ISSN 1070-3632, Russian Journal of General Chemistry, 2007, Vol. 77, No. 3, pp. 325–335. © Pleiades Publishing, Ltd., 2007.
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Grafted Surface Compounds in Chemical Sensors and Biosensors

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Received November 8, 2006

Abstract — The use of grafted surface compounds for covalent immobilization of molecules and nanoparticles on the surface of sensors of various types is discussed. The classification of immobilization methods is based on the chemical nature of the surface of the material used in the sensitive element of a sensor: noble metals; inogranic oxides and salts; carbon and polymeric materials.
DOI: 10.1134/S1070363207030024

Chemical sensor is a device used for qualitative detection or quantitative determination of the concentration of a given substance or a group of substances using a specific chemical reaction.

Each chemical sensor consists of a receptor interacting with a substance to be determined (analyte) and a detector (physical converter). A chemical reaction with a reagent immobilized on the receptor surface is accompanied by changes in the physical and physicochemical properties of a system (optical, electrical, or acoustic properties; weight) or by absorption or emission of heat or radiation. The detector responds to these changes and converts them to an analytical signal that can be presented in the form of numerical data on the analyte content [1]. Sensors in which the reagents for determining the analyte are components of specifically interacting couples of biologically active molecules (e.g., antibodies and their complementary antigens or haptens; enzymes and their substrates) are termed biosensors.

The receptor selectivity is determined by the presence on its surface of a tightly fixed layer of functional groups or molecules capable of specific and desirably reversible interaction with an analyte. Forming such a layer on the receptor surface as a necessary condition for making an efficient sensor [2]. Depending on the service conditions and chemical nature of the analyte to which the sensor should respond, it is necessary to immobilize on the receptor surface diverse compounds: relatively simple organic compounds containing a single functional group, polyfunctional macromolecules of proteins and nucleic acids, and also their complexes with low-molecular-weight compounds. In the recent 10–15 years, there has been a considerable progress in nanotechnologies, a new field of science developing at the interfaces between physics, chemistry, materials science, and molecular biology. The development of new methods for studying physicochemical properties of nanoparticles, in combination with strong financial support of this research field, stimulated active studies concerning applications of nanoobjects in diverse devices and units, including chemical sensors and biosensors [3]. Although application of nanoparticles in chemical and biochemical analyses is not always justified by objective reasons, in many cases this approach, indeed, improves the analysis efficiency [4]. In this connection, it becomes necessary to immobilize reagent molecules on the surface of nanoparticles and to immobilize nanoparticles on the receptor surface. The first problem is solved by classical methods of surface chemistry; therefore, in this paper we consider jointly the methods for immobilization of reagent molecules on the receptor surface and on nanoparticles, whereas methods for immobilization of nanoparticles on a receptor support are discussed in a separate section.

To ensure reliable operation of a sensor for a long time without changes in its characteristics, it is necessary to bind the reagent to the receptor surface as tightly as possible. There are three main approaches to immobilization of a reagent on the receptor surface: adsorption, mechanical incorporation into the receptor material or into a film on the receptor surface, and covalent binding with the receptor surface. If a sensor is intended for operation in the gas phase and reagent molecules are nonvolatile under the operation conditions, all the above methods are acceptable from the viewpoint of stability of the sensor operation. If a

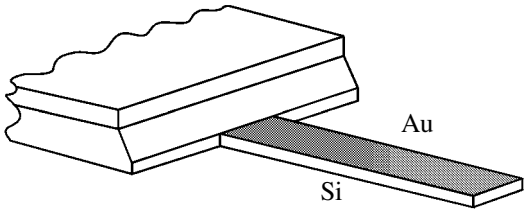

Fig. 1. Cantilever: a sensitive element of micromechanical sensors.

sensor is used in a liquid medium, only covalent binding is reliable; otherwise, the reagent is gradually washed out from the receptor surface in the course of operation and regeneration, which results in the sensitivity loss and shortening of the service life and makes it necessary to perform regular repeated calibrations. For example, the mean service life of biosensors in which a reagent is adsorbed on the surface or mechanically incorporated into the receptor material ranges from several days to a month; that of sensors with a reagent incorporated into a film, gel, or membrane reaches several months; and that of sensors with a covalently bound reagent reaches a year and over [5].

MAIN TYPES OF SENSORS

Traditional chemical sensors and biosensors can be subdivided into four groups with respect to the type of the detector-converter.

(1) Electrochemical sensors in which the reaction on the receptor surface alters the electrical characteristics: potential (ion-selective electrodes and field transistors, potentiometric cells), current at a given potential (voltammetric sensors), conductivity (conductometric and semiconductor gas sensors), or capacity of the receptor. Electrochemical sensors can be realized as an electrochemical cell consisting of the main electrode with a reagent immobilized on its surface and a reference electrode. In semiconductor and capacitive sensors, the receptor is a thin oxide film contacting with the gas phase. Operation of semiconductor gas sensors requires heating to 400°C and over. At such temperatures, grafted organic layers decompose, which strongly restricts the range of substances that can be immobilized on the surface of such sensors. It was shown recently that, with nanodispersed tin dioxide used as receptor, the operation temperature of the sensor can be decreased to 120–140°C [6] and even to room temperature [7]. In this case, chemical modification of the receptor surface becomes possible [8, 9].

