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(54) **BIOLOGICAL SENSOR AND A METHOD OF THE PRODUCTION OF BIOLOGICAL SENSOR**

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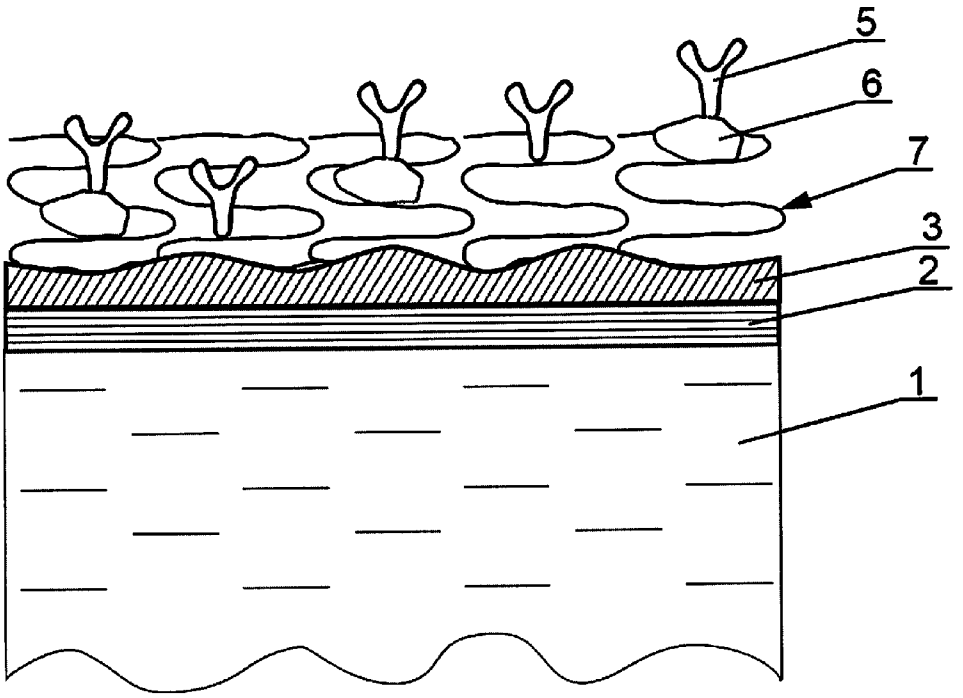
CPC ***G01N 33/553*** (2013.01); ***C23C 14/30*** (2013.01); ***G01N 33/54373*** (2013.01)

(57) **ABSTRACT**

The invention is related to the field of biotechnology, specifically to the investigation of biomolecular interactions and sensing of biomolecules using a surface plasmon resonance. The biological sensor and a method of its production based on the thin films of graphene, graphene oxide, or single-walled or multi-walled carbon nanotubes are described.

The technical results of the invention are a high sensitivity of the biosensor in combination with a high biospecificity; an expansion of the range of device applications; the protection of the metal film from an environmental exposure; the possibility to detect large biological objects.

The proposed device and method of its production can be used for monitoring and recording of the concentration of chemical and biochemical substances and for the definition of parameters of biomolecular reactions in different industrial processes using biological materials, the invention can be also used in the pharmaceutical industry for the investigation of pharmacological properties and for the determination of a chemical composition of developing drugs, and also it can be used in processes of quality control of food products.



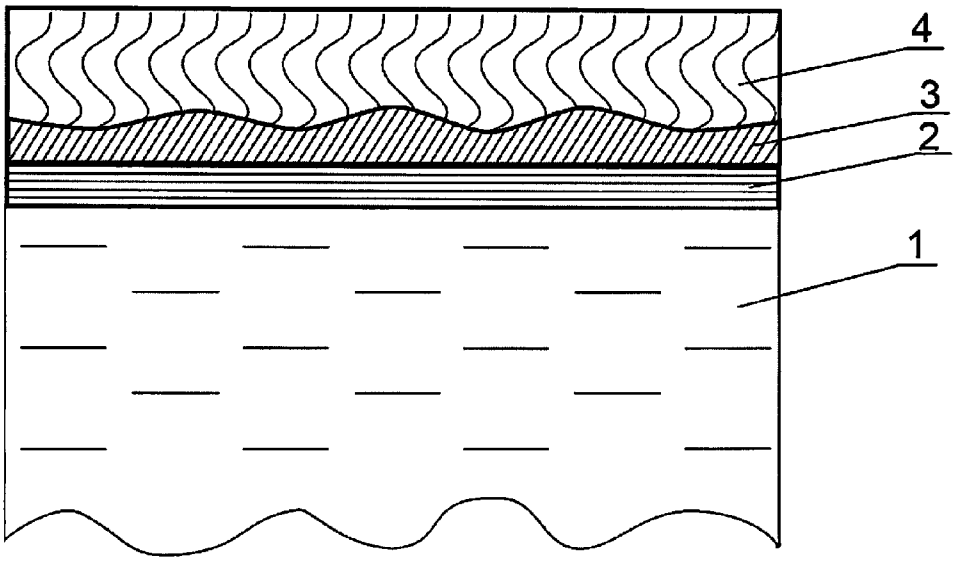


Fig. 1.

