Chapter 6
Biosensors for Virus Detection

Olga I. Guliy, Boris D. Zaitsev, and Irina A. Borodina

Abstract  Viral infections today remain one of the global problems. Despite the significant number of developed methods for the determination and study of viral particles, their practical use is difficult due to the complexity and a small number of existing measuring instruments. In addition, in a number of devices, there is a high proportion of manual operations that require highly qualified staff. Therefore, the problem of the development of new rapid methods for diagnosing viruses that allow getting accurate results in a short time in automatic mode is urgent. Instrumentation implementation of these methods should ensure high accuracy of measurements. One of the most sought-after areas is the development of fast and sensitive methods for determining viruses based on methods of electrophysical analysis. These methods can significantly reduce the analysis time and simplify the preparation of the investigated materials. The chapter provides a brief overview of modern biosensor methods for the virus’s detection.

Keywords  Biosensors · Virus detection · Bacteriophages · Electrochemical biosensors · Optical sensors · Acoustic biosensors · Microwave resonator · Cantilever · Antibodies

Nomenclature

Ab  Antibody
Ab-AuNPs  Antibody-conjugated gold nanoparticles
Abs  Antibodies
DNA  Deoxyribonucleic acid
ELISA  Enzyme-linked immunosorbent assay
EO  Electro-optical

O. I. Guliy (*)
Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences, Saratov, Russia
e-mail: guliy_olga@mail.ru

B. D. Zaitsev · I. A. Borodina
Kotelnikov Institute of Radio Engineering and Electronics, Russian Academy of Sciences, Saratov Branch, Saratov, Russia

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### 6.1 Introduction

Viral infections occupy one of the leading positions among human and animal diseases. Therefore, the diagnosis of viruses is the main strategy for combating viruses and eliminating them.

Various approaches are used to detect and identify viruses, such as microbiological and biochemical tests, genetic engineering, and immunological methods. Existing methods for identifying viral particles can be divided into the following groups:

1. Detection (identification, determination of concentration, size, and other physicochemical properties) of viral particles (virions) and determination of viral infectivity.
2. Determination of viral antigens.
3. Determination of viral nucleic acids (Carter and Saunders 2007).

Despite the significant number of developed methods for detecting viral particles, their practical use is difficult due to the complexity, high cost, and the small number of existing measuring instruments. In addition, in a number of devices, there is a high proportion of manual operations that require highly qualified staff.
For example, traditional methods for identifying viruses have drawbacks, primarily due to insufficient sensitivity (immunofluorescence methods), long duration of analysis, tedious, and lack of the universality of the procedure (culture method). The detection of antibodies to viruses in the serum of patients using enzyme-linked immunosorbent assay (ELISA) is a retrospective method because it does not detect the pathogen itself, but records the body’s response to infection. ELISA detection of viral antigens in clinical samples is limited by the lack of sensitivity of the method. The use of immunochemical and serological methods also complicates the high antigenic diversity of some groups of viruses. Traditional methods, due to the limitations listed above, do not allow simultaneous and highly sensitive detection of the main virus groups in clinical samples.

The molecular approaches, including the polymerase chain reaction (PCR) and its various modifications, are widely used in the laboratory diagnosis of viral infections. It should be noted that the real-time PCR is also widely used (Coiras et al. 2004; Syrmis et al. 2004; Templeton et al. 2004). In recent years, such high-tech technology as DNA microarray technology (Lin et al. 2007; Mehlmann et al. 2007; Huguenin et al. 2012; del Pilar Martinez Viedma et al. 2019) has been increasingly used for diagnostics, for example, acute respiratory viral infections. The advantages of molecular methods are high specificity, sensitivity, universality of the procedure, short analysis time, process automation, and the ability to identify several pathogens at once.

Viruses are dispersed and usually exist in various morphological forms, the size of which usually varies from 20 to 900 nm (Passi et al. 2015; Rojek et al. 2017; Shawky et al. 2017). Their intact, mature infectious particles usually consist of certain units of proteins and nucleic acids that self-assemble to form nanoparticle structures called virions. Viral proteins are usually located in the surface layer, called the capsid, and, sometimes, in the outer shell surrounding the inner nucleic acid nucleus. This core of viral nucleic acids can be single or double-stranded (ds), DNA or RNA, one or more linear or circular molecules, ranging in size from several thousand nucleotides to one million base pairs.