(2) Optical sensors in which a chemical reaction affects the capability of a receptor to reflect, emit, absorb, or scatter light. The receptors in these sensors are cells with a reagent, membranes fixed at the end of an optical waveguide, the waveguide surface itself, or a reflecting metal surface on which a reagent is immobilized (in sensors based on the surface plasmon resonance phenomenon).

(3) Mass-sensitive (piezoelectric microbalance and sensors on surface acoustic waves), recording a change in the mass of a sensitive layer upon binding of an analyte with reagent molecules.

(4) Heat-sensitive (calorimetric), in which the thermal effect of a chemical reaction involving an analyte is recorded with a thermometer. Operation of calorimetric sensors in the gas phase requires heating to ~500°C. Heat-sensitive sensors for operation in a liquid phase at 10–40°C are also available; the detectors-converters in such sensors are thermistors, sensitive thermometers made of ceramic materials whose conductivity strongly depends on temperature. A ceramic ball is coated with a protective glass layer on which the reagent molecules are immobilized [1].

Recently sensors of a principally new class, micromechanical sensors based on cantilevers for atomic-force microscopy [10], have been developed. Cantilever is an elastic silicon or silicon nitride beam fixed at one end on a massive support (Fig. 1).

Forces applied to a cantilever cause its bending. The extent of bending can be measured with a high accuracy using optical or other detection systems. In the majority of atomic-force microscopes, this is done using optical transducers in which a laser beam impinges on the upper mirror surface of the cantilever at a certain angle and is reflected to the center of a positional photodiode. Bending of the cantilever gives rise to a difference in the illumination of different areas of the photodiode, from which the extent of bending can be determined. To ensure the possibility of optical detection, one side of the cantilever beam is coated with a reflecting layer (usually with gold).

The extent of static bending of a cantilever in a homogeneous liquid or gas phase is determined by the difference between the surface tensions on the opposite sides of the cantilever beam. The beam surface adsorbs components from the gas or liquid medium in which the cantilever is placed. In the process, if opposite sides of the cantilever beam differ in the chemical nature, their surface tension changes upon adsorption to a different extent, and the beam bends. To ensure selective sorption of an analyte, a reagent binding the analyte is immobilized on one of the cantilever beam surfaces.

CHOICE OF RECEPTOR SUPPORTS

The choice of a method for immoblization of molecules of the sensitive layer is mainly governed by the

receptor material. Despite diversity of chemical and sensors biosensors developed, materials used in the receptors of modern sensors can be subdivided into five large groups with respect to the chemical nature.

(1) Noble metals (Au, Ag, Pt) are used as electrodes in electrochemical sensors, piezoelectric microbalances, sensors based on the surface plasmon resonance phenomenon, and also as reflecting coatings in micromechanical sensors. Also, gold nanoparticles, thanks to their high electrical conductivity, capability to catalyze certain electrochemical reactions, and high sensitivity of optical properties to aggregation and to changes in the chemical surrounding, are among the most popular nanoparticles for sensors.

The main presently used method for modification of metal surface is the reaction with organosulfur compounds: thiols and disulfides (Fig. 2).

(2) Oxide and ceramic materials (silicon and tin oxides, oxide glasses). Conducting oxide glasses are used as electrodes in electrochemical sensors. UV-transparent waveguides for optical sensors are made of quartz, and thermometers for calorimetric sensors (thermistors), of ceramic materials. Silicon dioxide is used in ion-selective field transistors, and tin dioxide, in semiconductor sensors. Micromechanical sensors are made of silicon or silicon nitride, which undergo surface oxidation in the presence of oxygen. Therefore, receptor molecules are immobilized on their surface via the natural oxide layer. The surface of oxide materials contains hydroxy groups which can react with a wide range of organic modifiers (Fig. 3) [2].

Therefore, in some cases when metals (Au, Ag, Pt) are used as receptor materials, is it appropriate to deposit onto the receptor surface a thin layer of an oxide (usually SiO_2) and thus to reduce the problem of modification of a metal surface to modification of hydroxy groups of the surface oxide [11, 12].

The most commonly used modifiers for oxides are organosilicon compounds.

(3) Inorganic layers in membranes of ion-selective electrodes are used without chemical modification. The problem of chemical modification of salts arises when nanoparticles of metal sulfides (CdS, ZnS) exhibiting unique optical and electrocatalytic properties are used in sensors. Chemical modification of sulfide surface is performed with organosulfur compounds: thiols and disulfides.

(4) Carbon materials (most frequently graphite and its composites) are used as electrodes in electrochemical sensors. Covalent immobilization of receptor molecules on carbon materials is not performed commonly, because mechanical mixing of receptor components is quite sufficient for preparing, e.g., a disposable carbon-paste printed electrode; however, biosensors with enzymes immobilized on the preoxidized graphite surface have also been reported [13].

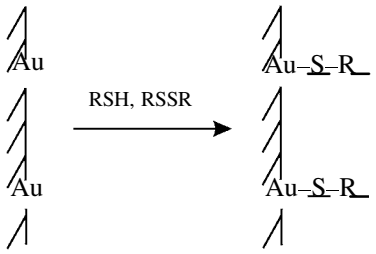

Fig. 2. Modification of the gold surface with thiols and disulfides.

(5) Organic polymers used for preparing waveguides and membranes for optical sensors, and also membranes for ion-selective electrodes. The method of modification of a polymeric material is primarily governed by its functional groups.