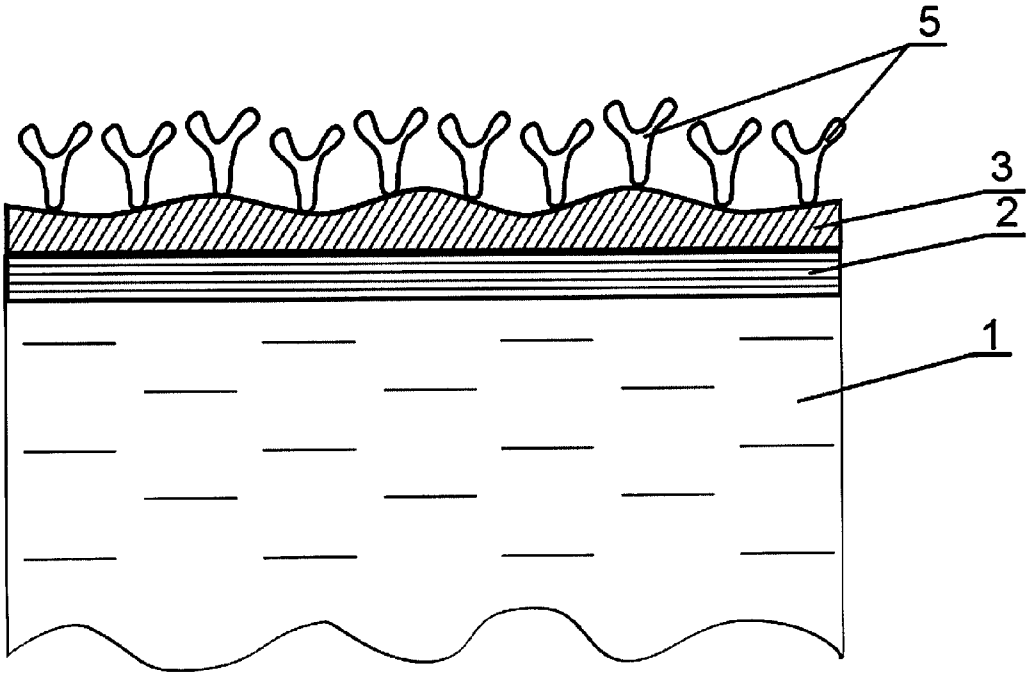


Fig. 2.

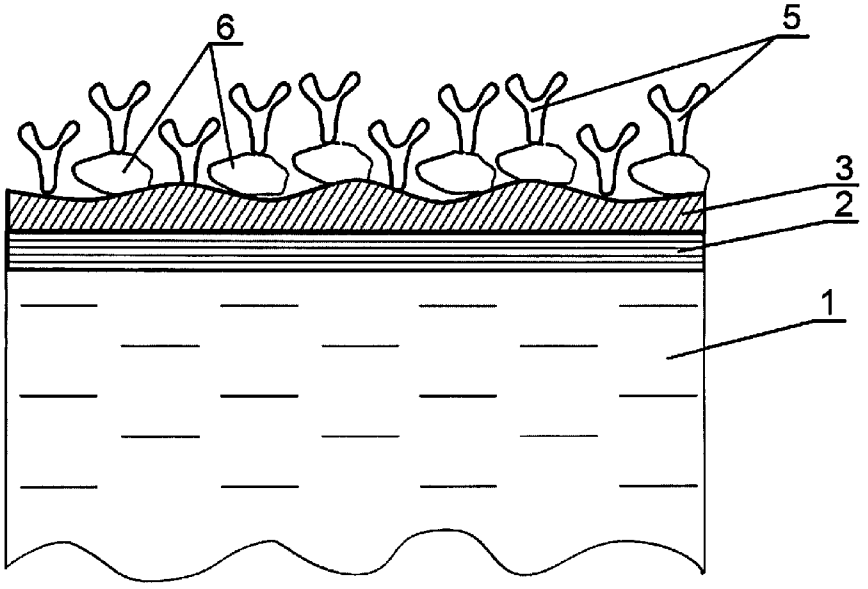


Fig. 3.

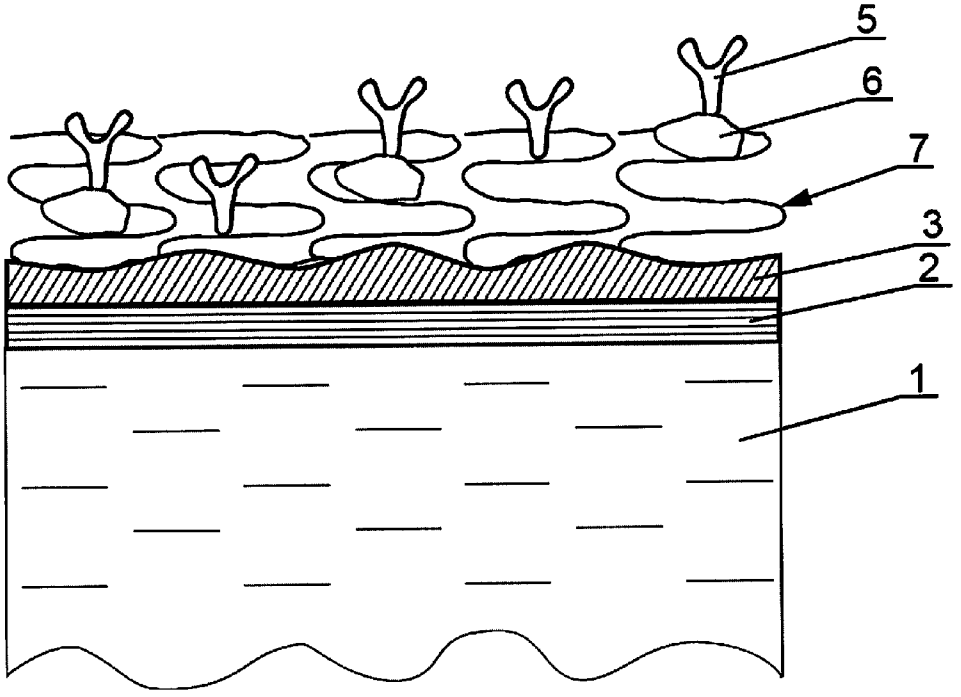


Fig. 4.