Features of the structure and activity of viruses are taken into account when developing methods for their analysis. However, it is important to consider that all viruses evolve rapidly, which is facilitated by the world-wide migration processes that have intensified in recent years. Under these conditions, humanity is increasingly encountering the emergence of new viruses or strains that are well-known but distinguished by increased pathogenicity (e.g., SARS coronavirus, A/H5N1 avian influenza virus). Therefore, a very important area is the development of express methods for determining viruses. One of the most promising tools for detecting viruses is biosensor analysis methods. Instrumentation of these methods should ensure high accuracy of measurements, and measurements should be carried out automatically by mid-level personnel.

An important criterion in the development of new methods for determining viruses is their versatility and the ability to use for various objects. Therefore, the
development of methods for the electrophysical analysis of viral particles occupies one of the leading positions in this direction. The chapter provides a short overview of modern biosensor virus detection methods.

6.2 Biosensor Methods for Virus Detection

Initially, methods of electrophysical analysis were used to study the electrophysical properties of the cell. A systematic study of these issues began in the 30s of the last century, when they began to study one of the electrical characteristics of cells—surface charge. Later, in the 60s, investigations on the study of the dielectric constant and electrical conductivity of tissues, cells, and cellular organelles began to carry out. Recent years have been characterized by an increased interest in the electrical characteristics of not only cells, but also viruses. With the advent of biosensors, the traditional approaches to methods for determining viruses are changing significantly. The development of biosensor virus detection technologies will be extremely useful for the early detection of diseases and the timely provision of medical care (Chandra, 2016).

Biosensors are biochemical-physical systems consisting of two components: a sensitive biological element and a detection system, allowing to record the concentration or activity of various analytes presenting in the sample. The biological element can be catalytic and non-catalytic. Catalytic elements include enzymes and tissues. Non-catalytic elements are receptors and nucleic acids. The detection system can be optical, calorimetric, acoustic, electrical, etc. There is a possibility of combining all these systems, which indicates the possibility of creating a huge number of various biosensor systems (Turner 1987).

Biosensor methods of analysis began to be used to determine glucose by Clark and Lyons in 1960 and have now become an integral part of clinical diagnosis and environmental monitoring (Brooks et al. 1988). Biosensors can significantly reduce the analysis time, due to the relative simplicity of the procedures, and as the published data show, they are quite sensitive and require minimal preliminary processing of the test material. For the detection of viral particles, biosensors with different designs and mechanisms of operation are increasingly being used. The key problem in the development of a biosensor is the process of introducing and fixing the bioreceptor on the surface of the carrier (transducer), that is, an immobilization process that allows the development of a selective, reproducible, sensitive, and stable biosensor (Leca-Bouvier 2010; Moreira 2014).

Therefore, one of the main points for the classification of sensors is to use the immobilization of analysis components on the surface of the sensor or to conduct studies directly in the liquid without immobilizing the analysis components.

Depending on the detection method, the sensors are divided into electrochemical, optical, acoustic, and cantilever sensors for viruses and bacteriophages.
6.2.1 Electrochemical Biosensors

Electrochemical biosensors were created with the aim of combining the sensitivity of the electrochemical detector and the specificity of the active layer during antigen–antibody interaction. Electrochemical biosensors include:

- Potentiometric sensors in which the cell potential is measured at zero current;
- Voltametric (or amperometric) sensors, in which the oxidation or reduction current of electroactive particles is measured at applying a given potential difference between the electrodes;
- Conductometric sensors in which the conductivity of the contents of the container is measured using a conductivity bridge (Budnikov 2010; Evtugin 2013).

Combining the principles of voltammetry with immunological reactions allows creating inexpensive and selective analytical devices—amperometric immunosensors, which combine high sensitivity of amperometric detection and the specificity of immune interactions. The immunosensor operation scheme is presented in Fig. 6.1(a).