METHODS FOR DIRECT IMMOBILIZATION OF A REAGENT AND CHEMICAL ASSEMBLY ON THE SURFACE

Modification of the receptor surface can be performed in one or several steps. One-step modification process involving compounds that contain functional groups capable to form chemical bonds with the support surface is termed immobilization [2]. The main advantages of the immobilization method are simplicity (one-step process), large amount of the grafted target product, and uniformity of sorption centers. Direct immobilization of molecules imposes certain limitations associated with the compatibility of the functional groups of the reagent molecule with the anchor group interacting with the surface, and also with the conditions of the reaction of the modifier with the receptor surface. Also, introduction of the required functional groups into the reagent molecule often involves fine and complicated organic synthesis and the use of difficultly available chemicals.

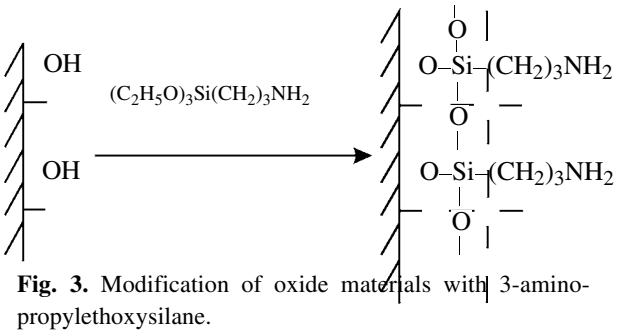

Fig. 3. Modification of oxide materials with 3-aminopropylethoxysilane.

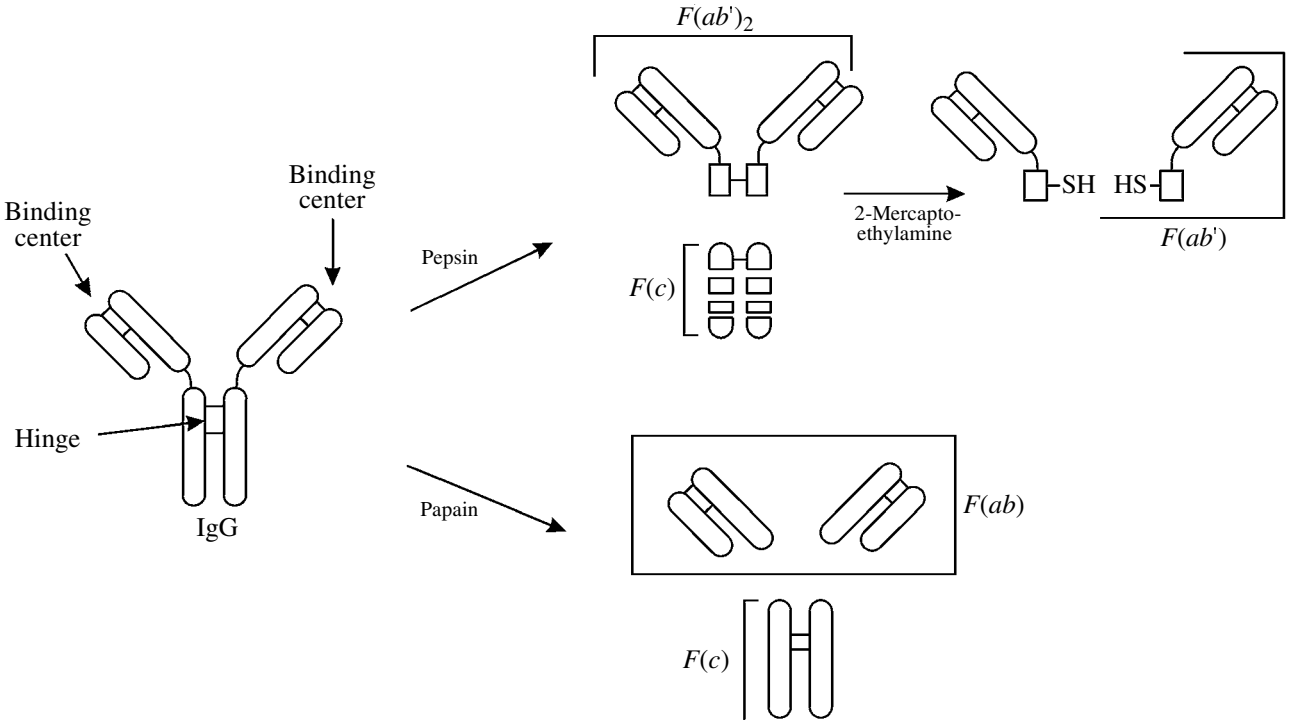

Fig. 4. Preparation of fragments [designated as $F(ab')$, $F(ab')_2$, $F(ab)$, $F(c)$] of antibodies (IgG) for immobilization on the gold surface.

In such cases, the surface assembly is used. The first step in this method is immobilization of a relatively simple compound containing a reactive fragment that does not react with the support surface. In subsequent steps, this fragment is transformed in the required direction by performing simple chemical reactions. The disadvantage of surface assembly is that reactions on the surface, as a rule, do not give a quantitative yield. Unchanged functional groups remaining on the receptor surface can participate in side reactions with reagent or analyte molecules or with other components with which the sensor is in contact in the course of fabrication and operation. To eliminate these reactions, the unchanged groups are blocked with an appropriate reagent.

In modern sensors, these strategies can be realized both separately and in combination. For example, additional functional groups can be introduced into a reagent molecule by homogeneous chemical reactions, and the modified molecule can be immobilized by chemical assembly.