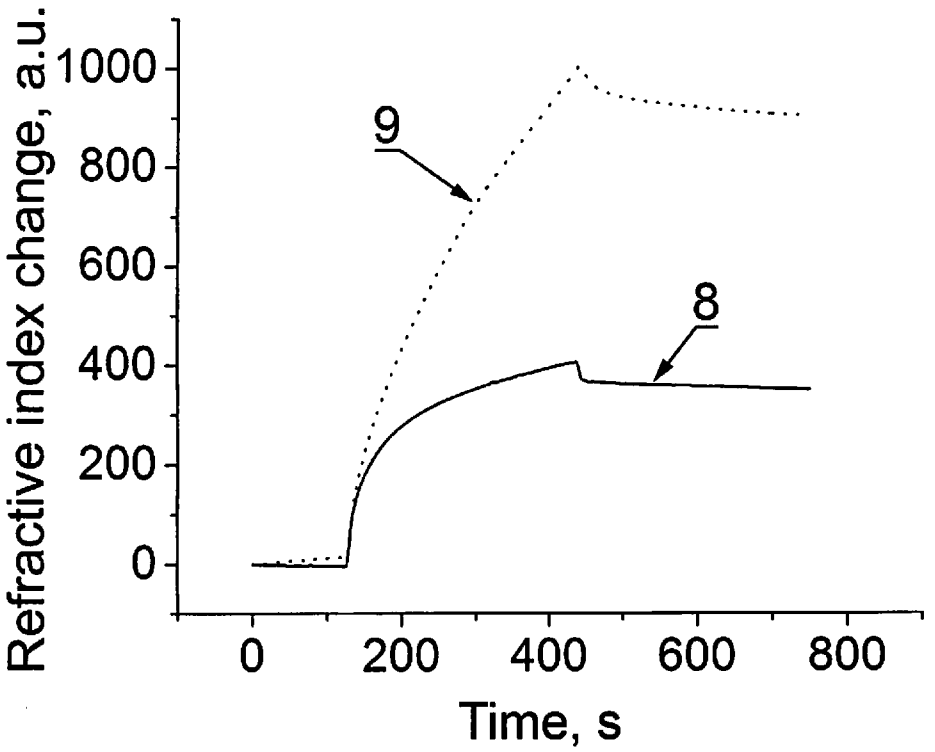

Refractive index change, a.u.
1000
800
600
400
200
0
9
8
0
200
400
600
800
Time, s

Fig. 5.

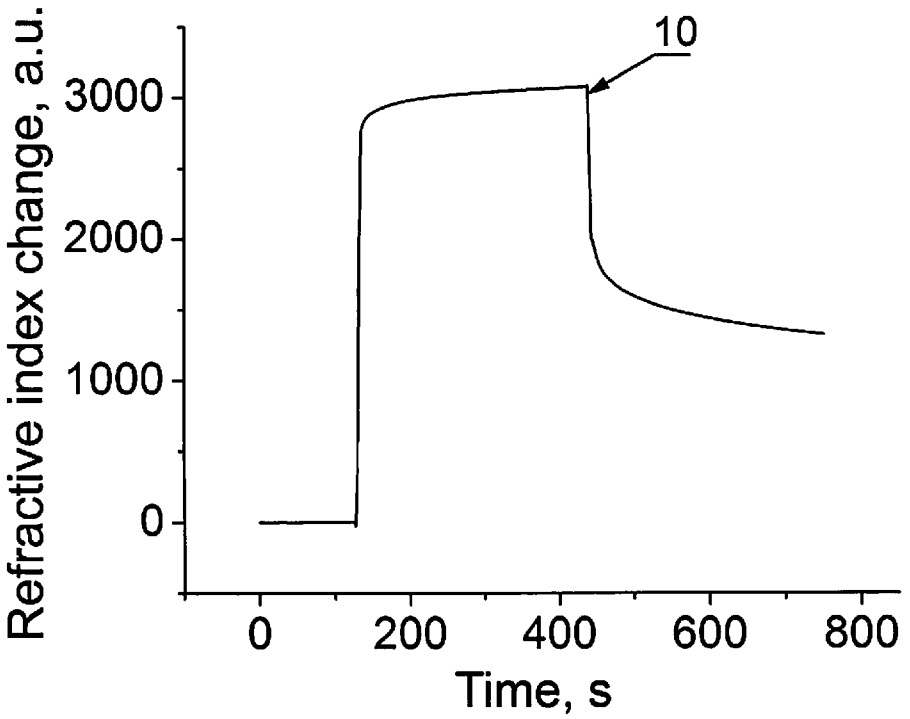

Refractive index change, a.u.
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2000
1000
0
0
200
400
600
800
Time, s
10