In biosensors of the electrochemical type, in combination with potentiometric electrodes, enzymes, receptors, microorganism cells, plant and animal tissues, and antibodies labeled with enzymes are used. In combination with amperometric electrodes, the use of enzymes, microorganisms, plant and animal tissues, and antibodies labeled with enzymes is known.

For example, an amperometric immunosensor was developed without the use of labels for the sensitive determination of antibody (Ab) to the Japanese encephalitis B virus. In order to obtain a biosensitive part of the sensor, the antiserum was immobilized to an o-phenylenediamine film modified with gold nanoparticles (GNPs) and Prussian blue on the surface of a platinum electrode. The formation of the analytical signal of the sensor is based on a change in the behavior of the mediator—Prussian blue (Fe²⁺/Fe³⁺)—during immunochemical interaction, which was recorded using the method of cyclic voltammetry. The detection limit was $6 \times 10^9$ (Ig particles)/Mn.

Los et al. (2006) proposed the use of an electrochemical biochip to quickly detect phage infection and the phenomenon of lysogeny. The principle of the method is to capture target molecules (either nucleic acids or proteins) on a chip using a probe that is connected to an enzyme that catalyzes the oxidation–reduction reaction. The electrical signal resulting from this reaction is measured using a microelectrode. Two types of biochips were used in the work: for detecting nucleic acids of bacteriophages (using DNA probes) and for detecting virions (using specific Ab). The authors demonstrated the ability to detect phages M13, P1, T4, and λ in a short time (25–50 min) and in an amount of $10^4$ to $10^7$ particles/ml.

Using the detection of T7 bacteriophage as an example, the use of a colorimetric immunosensor made on the basis of gold nanoparticles covalently associated with specific antibodies to the T7 bacteriophage was proposed (Lesniewski et al. 2014).
Fig. 6.1 (a) The general scheme of the immunosensor. (b) The structure of the gas-sensitive electrochemical sensor: M1 and M2—internal and external metal electrodes; E1 and E2—internal and external electrolyte; P—porous septum; GM—gas-permeable membrane. (c) Diagram of the SPR biosensor based on a dielectric prism (like Kretschmann scheme)
The described model of the immunosensor, due to the formation of immune complexes of the bacteriophage with Ab and gold nanoparticles, allows us to detect quickly, simply, and selectively the virus. As a result, one can observe a change in the color of the solution from red to purple with the naked eye. At that, the authors conclude that this method can be used to detect almost any virus.

The study by Grabowska et al. (2014) provides data on the use of an electrochemical biosensor (genosensor and immunosensor) for determining the bird flu virus.

A barcode lateral flow immunoassay (LFIA) based on magnetic nanoparticles with a controllable cut-off level was developed for the first time to detect potato virus X in the leaf extracts. To obtain specific conjugates, monoclonal antibodies were covalently immobilized on the magnetic nanoparticles’ surface. The application of magnetic concentration leads to a six-fold reduction in the first cut-off level (0.5 ng/mL) in comparison with magnetic LFIA without the concentration stage (Panferov et al. 2017).

The joint use of magnetic nanoparticles (MNPs) and gold nanoparticles (GNPs) double enhancement in a LFIA for potato virus X detection was shown in Razo et al. (2018). The study realizes two types of enhancement: (1) increasing the concentration of analytes in the samples using conjugates of MNPs with specific antibodies and (2) increasing the visibility of the label through MNP aggregation caused by GNPs. The double-enhanced LFIA achieved the highest sensitivity, equal to 0.25 ng/mL and 32 times more sensitivity than the non-enhanced LFIA (detection limit: 8 ng/mL).