METHODS FOR COVALENT IMMOBILIZATION OF A REAGENT ON THE RECEPTOR SURFACE

Immobilization of receptor molecules on the gold and silver surface. Chemical modification of gold and silver surfaces is often performed through formation of self-assembling (regular) monolayers of organic thiols and sulfides. The chemisorption of thio compounds is performed for 4–24 h from a 1×10^{-6}–1×10^{-1} M solution of a thiol or disulfide in water, methanol, or ethanol. In the process, a highly organized monolayer of gold or silver thiolate, stable in both acidic and alkaline media, is formed. The sensitive layer of a sensor can be prepared by chemisorption of organosulfur compounds using both alternatives: direct immobilization of molecules selectively binding with an analyte and chemical assembly on the receptor surface.

In the first case, the receptor molecule is additionally functionalized with thiol groups by methods of classical organic chemistry. Examples of direct immobilization can be found in [14, 15], and also in studies [16–19] involving immobilization of oligonucleotides with thiol groups at the 3'- or 5'-termini on the surface of a gold electrode of a piezoelectric crystal. In some cases, the thiol or disulfide group is already present in a molecule to be immobilized on the receptor surface. For example, molecules of antibodies contain disulfide bonds which can be cleaved to obtain thiol groups by treatment with mercaptoethylamine; the resulting fragments of antibodies can be directly used for treating the gold surface [20] (Fig. 4).

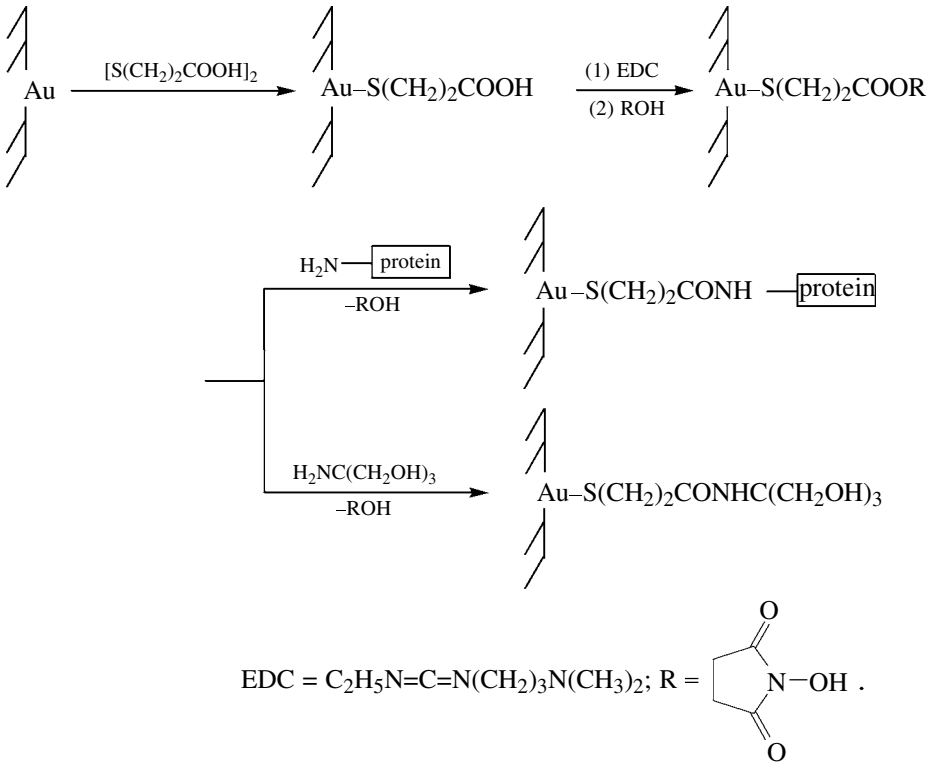

Fig. 5. Immobilization of a protein on a monolayer of thio acids on the gold surface.

To be suitable for chemical assembly, thiol or disulfide molecules should contain reactive functional groups (e.g., carboxy or amino groups) capable of subsequent reaction with molecules to be immobilized on the receptor surface.

Among organosulfur compounds with a carboxy functional group, it was suggested to use in sensors 3,3'-dithiopropionic acid and its *N*-hydroxysuccinimide ester [21, 22], thiosalicylic [23], thioacetic, 3-thiopropionic, 6,8-dithiooctanoic, and 11-thioundecanoic acids [24]. Modification with thio acids is performed by procedures general for thiols; with the *N*-hydroxysuccinimide ester, the modification is performed from solution in DMSO for 0.5–2 h [21, 22].

Covalent immobilization of protein on the monolayer of thio acids is provided by formation of a peptide bond between the surface carboxy groups and amino groups of the protein molecule. Since carboxylic acids in aqueous solutions at physiological values of pH and temperature are insufficiently reactive toward aliphatic amines, the carboxy groups should be preliminarily activated. This is attained by successive treatment of the surface with water-soluble derivatives of carbodiimide, e.g., with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, and with *N*-hydroxysuccinimide. Treatment is performed with aqueous solutions of the reagents for 1 h [24]. Then the activated surface is treated with a solution of a protein in a buffer solution with pH close to 7 for 30–60 min. After that, to avoid side reactions in the course of sensor operation, the unchanged ester groups are blocked by the reaction with tris(hydroxymethyl)aminomethane (Tris) or glycine under the same conditions as for the reaction with the protein (Fig. 5). This procedure is also used for immobilization of an oligonucleotide into which an aminoalkyl group is introduced in the synthesis step [25].