Fig. 6

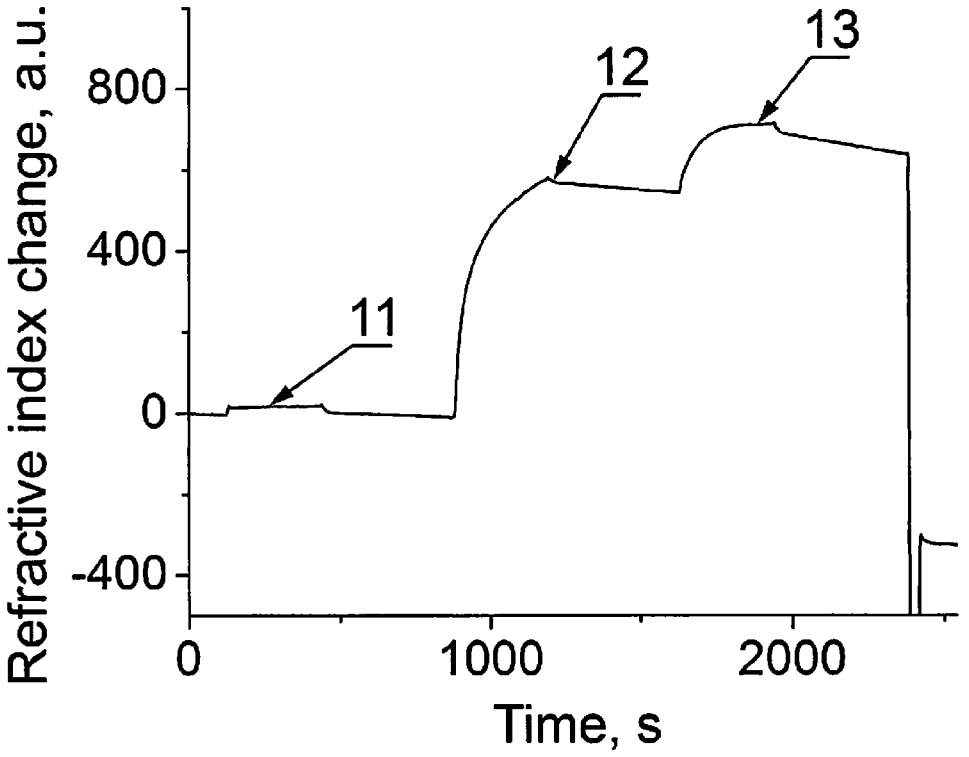

Refractive index change, a.u.
800
400
0
-400
0
1000
2000
Time, s
11
12
13

Fig. 7

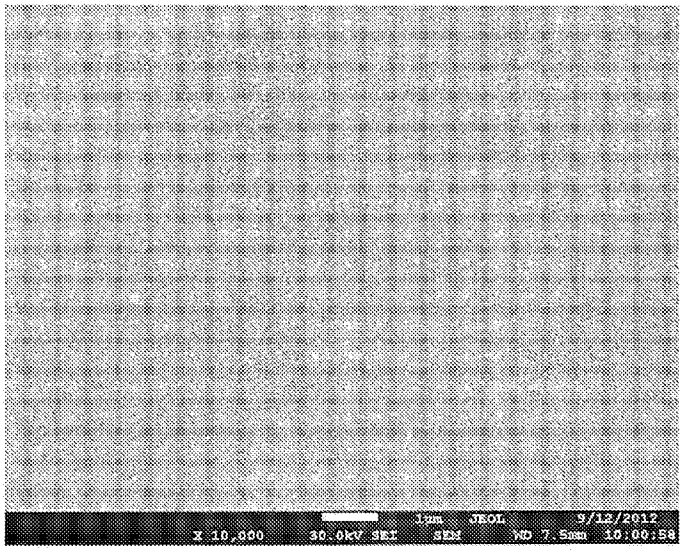


Fig. 8

Comparison of the biological sensors comprising the film of hydrogel and the film of graphene oxide as an intermediate binding layer		
Substrate	Borosilicate glass (thickness of 0.4 mm) with deposited thin film of titan (thickness of 2 nm)	Borosilicate glass (thickness of 0.4 mm) with deposited thin film of titan (thickness of 2 nm)
Metal film	Gold (thickness of 40 nm)	Gold (thickness of 40 nm)
Intermediate binding layer	Hydrogel (thickness of 150 nm)	Graphene oxide (thickness of 20 nm)
Biospecific layer	Streptavidin	Streptavidin
Analyte	Biotinylated DNA	Biotinylated DNA
Response, a.u.	409	570

Fig. 9

BIOLOGICAL SENSOR AND A METHOD OF THE PRODUCTION OF BIOLOGICAL SENSOR

TECHNICAL FIELD

[0001] The invention relates to the field of biotechnology, namely to the devices for the investigation of biomolecular interactions and for the sensing of biomolecules using a surface plasmon resonance and to the methods of their production. Surface plasmon resonance is a phenomenon of excitation of surface plasmons under the influence of light. It occurs near the metal surface under the condition of attenuated total reflection. The term of "surface plasmon resonance" is related to the optical phenomena allowing to analyze interactions in real time sensing the properties of analyzing media on a matrix and their changes.

[0002] The method of biosensing using surface plasmon resonance have several advantages comparing to existing methods such as label-free biosensing without using of radioactive and fluorescent labels, and makes possible to gain a high sensitivity of biosensors based on this method and a high rate of conducted measurements. The proposed invention is related to the devises with sensing surfaces for chemical reactions.

DESCRIPTION OF PRIOR ART

[0003] Several technical solutions are known from the prior art.

[0004] For example the biological chips for biosensor manufacturing and analysis of biological interactions are known according to U.S. Pat. No. 5,242,828 and consists of three layers: a substrate, a metal film, and a monolayer of biomolecules for adsorbing of a binding partner of an analyte. These biological chips can be used for biosensors based on a surface plasmon resonance. Used in this case biomolecules have the special structure. The limitation of this method is a low number of active centers for biomolecule adsorption due to the planar structure of the biolayer. Also the limitation is the complexity of this device production due to low availability of necessary biomolecules on the market, and processes of their synthesis include many steps and need many reagents. Also the limitation is complexity of biosensors construction based on these devices because for adsorption molecules of a binding partner of an analyte must possess specific functional groups, therefore in every case methods of activation must be developed that limits the class of analyzing molecules.

[0005] Moreover, biological sensor according to patent GB 2459604 is known and consists of the following layers: a substrate, a metal film, a film based on amorphous carbon, and a layer of biomolecules. This biosensor allows realizing photolithographic process of molecule organizing. The patent also includes the method of biosensing using this devise and the method of its creation. The limitation of this device is a decrease in sensitivity due to the influence of the carbon film on electromagnetic properties of surface plasmon waves and their adsorption. Also the primary method of binding of biomolecules with films of amorphous carbon is the formation of C—C chemical bonds due to absence of a crystal lattice that limits available for analysis biomolecules and requires development of activation methods for every case.

[0006] Also biosensor is known from a prior art according to EP 2216642 A1, which consists of the metal layer with embedded diamond particles. The limitations of this device are complexity of its manufacturing due to usage of complex composite structures and reduction of sensitivity due to low surface area for biomolecule adsorption defined by area of opened diamond particles.

[0007] Moreover, multilayer structure is known according to the article "Graphene-based high-performance surface plasmon resonance biosensors" and consists of a metal film covered with a thin layer of graphene. This structure allows to investigate the reaction between biological molecules and graphene, however it is not possess the property of bioselectivity, that makes it unsuitable for the investigation of chemical reactions. In this article graphene film is used as an external surface which interacts with all types of biomolecules in a solution.