Although LFIAs have many advantages including speed and ease of use, their sensitivity is limited without specific equipment. Furthermore, their response cannot be enhanced through enzymatic reactions. Owing to these limitations, LFIAs have not yet been generally adopted as the standard protocol for in vitro analysis of infectious pathogens. Noh et al. (2019) described a novel pipetting-based immunoassay using a removable magnetic ring-coupled pipette tip. The “magnetic bead-capture antibody-targeted protein complex” was simply purified by pipetting and quantified by enzymatic color development or using a lateral flow system. This pipetting-based immunoassay was applied to detect the nucleoprotein (NP) of the influenza A virus. Using an HP-conjugated monoclonal antibody as a probe, the assay allowed for specific and sensitive detection. Khoris et al. (2020) described an efficient and quick monitoring system for Hepatitis E virus (HEV) detection. The advanced platform for immunoassay has been constructed by a nanzyme that constitutes anti-HEV IgG antibody-conjugated gold nanoparticles (Ab-AuNPs) as core and in situ silver deposition on the surface of Ab-AuNPs as outer shell. The virus has been entrapped on the nanocomposites while the silver shell has decomposed back to the silver ions (Ag+) by adding a tetramethylbenzidine (TMBZ) and hydrogen peroxide (H2O2) which indirectly quantifies the target virus concentration. Most importantly, the sensor performances have examined in clinically isolated HEV from HEV-infected monkey over a period of 45 days which successfully
correlated with their standard RT-qPCR data, showing the applicability of this immunoassay as a real-time monitoring on the HEV infection.

By using as an example the bacteriophage PhiX17 and *Escherichia coli* WG5 sensitive to this phage, a fast method for detecting viral particles in a sample using a whole-cell biosensor was demonstrated. A biofilm of *Escherichia coli* cells was formed on the surface of the metal electrode. The infection of biofilm cells with a specific bacteriophage leads to their lysis and a change in impedance on the surface of the microelectrode. This change was recorded using impedance spectroscopy. The method is simple and allows to detect bacteriophages as long as there are sensitive cells on the chip (Muñoz-Berbel et al. 2008).

Constant outbreaks of infectious diseases have shown that in some cases there are no quick non-invasive methods for their diagnosis. In modern realities, a patient sometimes cannot detect, for example, the flu virus without assistance. Therefore, in most cases, a person has no idea whether he is infected until certain symptoms appear, and most often this moment comes too late. One example of a virus definition is the so-called “gas-sensitive electrodes.” The principle of their operation is shown in Fig. 6.1 (b). An important structural element of the potentiometric gas-sensitive sensor is the permeable gas membrane (GM), which separates the electrolyte of the “external” half cell from the surrounding atmosphere. The membrane is impervious to the electrolyte, but permeable to molecules of controlled gases. The higher the concentration of such gases in the surrounding atmosphere, the more their molecules penetrate through the GM membrane into the electrolyte and dissolve in it. They can enter into chemical reactions, and this violates the dynamic equilibrium in the near-electrode zone and changes the potential difference between the electrodes. For example, Gouma et al. (2017) created a device resembling a breathalyzer and able to detect the influenza virus in the early stages. The main difference from breathalyzers or household carbon monoxide detectors is the specificity of sensors that detect gas.

The measurement of the amount of heat released during the interaction of the analyte with the bioreceptor material is used in biosensors of the calorimetric type. In this case, the basis of the bioreceptor can serve as enzymes, cells of microorganisms and animals. For a more detailed acquaintance with various types of biosensors, you can refer to the monographs (Turner 1987; Buerk 1993; Harsanyi 2000).

Electrochemical biosensors have advantages over many alternative approaches, in particular, optical sensors, for which the turbidity of the solution or its color can significantly limit the scope of potential applications. The current stage of development of electrochemical biosensors is characterized by an explosive increase in interest in additional factors determining their selectivity and sensitivity. These include modification of the surface of the electrode as a primary signal transducer, and the substrate for the localization of the biochemical receptor. The disadvantages of electrochemical biosensors include sensitivity to radiation, as well as to fluctuations in temperature and pH of the solution.
6.2.2 Optical Biosensors

In optical biosensors, the analytical signal is caused not by the chemical interaction of the component being determined with the sensitive element, but by the measured physical parameters—the absorption and reflection of light, the luminescence intensity of the object, etc. The principle of operation of optical biosensors is based on recording changes in the optical properties of the medium: optical density (densitometric biosensors), color (colorimetric biosensors), turbidity (turbidimetric biosensors), medium refractive index (refractometric biosensors), and other properties as a result of the presence of a biological agent. Currently, optical biosensors are most developed, based on a change in the direction of propagation of the light flux passing through an optical fiber or a triangular prism coated with a thin metal film. They are based on the effect of surface plasmon resonance (Deisingh 2003).

