂, anhydride or other reactive groups. Nowadays, plasma polymers were already employed for the biomolecular immobilization and cell adhesion improvement [71]. Compared to SAMs, these coatings will be stable in a wider range of conditions, are independent of the substrate material and will be deposited significantly faster and without use of solvents. Then, on the plasma-coated Au surface the antibody can be immobilized and the SPR biosensor will be able to detect the analyte. Tested in the work were the (plasma polymer)-treated Au SPR chips for stability in different buffers, the sensory element with self-assembled monolayer was used for comparison [72]. The tested sensor is slightly unstable in time. It seems that the layer is partially degraded or the non-reacted monomer/oligomer molecules became gradually washed out. Considering very similar behavior in the reference flow channel, one can conclude that the polymer can be stable in all tested buffers, and the gradual loss of signal level is not dependent on pH within the tested interval.

Amongst nano structured gas sensing systems, nano-carbon based materials proved to be promising due to their intrinsic electrical properties that are highly sensitive to the changes in chemical environment. Further, the high surface area, high chemical and thermal stability and functionalization capability of carbon-based nanostructures make them suitable for high performance label free chemical sensing. Recently, graphene, a carbon allotrope has attracted a great deal of interest due to its extraordinary electronic, chemical, mechanical, thermal and optical properties. Graphene materials are used as sensing materials due to its high specific surface area and unique electrical properties such as high mobility and low electrical noise. Wide range of chemicals, biomolecules and gases/vapors has been detected using graphene based sensors [73].

Conclusion

Adduced in this paper are statistical data, in accord with which the number of publications in scientific journals devoted to the SPR sensor topic is permanently increased by the exponential law and to the end of 2015 reached approximately 6,000. It is indicative of topicality of further investigations aimed at development and creation of new analytical devices based on the SPR principle. Considered in the paper are the main directions to develop technology and construction of the available SPR sensors to increase their sensitivity and accuracy of measurements. It has been shown that lowering the roughness of the plasmon carrying layer and application of an additional dielectric layer with developed surface enables to increase more than two-fold the sensitivity due to increased surface of interaction between the sensitive element and studied substance.

The main technical way to lower the surface roughness is thermal annealing, and the best result can be reached at the annealing temperature 120 °C. The promising technological way to enhance the accuracy and sensitivity of measurements is to narrow the reflection characteristic R(θ), which can be realized by decreasing the roughness of the SE metal layer due to changing the geometry of mutual arrangement of the substrate and evaporator. It was
The presence of this additional dielectric layer enables to lower the detection limit when measuring the changes in refraction index of the studied substance down to the level $1.10^{-8}$, which corresponds to binding the complementary pair antigene–antibody with the concentration of antigene $1$ fg/mL. This value of detection limit is by one order lower than that in available commercial analytical devices based on the SPR phenomenon (for instance, $3.10^{-8}$, which is typical for commercial Biacore T200). Considered in this review are promising directions for development of sensorics based on SPR, such as application of multilayer graphene coatings and polymer layers prepared using polymerization in high-frequency plasma of inert gas.

In our opinion, further development of SPR sensors will be directed to increasing their selectivity, wear resistance of the sensitive element surface, as well as to the methods of receptor regeneration for multiple using the sensitive elements.

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