Another method for immobilization of molecules containing aliphatic amino groups on the gold or silver surface of a receptor or on metal nanoparticles involves preliminary formation of a layer of amino groups on the receptor surface, followed by cross-linking with amino groups of the receptor molecules using a dialdehyde (Schiff base formation). Functionalization of the surface with amino groups is performed with cystamine [22, 26, 27] or 4-aminothiophenol (4-ATP) [28]. The reaction of cystamine with the receptor surface is performed from a 1–100 mM solution in a phosphate buffer (pH 7) for an hour. In case of 4-aminothiophenol, the surface is treated with a 1–100 mM solution of 4-ATP in alcohol for 12 h. Glutaraldehyde $OHC(CH_2)_3CHO$ is commonly used as cross-linking agent. To avoid binding of receptor molecules with each other, the cross-linking is per-

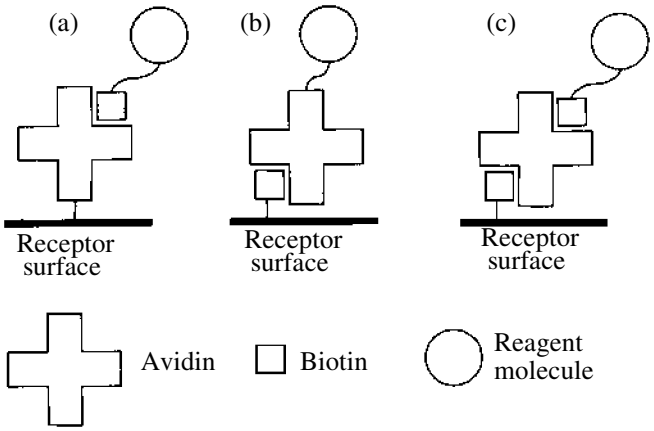

Fig. 6. Avidin–biotin methods for immobilization of reagent molecules via surface-immobilized (a) avidin or (b) biotin, or (c) via avidin–biotin complex.

formed in two steps: The receptor surface functionalized with amino groups is treated with a 5–10% aqueous solution of glutaraldehyde for 30–60 min and then with a solution of a protein or oligonucleotide derivatized with an aminoalkyl group. The reaction is performed in a buffer solution with pH 7, also for 30–60 min. The unchanged aldehyde groups on the surface are blocked by treatment with Tris or glycine as described above.

Cross-linking of reagent molecule with the sensor surface is often performed using a highly specific reaction of avidin glycoproteide (contained in albumen of bird and reptile eggs) or its analog streptavidin (from Streptomyces avidinii) with a low-molecular-weight compound biotin to form a stable complex. Avidin [16] or biotin [29] is immobilized on the receptor surface via organosulfur compounds, and the grafted layer is treated with the conjugate of the reagent molecule with biotin or avidin, respectively, prepared beforehand. Avidin has four sites of biotin binding; therefore, its immobilization on the surface can also be provided by the reaction with biotin immobilized on the surface (Fig. 6).

The method for determination of streptavidin is based on this principle [30]. A conjugate of albumin and biotin was immobilized on the surface of a carbon-paste electrode. The streptavidin to be determined was bound to biotin on the electrode surface. The capability of streptavidin to bind more than one biotin molecule allows further modification of the electrode surface with biotin-modified gold nanoparticles. This is followed by electrochemical deposition of silver on the sandwich complexes obtained. The electrode potential is varied to control the amount of the deposited silver. With this method, the level of streptavidin concentrations accessible for the determination can be made as low as 10^{-15} M.

In some cases, other methods can be used for immobilization of a receptor molecule on the surface containing amino groups; their applicability is determined by the presence of specific functional groups in the receptor molecule or ease of their introduction. For example, in [22], for immobilization of fluorescein, a gold electrode of a piezoelectric crystal was successively treated with cystamine and an aqueous solution of fluorescein isothiocyanate. The same method was used in [22] for immobilization of α-D-mannopyranose (Fig. 7).

In [31], hexachlorocyclopentadiene was immobilized on an aminated surface from a 0.25 M solution of C_5Cl_6 in absolute diethyl ether (Fig. 8).

Immobilization of receptor molecules on the surface of inorganic oxides. The most widely used chemicals for modifying oxide surfaces in modern chemical sensors are silanes containing chlorine atoms or alkoxy groups as anchor groups: $RSi(CH_3)_x(OCH_3)_{3-x}$, $RSi(CH_3)_x(OC_2H_5)_{3-x}$, and $RSi(CH_3)_xCl_{3-x}$.

Immobilization of silanes on oxide surfaces is usually performed in nonpolar solvents (toluene, xylene); the reaction time is varied from 15 min to several hours, and the temperature, from room temperature to 100°C.

Unmodified metal oxides, such as Ta_2O_5, SnO_2, RuO_2, TiO_2, PtO, etc., are often used as sensitive elements of electrodes. To control the selectivity of electrodes based on them and improve the analytical characteristics, the surface of these oxides was subjected to chemical modification with relatively simple functional groups, which was done by treating the oxide surface with 3-aminopropyltriethoxysilane (APTES), 2-pyridylethyltriethoxysilane, 3,3-dichloropropyltriethoxysilane, or 3-(2-aminoethylamino)propyltriethoxysilane [32–34]. To enhance the selectivity of semiconductor gas sensors based on SnO_2 to NO_2 [8] and H_2 [9], the SnO_2 surface was modified by immobilization of APTES and dimethyldiethoxysilane, respectively.