Optical biosensors, including planar waveguide sensors, were developed simultaneously with the first electrochemical devices, but for a long time, they did not receive proper attention (Evtugyn 2013). Optical fiber sensors can be divided into two large groups: internal and external sensors. Internal sensors include ones in which the transit time, intensity, or polarization of light propagating along the fiber can be modulated by an external force acting on the fiber. In external sensors, fiber is used primarily as a means of transmitting light to the substance being determined, where the properties of light (intensity, length, wave polarization) are modified, and then the modified light is removed from the measuring element through the fiber (Turner 1987).

Most optosensors are optical fibers modified with various auxiliary chemicals and biocomponents. The optical fiber is a flexible transparent layer (core) of glass (silicon dioxide, plastic) with a refractive index $n_1$, coated by the shell with a refractive index $n_2$ ($n_2 < n_1$). Due to total internal reflection, optical fibers are used as waveguides for light. Optical biosensor fibers can be used in combination with various spectroscopic methods, for example, with fluorescent ones. Chemically and bioluminescent biosensors, as well as electroluminescent sensors, provide very sensitive detection of specific substrates. The only problem existing in the detection of luminescence of a biochemical signal is the achievement of the selectivity of the system due to the numerous light interferences. Optosensors have several advantages, namely: each type of analyte can be determined using appropriate spectroscopic methods. At that, there is the possibility of remote monitoring and the implementation of non-invasive formats of biomedical sensors. The widespread use of optical biosensors is also due to the fact that they allow the analysis of very small quantities of substances and can be adapted to the analysis and detection of a large range of various biological and chemical objects (Erickson et al. 2008; Fan et al. 2008). However, some disadvantages make their development difficult. These include interference with ambient light, possible photobleaching of dyes and other auxiliary components, the high optical density of the background, the fluorescence of the fiber, a fairly long measurement time, and the limited availability of accessories.
An optical immunosensor has also been developed to detect the biomarker of non-structural protein 1 (NS1) dengue in clinical samples obtained in the early stages of infection. The principle of operation is based on the determination of the NS1 antigen by immunofluorescence using fluorescein isothiocyanate (FITC) conjugated to an IgG antibody. The sensor is characterized by high reproducibility (relative standard deviation of 2%) and good stability for 21 days at 4 °C with a detection limit of 15 ng/mL (Darwish et al. 2018).

The fluorescence method has made great progress in the construction of sensitive sensors, but the background fluorescence of the matrix and photobleaching limit its broad application in clinical diagnosis. Wu et al. (2019) proposed a digital single virus immunoassay for multiplex virus detection by using fluorescent magnetic multifunctional nanospheres as both capture carriers and signal labels. The super-paramagnetism and strong magnetic response ability of nanospheres can realize efficient capture and separation of targets without sample pretreatment. Due to their distinguishable fluorescence imaging and photostability, the nanospheres enable single-particle counting for ultrasensitive multiplexed detection. Based on multifunctional nanospheres and digital analysis, a digital single virus immunoassay was proposed for simultaneous detection of H9N2, H1N1, and H7N9 avian influenza virus without complex signal amplification, whose detection limit was 0.02 pg/mL.

The highly efficient detection of the Human herpes simplex virus type 1 (HSV) UL27 gene through the programmed assembly of superparamagnetic (SPM) nanoparticles based on oligonucleotide hybridization was demonstrated (Li et al. 2020). The state of assembly of the SPM nanoparticles was determined by optical signature of the synchronized motion on the beads on a micromagnetic array (MMA). This technique has been used to identify <200 copies of the HSV UL27 gene without amplification in less than 20 min.