[0008] The device described in the patent U.S. Pat. No. 5,763,191 is chosen as a prototype of the proposed invention. This is the universal binding film which is used for analysis of specific biological interactions and consists of a metal film or a film based on metal oxide, and a layer of biological reagent attached to the surface of metal or metal oxide by thiol, disulfide, or phosphine groups of the binding molecule. This biological layer is capable chemically interact with other biological molecules and it can be used for manufacturing of biological sensors based on a surface plasmon resonance. Also this patent includes the method of the analysis using the considered device and the method of its manufacturing.

[0009] The limitation of the prototype is the complexity of manufacturing of the layer of biomolecules requiring the synthesis of compounds comprising necessary functional groups and capable to attach to gold surface. Also the limitation is the complexity of attachment of molecules of binding partner of analyte to this layer requiring the development of special methods of activation with focus on the reaction through certain functional groups. This implies that the activation method will work only with certain class of analytes that limits possible applications of the device. Besides, the surface of the metal film of the proposed device is exposed to the influence of an external environment that imposes restrictions on work conditions and chemical reagents used in biosensing. All these limitations cannot provide a high sensitivity together with a specificity of biosensing.

SUMMARY OF THE INVENTION

[0010] Technical problem which is solving in the present invention is the creation of a highly sensitive and universal biological sensor with high specificity for biosensing based on a surface plasmon resonance.

[0011] This technical problem is solved by the biological sensor (FIG. **1-4**) for use in biosensing based on surface plasmon resonance. The biosensor consists of the multilayer structure, which includes a substrate (**1**), that covered with a thin metal film (**2**), on the external surface of which an intermediate binding layer (**3**) is deposited. The intermediate binding layer (**3**) is performed from the thin film of graphene with the thickness of 0.3-2000 nm, or the thin film of single-walled or multi-walled carbon nanotubes with the thickness of 0.4-2000 nm, or the thin film of graphene oxide with the thickness of 0.7-2000 nm. The biospecific layer (**4**) is deposited conformally and homogeneously on the surface of the intermediate binding layer (**3**). The biospecific layer (**4**) is capable of specific chemical reacting with a certain type of biological molecules of an analyte.

[0012] The metal film may be produced from such metals as gold, silver, copper, and aluminum, and its thickness can be

equal 10-150 nm. The biospecific layer (**4**) may contain molecules of a binding partner of an analyte (**5**). Also the biospecific layer (**4**) may contain molecules of a binding partner of an analyte (**5**) and molecules with a high affinity to a binding partner of an analyte (**7**) and forming a chemical bond with them. Moreover, the biospecific layer may contain the hydrogel (**7**) with pre-immobilized molecules of a binding partner of an analyte (**5**). Also the biospecific layer can contain the hydrogel (**7**) with pre-immobilized molecules of a binding partner of an analyte (**5**) and the molecules with a high affinity to a binding partner of an analyte (**7**) and forming a chemical bond with them. The hydrogel of the biospecific layer (**4**) can be a polysaccharide. The polysaccharides can consist of agarose, alginic acid, dextran, carrageenan, starch, cellulose or derivatives thereof. The derivatives of dextran in the biospecific layer can consist of for example a carboxymethylated dextran. Also the molecules with a high affinity to the molecules of a binding partner of an analyte in the biospecific layer can contain avidin, streptavidin, and deglycosylated avidin, in this case the molecules of a binding partner are biotinylated. The pairs of an analyte and a binding partner to it can consist of the pairs of receptor-ligand, antigen-antibody, enzyme-substrate. The binding parent of an analyte may be an antibody, and a fragment of an antibody to an analyte, and a receptor of an analyte. Moreover, the binding partner of an analyte can be the binding partner of proteins, lipids, DNAs, RNAs, viruses, cells, bacterias, and toxins, and also the modifications of these substances.

[0013] The usage in the proposed device of the thin films of graphene, graphene oxide, single-walled and multi-walled carbon nanotubes performing the function of an intermediate binding layer allows the adsorption of a large class of biological molecules, that makes possible the usage of the considered devise for different applications and protects the metal surface from harmful effects of the environment. So in biosensing reagents which can damage the surface of a metal can be used, and also such plasmonic materials as silver can be used.

[0014] The inventive method of the production of the biosensor is that the method comprises the following steps:

[0015] a) the step of deposition of the metal film (**2**) on the substrate (**1**);

[0016] b) the step of applying to the outer surface of the metal film of the intermediate binding layer (**3**) performed as the thin film of graphene with the thickness of 0.3-2000 nm, or the thin film of single-walled or multi-walled carbon nanotubes with the thickness of 0.4-200 nm, or the thin film of graphene oxide with the thickness of 0.7-2000 nm;

[0017] c) the step of the biospecific layer (**4**) deposition, which is conformally and homogeneously adsorbed on the surface of the intermediate binding layer (**3**) due to the chemical interaction between molecules of the intermediate binding layer (**3**) and the molecules of the biospecific layer (**4**). This interaction is due to the stacking interaction or the interaction between the molecules of the biospecific layer (**4**) with the functional groups of graphene, single-walled or multi-walled carbon nanotubes, or graphene oxide, wherein during the adsorption a large number of the adsorption centers is created on the surface of the intermediate binding layer (**3**) with the 15-100% degree of filling of the area of the intermediate binding layer by the molecules of the biospecific layer.

[0018] The metal film (**2**) can be the film of gold, silver, copper, or aluminum with the thickness of 10-150 nm.

[0019] The biospecific layer (**4**) can consist of the layer of the molecules of a binding partner of an analyte.

[0020] Also the biospecific layer (**4**) can consist of the layer of the molecules of a binding partner of an analyte and the molecules with a high affinity to the molecules of a binding partner of an analyte and forming a chemical bond with them.

[0021] Also the biospecific layer (**4**) can consist of the layer of the hydrogel (**7**) with immobilized molecules of a binding partner of an analyte (**5**).

[0022] Also the biospecific layer (**4**) can consist of the layer of the hydrogel (**7**) with immobilized molecules of a binding partner of an analyte and the molecules with a high affinity to the molecules of a binding partner of an analyte and forming a chemical bond with them.