Immobilization of proteins is usually performed with 3-aminopropyltriethoxysilane, with glutaraldehyde as cross-linking agent [11, 35–37]. In [11], antibodies to potato X-virus were immobilized on a thin layer of silicon oxide preliminarily deposited on a silver electrode of a piezoelectric sensor (Fig. 9). The weight of the oxide layer was chosen so that the resonance of the piezoelectric crystal was not distorted even upon subsequent binding of virus particles with the antibodies.

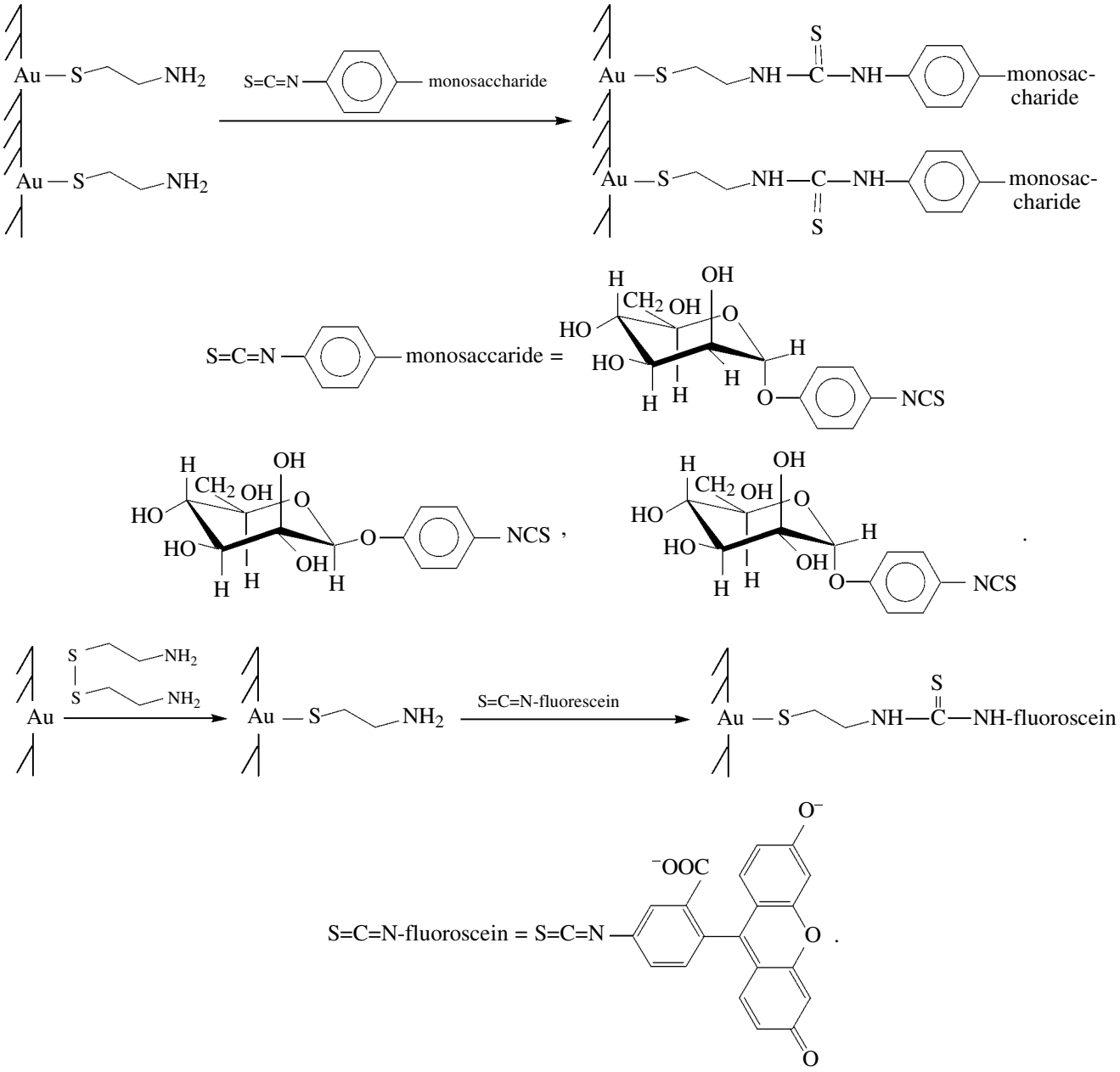

Fig. 7. Immobilization of organic compounds by the isocyanate method.

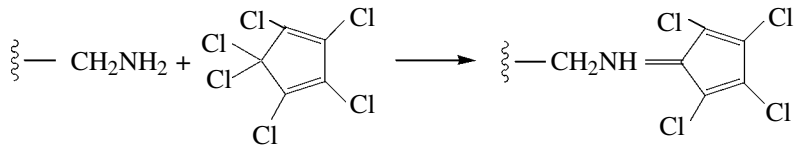

Fig. 8. Immobilization of hexachlorocyclopentadiene on a layer of amino groups.

As shown in [12], glutaraldehyde can be immobilized directly on the hydroxylated silica film (Fig. 10).

Immobilization of nanoparticles on the receptor surface. Covalent cross-linking of nanoparticles with the receptor surface is possible if on the surface of the receptor and nanoparticle there are functional groups capable to react with each other to form a "bridge" between the nanoparticle and receptor [38, 39].