In the late 60s of the twentieth century, E. Kretschmann showed the possibility of excitation of surface plasmons by polarized light, which served as an impetus for the development of the method of surface plasmon resonance (SPR). After 10–20 years, using the phenomenon of plasmon resonance, a number of researchers have shown the possibility of using the method to study biological objects, including viruses. The advantage of this technology is the ability to observe almost any intermolecular interactions in real-time, without using special tags (Homola 2006; De Mol and Fischer 2010).

SPR is a phenomenon that occurs at the phase boundary, for example, a glass prism—a metal film. Part of a light passing through a prism and falling at a certain angle on the metal surface propagates in a metal film in the form of a damped electromagnetic wave, which causes collective oscillatory movements of free electrons. The connection of the studied object with the surface of the metal film leads to a change in the dielectric constant and, consequently, to a change in the angle of spatial resonance. The change in the angle of spatial resonance can be monitored in real-time, obtaining information on the kinetics of interactions that occur on the surface of a metal film (Garcia-Aljaro et al. 2008; De Mol and Fischer 2010).

In the majority of biosensor SPRs used today, surface plasmons are excited using a prism scheme, which is widespread due to the simple implementation and the
possibility of using methods with various types of signal modulation (see Fig. 6.1c) (Kretschmann et al. 1968; Kanso et al. 2008; Mamichev et al. 2012).

Biosensors of this type allow the study of a variety of intermolecular ties and affinity. In general, SPR can be attributed to express methods. For example, in the work (Garcia-Aljaro et al. 2008) showed the possibility of detecting bacteriophages of *Escherichia coli* using a two-channel microfluidic SPR sensor in real-time. Biotinylated *Escherichia coli* WG5 cells were applied to a gold film using avidin as a ligand. Bacterial cells immobilized in this way were used as targets for the selective detection of coliphages. After adding bacteriophages isolated from wastewater to this system, their binding to cells was observed. The sensitivity of this method was $10^2$ plaque-forming units (PFU/ml), with an incubation time of 120 min.

Biosensors are widely used, the effect of which is based on giant Raman scattering (GRS) (Monzon-Hernandez and Villatoro 2006; Liu 2007). One of the most promising applications of GRS is to study the structural and functional features of various biological molecules, since this method is non-destructive and allows you to quickly obtain information about the chemical and structural properties of biomolecules.

Two methods for detecting biomolecules by means of GRS are most common: homogeneous—the target molecule forms a bond with metal nanoparticles in the solution, which play the role of cattle “amplifiers” (Fig. 6.2a), and heterogeneous—the solution of the analyzed molecules is placed on the surface with GRS active centers (Fig. 6.2b). The advantages of the first method are the high reaction rate and the relative ease of implementation, as well as the uniformity and repeatability of the obtained amplification of the GRS signal, since nanoparticles can be synthesized with a high degree of repeatability of their parameters. In such GRS systems, metal nanoparticles with various types of shells (Jackson et al. 2003) and nanorods (Nikoobakht 2003) are often used as GRS-active substrates.

The GRS technique is used for various biochemical analyzes, including immunoassay, the purpose of which is to detect a specific interaction between antibodies and antigens. In Xu et al. (2004), hepatitis B virus antigen was detected by using gold nanoparticles equipped with immunolabels that were adsorbed on a GRS-active silver substrate.

Progress in the use of an electro-optical (EO) sensor for transmissible gastroenteritis virus (TGEV) detection with help of specific antibodies without their immobilisation on the sensor surface was described in Guliy et al. (2020). The EO signal is an information parameter that characterizes the change in the electrical characteristics of suspended particles (viruses) under the influence of an electric field. During the surface interaction of viral particles with antibodies, the surface electrical properties change, which leads to a change in particle polarization, orientation, and optical response. In the method used for the optical recording of interaction effects, the response is used in the form of a change in the attenuation of monochromatic unpolarized light when the virus-specific antibody complexes are oriented. The limit for reliable virus detection is $10^4$ virus particles/ml, and the time of analysis is 10–15 min.
Rossi et al. (2007) demonstrated the possibility of detecting the bacteriophage MS2 using a biosensor based on thin films of nanoporous silicon. Due to the advantages of nanoporous silicon—the simplicity of the technology and extremely high surface area—it is an ideal basis for the manufacture of sensors. Antibodies were immobilized on porous films by covalent bioconjugation and then dye-labeled.