[0023] Also polysaccharides is appropriate to use as the hydrogel (**7**). Agarose, alginic acid, dextran, carrageenan, starch, cellulose or derivatives thereof is preferable to use as hydrogel (**7**). As derivatives of dextran the biospecific layer can contain a carboxymethylated dextran. As the molecules with a high affinity to the molecules of a binding partner of an analyte the molecules of avidin, streptavidin, and deglycosylated avidin can be deposited, in this case the molecules of a binding partner are biotinylated.

[0024] The interaction of the molecules of the biospecific layer (**4**) with the functional groups of graphene, single- or multi-walled carbon nanotubes, or graphene oxide can be performed by the interaction with the functional groups such as epoxy, hydroxyl, carbonyl or carboxyl groups. As a binding partner of an analyte an antibody, an antibody fragment to an analyte, or an analyte receptor can be used. Furthermore, as the binding partner of a analyte can consist of the binding partner of proteins, lipids, DNA, RNA, viruses, cells, bacteria or toxins, as well as chemical modifications of the above substances.

LIST OF FIGURES

[0025] On FIG. **1** the general view if the biological sensor (side view) is shown.

[0026] On FIG. **2** the biological sensor with the biospecific layer (**4**) containing the molecules of a binding partner of an analyte (**5**) is shown.

[0027] On FIG. **3** the biological sensor with the biospecific layer (**4**) containing the molecules of a binding partner of an analyte (**5**) and the molecules capable of forming a chemical bond with the molecules of a binding partner (**6**) is shown.

[0028] On FIG. **4** the biological sensor with the biospecific layer (**4**) containing the hydrogel with the immobilized molecules of a binding partner of an analyte (**5**) and the molecules capable of forming chemical a bond with the molecules of a binding partner (**6**) is shown.

[0029] On FIG. **5** the kinetic curve of adsorption of the biotinylated oligonucleotide molecules adsorption on the surface of the thin film of graphene oxide and on the surface of biological sensor comprising of three layers: the substrate, the metal film, and the carboxymethylated dextran with the immobilized molecules of streptavidin is shown.

[0030] On FIG. **6** the kinetic curve of adsorption of the molecules capable of forming a chemical bond with the molecules of a binding partner of an analyte on the biological sensor based on the thin film of graphene oxide is shown.

[0031] On FIG. **7** the kinetic curve of adsorption of the oligonucleotides on the surface of the biospecific layer with the immobilized molecules of streptavidin is shown.

[0032] On FIG. **8** the raster electronic microscopy image of the thin film of graphene oxide deposited on the surface of the metal film is shown.

[0033] On FIG. **9** the comparative table of experimental data obtained by the biological sensors containing as the intermediate binding layer thin film of the hydrogel and the thin film of graphene oxide is shown.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0034] The biological sensor (FIG. **1**) consists of a substrate (**1**), a metal film (**2**), which is covered by the intermediate binding layer (**3**) made of the thin film of graphene, the thin film of graphene oxide, or the thin film of carbon nanotubes. The biospecific layer (**4**) is conformally and homogeneously adsorbed on the surface of layer (**3**). The layer of the molecules of a binding partner of an analyte (**5**) (FIG. **2**) or the layer of the complex of the molecules capable chemically bind with the molecules of a binding partner of an analyte and chemically bond with them (FIG. **3**) can be used as the biospecific layer. Also the hydrogel (**7**) (FIG. **4**) with the immobilised molecules of the molecules of a binding partner of an analyte (**5**) and/or the complex of the molecules of a binding partner of an analyte and the molecules capable of chemically bind with them (**6**) can be used as the biospecific layer. FIG. **5** shows the kinetic curve of adsorption of the biotinylated oligonucleotides on the surface of the intermediate binding layer of the biosensor based on the thin film of graphene oxide (curve **8**) and on the surface of biological sensor consisting the following layers: the substrate, the metal film, and the biospecific layer with the hydrogel (carboxymethylated dextran) and streptavidin molecules (curve **9**). The horizontal axis is time, the vertical axis is the change of the refractive index of the medium near the adsorption surface, which is proportional to the mass of molecules adsorbed on the surface. Therefore, we can conclude that the film based on graphene oxide has better adsorption properties than the layers containing hydrogel. FIG. **6** shows the graph of streptavidin molecule adsorption on the biological sensor based on the thin film of graphene oxide.

[0035] FIG. **7** shows the graph of oligonucleotide adsorption on the biological sensor comprising the substrate made of the borosilicate glass with the thickness of 0.4 nm which surface is covered by the titan film with the thickness of 2 nm. The substrate is covered by the gold film with the thickness of 40 nm. The intermediate binding layer of graphene oxide with the thickness of 20 nm and the biospecific layer are deposited on the gold film. The biospecific layer consists of streptavidin molecules, which form a stable complex with the molecules having a biotin residue. Streptavidin was adsorbed during 10 minutes from the solution with the concentration of 50 ug/ml on the surface of the intermediate binding layer in the flow cell. Three peaks on graph correspond to the adsorption of oligonucleotides: **11**, **13**—without biotin residue, **12**—with biotin residue. Oligonucleotides used in the cases **11**, **13** and in the case **12** are complimentary and can form a bind with each other. Smallness of the peak **11** indicates a high specificity of the obtained biological sensor, which means that the biological sensor interacts only with certain types of molecules. FIG. **8** shows the image of the graphene oxide layer on the surface of the metal film, obtained using raster electron microscopy. The data in table (FIG. **9**) are based on the experimental results and compares biological sensors comprising the thin layer of hydrogel with the thickness of 150 nm and the thin layer of graphene oxide with the thickness of 20 nm as intermediate binding layers. The signal of the biological sensor comprising film of the hydrogel obtained during the sensing of biotinylated DNA and which is proportional to the change of the refractive index of the media near the surface of the biological sensor is 409 arbitrary units. In the case of the biological sensor comprising the film of graphene oxide the signal is 570 arbitrary units. Thus, the response and, therefore, the sensitivity of the biological sensor comprising the thin film of graphene oxide as the intermediate binding layer is 40% higher.