Operation of a sensor for organophosphorus compounds [38] that are acetylcholinesterase inhibitors

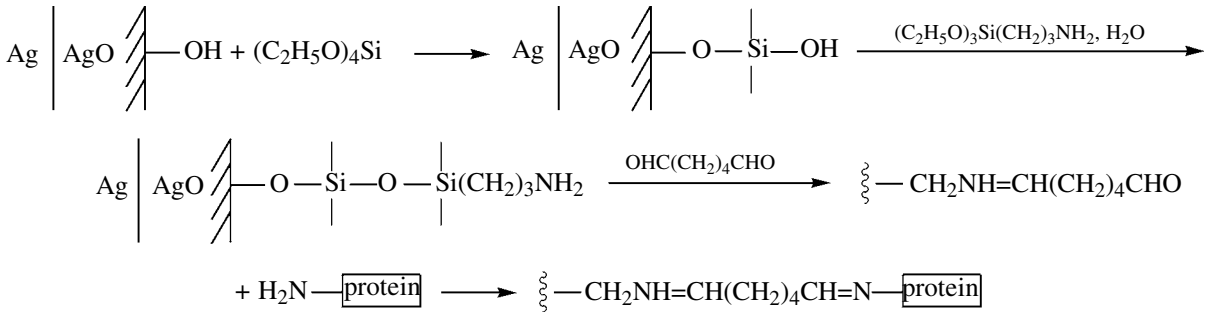

Fig. 9. Immobilization of antibodies on silicon dioxide deposited on a silver electrode.

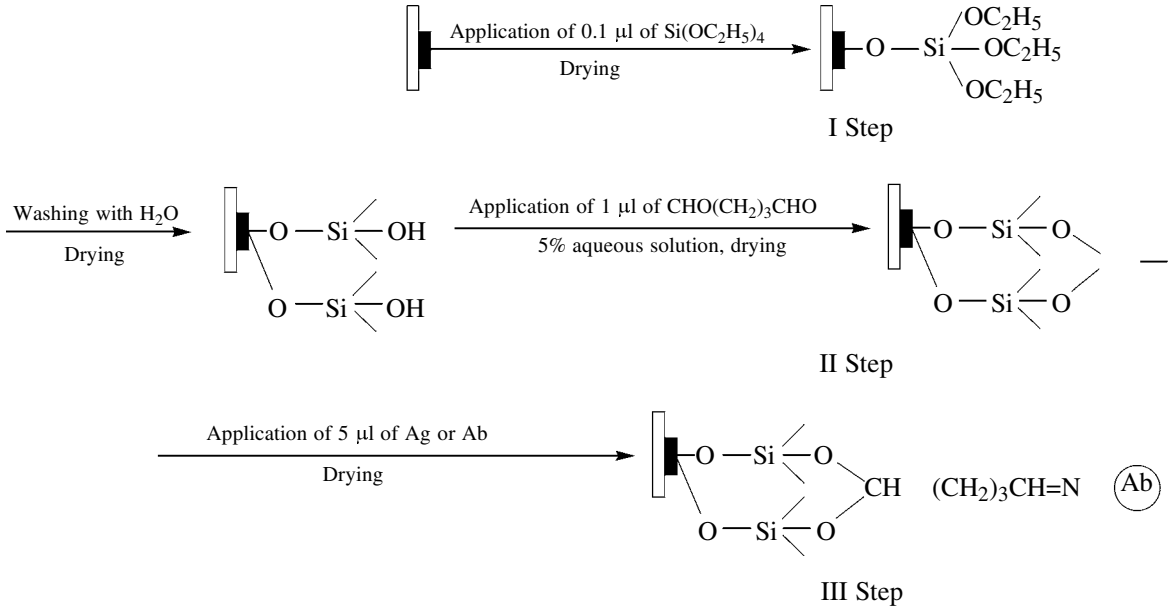

Fig. 10. Immobilization of antibodies (Ab) on silicon dioxide via glutaraldehyde.

is based on generation of a photocurrent upon oxidation of organic substances on the surface of CdS nanoparticles. The sensor was made by step-by-step assembly of a conjugate of acetylcholinesterase and cadmium sulfide nanoparticles on the surface of a gold electrode. In the first step, the electrode surface was treated with 3-thiopropionic acid *N*-hydroxysuccinimide ester, after which it was brought into reaction with amino groups of cystamine localized on CdS nanoparticles.The nanoparticles thus immobilized were then modified via cystamine amino groups with glutaraldehyde, after which they were treated with the enzyme molecules. If the solution being analyzed contains acetylthiocholine (enzyme substrate), acetylcholinesterase catalyzes its hydrolysis to thiocholine and acetate. Photoexcitation of nanoparticles of the CdS semiconductor generates electron–hole pairs in the conduction and valence bands, respectively. Thiocholine generated by the enzyme acts as an electron donor on holes in the valence band, which results in accumulation of electrons in the conduction band and their transfer to the electrode, i.e., in generation of a photocurrent. Addition of cholinesterase inhibitors results in deceleration or complete termination of the catalytic reaction; as a result, the photocurrent decreases. In [39], similarly to the above-described procedue, formaldehyde dehydrogenase was used for determination of its substrate, formaldehyde.

Functional groups can also be located only on one of the surfaces: receptor or nanoparticle surface [40–42].

In electrochemical sensors, the operation principle involving contact of redox enzymes with the electrode surface and hence transfer of an electron generated by a redox reaction to the electrode surface is being actively introduced into medical diagnostics and has numerous implementations in the form of biosensors [43, 44] and biological fuel cells [45–47]. However, in most cases it is difficult to ensure direct electron

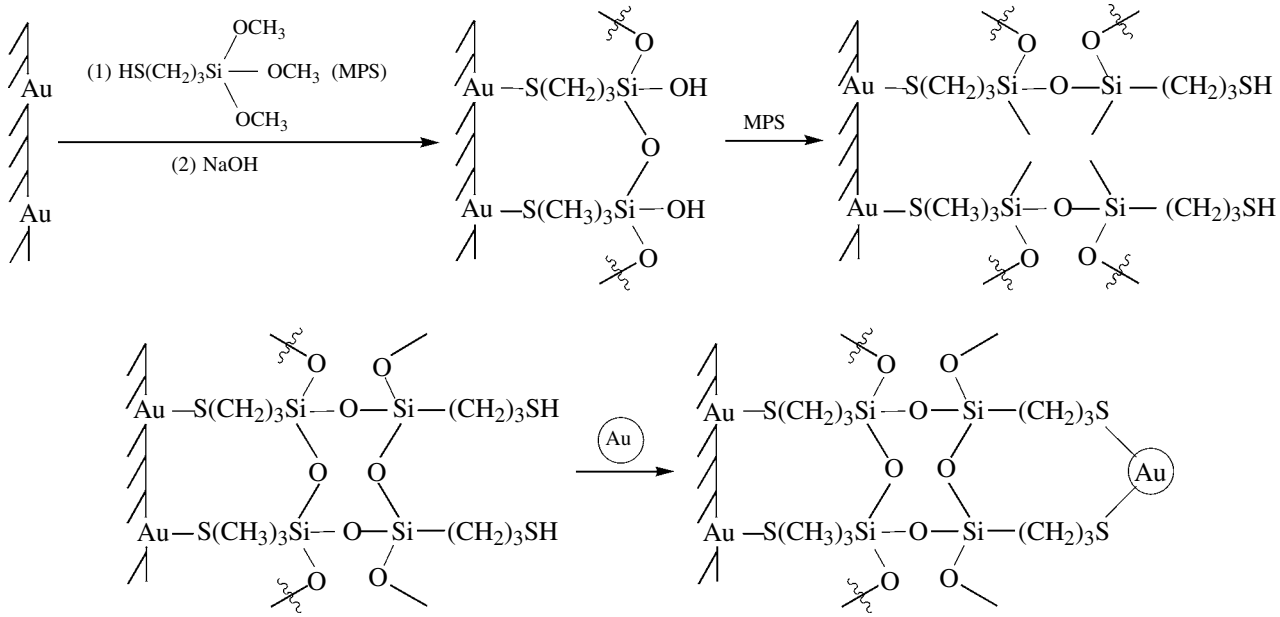

Fig. 11. Immobilization of gold nanoparticles on a two-dimensional polysiloxane layer.

transfer from an enzyme to an electrode without mediators. The use of conducting nanoparticles with an immobilized enzyme [48–51] allowed low-molecular-weight agents for electron transfer to the electrode surface to be dispensed with. In so doing, it is necessary that the electron transfer from a nanoparticle to an electrode involve no mediators either. For this purpose, gold nanoparticles are immobilized on a monolayer of dithiols $HS(CH_2)_nSH$ (n = 3, 6, 9) [40] or 1,4-benzenedithiol (dithiohydroquinone) [41], made beforehand on the surface of a gold electrode.

To immobilize gold nanoparticles, Xia et al. [42] first treated the surface of a gold electrode with 3-mercaptopropyltrimethoxysilane (MPS) and then hydrolyzed the methoxysilyl groups with a NaOH solution. The resulting two-dimensional polysiloxane layer was modified via hydroxy groups by the reaction with MPS. After that, the electrode was treated with gold nanoparticles, which were chemisorbed on the layer of thiol groups of the surface (Fig. 11).

The data given in this paper demonstrate successful use of synthetic methods of chemical modification of a surface for enhancing the selectivity, sensitivity, and stability of operation of chemical sensors and biosensors. Today the sensor technology is the major applied field of the chemistry of grafted surface compounds.

It should be noted that synthetic methods of chemical modification open prospects for development of new approaches to fixation of receptor molecules. Among possibilities that have not yet been realized are the use of phosphonic acids and their esters for immobilization of biomolecules on oxide surfaces and the use of organometallic compounds of transition metals for making metal centers on surfaces. Molecular layer deposition of volatile and readily hydrolyzable metal halides may be useful for enhancing the selectivity of semiconductor gas sensors, the more so as such grafted layers are relatively heat-resistant and can operate at temperatures of up to 300°C. For example, Belova [52] showed that vanadium oxychloride deposited as molecular layers on silica can be used as humidity sensor.

Much promise in immunoassay is shown by aptamers (sharply selective artificial antibodies, oligonucleotides). Sensors based on grafted aptamers allow determination of virtually any biological (thrombin) and organic (cocaine) compounds [53, 54]. For the time being, wide use of such sensors is restrained by the high cost of aptamers.

Finally, in our opinion, it seems promising to use physical actions (light, temperature, magnetic fields) on grafted receptor molecules for controlling the sensor selectivity. In this case, it is necessary to immobilize bistable molecules on the surface [55].

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