[0036] The device operates as follows. The solution of an analyte is supplied to the biospecific layer (**4**) of the biological sensor by means of a flow cell or a cuvette. Wherein, the chemical reaction is carried out between an analyte and the molecules of the biospecific layer (**4**) represented by the molecules of a binding partner of an analyte (**5**) attached to the surface of the intermediate binding layer directly or using the biological molecules (**6**) capable to form a chemical bond with the molecules of a binding partner of an analyte and/or the hydrogel (**7**) deposited on the surface of the biological sensor. Further, required parameters of this reaction are obtained using the method of biosensing based on a surface plasmon resonance. The essence of the method is to detect in various ways the changes of the resonant conditions of the surface plasmon excitation in the metal layer (**2**) caused by the changes of the effective refractive index of the media near the surface due to attaching of biomolecules. The most popular in commercial devices way of the surface plasmon excitation is proposed by Kretschmann [6]. According to this, a laser beam is falling under certain angle on the metal film (**1**) from the substrate side (**1**) and excites surface plasmons on the border of the metal film (**2**) and the media containing analyte. Wherein the optimal thickness of metal film (**2**) is in the range of 10-150 nm. The upper border is explained by the fact, that at higher values of the film thickness the failure in reflection is small, which greatly affects the sensitivity of the method. At the thicknesses of the film (**2**) less than 10 nm the form of the resonant curve corresponding to the surface plasmon resonance changes due to the change of the waveguide mode of the surface plasmon. Further, the information about the refractive index change of the media near the metal film is obtained basing on the value of the resonant angle, phase shift of the reflected beam, or the changes of the intensity of the reflected beam. It does not make sense to deposit the intermediate binding layer (**3**) with the thickness greater than 2000 nm on the surface of the metal film (**2**) because of the penetration depth of the electromagnetic field of the surface plasmon is about 500 nm, therefore, molecules located at a distance greater than 2000 nm have little effect on conditions of a surface plasmon excitation and hence it cannot be detected. The intermediate binding layer (**3**) with the thickness greater than 2000 nm, in turn, hinders the access of the analyte in the region, where it can be detected. The minimal thickness of the intermediate binding layer comprising graphene corresponds to the monomolecular layer which the thickness is assumed to be equal 0.3 nm [7]. For the intermediate binding layer (**3**) comprising graphene oxide the minimum possible thickness corresponded to the monomolecular layer equals 0.7 nm [8]. For the intermediate binding layer (**3**) comprising carbon nanotubes the minimum possible thickness equals the diameter of carbon nanotubes which can be equal to 0.4 nm [9]. Molecules of proteins, lipids, DNA,

RNA, viruses, cells, bacterias, and toxins can be used as analytes for the biological sensor.

[0037] The method of production of the biological sensor is realized as following:

[0038] The metal film (**2**) is deposited on the substrate (**1**) using for example electron beam deposition. So, for example, to deposit gold film with the thickness of 40 nm as a substrate the plate of borosilicate glass with the deposited titan film with the thickness of 2 nm is used. Further deposition of gold on the substrate is conducted in the vacuum chamber at the pressure of 10^{-7} Torr, the accelerating voltage of electrons of 4 kV, and the temperature of 150 degrees Celsius. The thickness and optical properties of the gold film are controlled by means of ellipsometric measurements.

[0039] Further the intermediate binding layer (**3**) in the form of the thin film of graphene, graphene oxide, or single-walled or multi-walled carbon nanotubes is deposited on the surface of the metal film (the image of the graphene oxide film obtained using rater electron microscopy is shown on FIG. **8**). A thin film of graphene, graphene oxide, or single-walled or multi-walled carbon nanotubes are deposited using the solution of the respective substance, which is further filtrated by the cellulose membrane. After the filtration process the membrane is placed on the surface of the metal film and dissolved in acetone leaving the thin film of graphene, graphene oxide, or carbon nanotubes. So for example for the deposition of the intermediate binding layer containing the thin film of graphene oxide with the thickness of 20 nm 1 ml of graphene oxide solution in water with the concentration of 5 ug/ml is used.

[0040] The next step of the biological sensor creation is the stage of biospecific layer (**4**) deposition on the intermediate binding layer in which such molecules comprising the biospecific layer as molecules of the partner of an analyte (**5**), the molecules capable chemically bind with the molecules of the partner of an analyte (**6**), or the hydrogel are deposited directly from the solution. The solution with biomolecules is brought in a contact with for example a flow cell or a cuvette. FIG. **6** shows the adsorption of the streptavidin molecules which are the binding partner of the molecules with the biotin residue using a flow cell. At the same moment a time of adsorption is selected so that biological molecules occupy large number of adsorption centers on the surface of graphene, graphene oxide, or carbon nanotubes eliminating in further nonspecific binding of analyte molecules with the surface of the biological sensor. Wherein usage of special substances except molecules themselves are not required for manufacturing of such films. So for example for adsorption of the biospecific layer containing streptavidin molecules on the surface of graphene oxide film these molecules are adsorpted from the solution with the concentration of 10 ug/ml using the flow cell during 10 minutes. Subsequently the quality can be checked by using a test sample, which is known that molecules from its structure should not interact with the obtained biological layer. The kinetic curve (**12**) of biotinylated DNA deposition on the obtained biosensor comprising streptavidin molecules is shown on the FIG. **7**. The smallness of the peak (**11**) reflecting the interaction of the nonbiotinylated molecules with the streptavidin layer shows a sufficient level of negligibility of nonspecific interactions.

[0041] The proposed device and method of its production provide in comparison with the known level of technique the following results: a high sensitivity of biosensor in combination with a high biospecificity; the protection of metal film from an environmental exposure that allows to use in the biosensing reagents that may damage the metal surface, and also to use such plasmonic materials as silver easily degrading under an environmental exposure; the possibility to detect large biological objects.

[0042] Thus the new relationship of known properties and a set of distinctive properties of the proposed biosensor and method of its creation allows creation of a highly sensitive and universal biological sensor for the biosensing based on the surface plasmon resonance.

[0043] The proposed device and a method of its production can be used for monitoring and recording of the concentration of chemical and biochemical substances and for the definition of parameters of biomolecular reactions in different industrial processes using biological materials.

[0044] The proposed invention can be also used in the pharmaceutical industry for the investigation of pharmacological properties and for the determination of a chemical composition of developing drugs.

[0045] Moreover, the developed device and a method of its production can be used in processes of a quality control of food products.

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1. A biological sensor comprising:

a substrate, wherein a metal film is deposited on a surface of the substrate, wherein an intermediate binding layer with a biospecific layer adsorbed on its surface is located on an outer surface of the metal film, wherein the intermediate binding layer is composed of a thin film of graphene with a thickness of 0.3-2000 nm, or a thin film of single-walled or multi-walled carbon nanotubes with a thickness of 0.4-2000 nm, or a thin film of graphene oxide with a thickness of 0.7-2000 nm and

wherein the biospecific layer is located conformally and homogeneously on the surface of the intermediate binding layer and is adapted for a specific chemical interaction with biological molecules to be analyzed.

2. The biological sensor of claim **1**, wherein a metal film is the film made of gold, silver, copper, or aluminum with a thickness of 10-150 nm.

3. The biological sensor of claim **1**, wherein the biospecific layer comprises molecules of a binding partner of an analyte.

4. The biological sensor of claim **3**, wherein the biospecific layer additionally comprises: (i) molecules which have a high affinity to the molecules of a binding partner of an analyte and which form a chemical bond with them; or (ii) a hydrogel having immobilized therein the molecules of a binding partner of an analyte; or (iii) a hydrogel having immobilized therein both the molecules of a binding partner of an analyte and the molecules which have a high affinity to the molecules of a binding partner of an analyte and which form a chemical bond with them.

5-6. (canceled)

7. The biological sensor of claim **4**, wherein, in (ii) or (iii), the hydrogel in the biospecific layer is comprises polysaccharides.

8. The biological sensor of claim **7**, wherein the polysaccharides, in the biospecific layer comprises agarose, alginic acid, dextran, carrageenan, starch, cellulose or derivatives thereof.

9. The biological sensor of claim **8** wherein the derivatives of dextran in the biospecific layer comprises carboxymethylated dextran.

10. The biological sensor of claim **4**, wherein the molecules which have a high affinity to the molecules of a binding partner of an analyte in (i) or (iii), the biospecific layer comprises molecules of avidin, streptavidin, and deglycosylated avidin, wherein molecules of a binding partner of an analyte are biotinylated.

11. The biological sensor of claim **3**, wherein the binding partner of an analyte is an antibody or a fragment of an antibody to an analyte; or wherein the binding partner of an analyte is a receptor of an analyte; or wherein the binding partner of an analyte is a binding partner of proteins, lipids, DNAs, RNAs, viruses, cells, bacteria, or toxins, or chemical modifications of these substances.

12-13. (canceled)

14. A method for producing the biological sensor according to claim **1**, the method comprising the steps of:

a) depositing a metal film on a substrate;

b) depositing an intermediate binding layer on an outer surface of the metal film;

c) depositing a biospecific layer onto a surface of the intermediate binding layer,

wherein a thin film of graphene with a thickness of 0.3-2000 nm, or a thin film of single-walled or multi-walled carbon nanotubes with a thickness of 0.4-2000 nm, or a thin film of graphene oxide with the thickness of 0.7-2000 nm is deposited as the intermediate binding layer on the outer surface of the metal film, and wherein the biospecific layer is adsorbed conformally and homogeneously on the surface of the intermediate binding layer due to chemical interaction forces between molecules of the intermediate binding layer and molecules of the biospecific layer, said chemical interaction being caused by stacking interaction or interaction of molecules of the biospecific layer with functional groups of graphene, single-walled or multi-walled carbon nanotubes or graphene oxide, wherein the adsorption provides creation of a large number of activation centers on the surface of the intermediate binding layer with a degree of filling of the surface by the molecules of the biospecific layer being 15-100% of the surface area of the intermediate binding layer.

15. The method of claim **14** wherein a film made of gold, silver, copper, or aluminum with a thickness of 10-150 nm is deposited as the metal film.

16. The method of claim **14**, wherein the biospecific layer comprises molecules of a binding partner of an analyte.

17. The method of claim **14**, wherein the biospecific layer additionally comprises: (i) molecules which have a high affinity to the molecules of a binding partner of an analyte and which form a chemical bond with them; or (ii) a hydrogel having immobilized therein the molecules of a binding partner of an analyte; or (iii) a hydrogel having immobilized therein both the molecules of a binding partner of an analyte and the molecules which have a high affinity to the molecules of a binding partner of an analyte and which form a chemical bond with them.

18-19. (canceled)

20. The method of claim **14**, wherein in (ii) or (iii), the hydrogel comprises polysaccharides.

21. The method of claim **20**, wherein the polysaccharides comprises agarose, alginic acid, dextran, carrageenan, starch, cellulose or derivatives thereof.

22. The method of claim **21**, wherein the derivatives of dextran, the biospecific layer comprises carboxymethylated dextran.

23. The method of claim **14**, wherein in (i) or (iii), the molecules which have a high affinity to the molecules of a binding partner of an analyte comprise molecules of avidin, streptavidin, and deglycosylated avidin, wherein the molecules of a binding partner of an analyte are biotinylated.

24. The method of claim **14**, wherein the interaction of molecules of the biospecific layer with functional groups of graphene, single-walled or multi-walled carbon nanotubes or graphene oxide is carried out by the interaction with functional groups such as epoxy, hydroxyl, carbonyl or carboxyl groups.

25. The method of claim **16**, wherein the binding partner of an analyte is an antibody or a fragment of an antibody to an analyte; or wherein the binding partner of an analyte is a receptor of an analyte; or wherein the binding partner of an analyte is a binding partner of proteins, lipids, DNAs, RNAs, viruses, cells, bacteria, or toxins, or chemical modifications of these substances.

26-27. (canceled)

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