Fig. 6.2 The principle of action of the biosensor for detecting biomolecules by means of giant Raman scattering (GRS). (a) the homogeneous variant (the target molecule forms a bond with metal nanoparticles in the solution, which play the role of cattle “amplifiers”) and (b) the inhomogeneous variant (the solution of the analyzed molecules is placed on the surface with GRS active centers). (c) General scheme of a sensor with an acoustic Love wave.

Rossi et al. (2007) demonstrated the possibility of detecting the bacteriophage MS2 using a biosensor based on thin films of nanoporous silicon. Due to the advantages of nanoporous silicon—the simplicity of the technology and extremely high surface area—it is an ideal basis for the manufacture of sensors. Antibodies were immobilized on porous films by covalent bioconjugation and then dye-labeled.
bacteriophages MS2 were introduced into the system. During the measurement of fluorescence, the possibility of detecting viral particles in the amount of $2 \times 10^7$ PFU/ml was established.

### 6.2.3 Acoustic Biosensors

Recently, piezoelectric resonators or delay lines with a propagating surface or plate acoustic wave have been widely used to create biosensors. Such biosensors are sensitive to changes in the mechanical or electrical properties of a biological object contacting with the surface of the waveguide. Acoustic biosensors are most often made on the basis of piezoelectric crystals such as quartz, lithium niobate, or lithium tantalate, since they are characterized by high chemical resistance. Acoustic waves excited in a piezoelectric medium may be used for creating a whole family of sensors characterized by high sensitivity, speed of analysis, low cost, and small sizes.

Some methods are based on the use of Abs or membranes as a receptor deposited on the surface of a piezoelectric waveguide or resonator (Ballantine et al. 1997). For example, using the immobilization of the corresponding antiviral antibodies on the surface of a piezoelectric resonator, an immunosensor was developed for the selective detection of herpes viruses in human blood (Koenig and Graetzel 1994), as well as in natural water reservoirs (rivers, sewers, wastewaters) without preliminary processing of the analyzed substrate (Bisoffi et al. 2008).

The work (Uttenthaler et al. 2001; Kurosawa et al. 2006) showed the possibility of detecting bacteriophages by biosensors representing a piezoelectric resonator based on crystalline quartz ($\text{SiO}_2$). The surface of the crystal is coated with antibodies (Abs) specific for the bacteriophage, and in the course of a specific reaction on the surface of the crystal, the resonant frequency of the vibrations changes, which is detected by the biosensor.

Tamarin et al. (2003), using elastic Love waves with horizontal shear polarization in a layered medium, showed the possibility of detecting bacteriophages M13 in real-time. Initially, irreversible immobilization of Abs specific for bacteriophage M13 was performed on a silicon oxide substrate. Then, an immunoreaction was carried out between the bacteriophage M13 and the immobilized Abs, and the resulting multilayer structure appeared on the surface of the waveguide, leading to a change in the velocity, and attenuation of the Love wave was analyzed. The general scheme of the sensor based on Love waves is shown in Fig. 6.2c. As a control for counting the bacteriophage titer, particles bound to Abs on the surface of the sensor were eluted by changing the pH of the solution. In this case, the number of PFU was evaluated by microbiological methods.

Matatagui et al. (2014) considered the possibility of using a Love-wave immunosensors in combination with a microfluidic chip to determine the bacteriophage M13. Along with the use of active layers, the possibility of studying biomolecular interactions in solutions in direct contact with the surface of the waveguide was shown. This significantly reduces the time required for detecting the test sample.
For example, a biosensor for detecting endotoxin in various solutions was created on the basis of a resonator with a longitudinal electric field (Muramatsu et al. 1989).

It has been shown that an acoustic sensor with a surface acoustic wave allows to detect the Ebola virus (Baca 2004). The absence of an active layer in the sensor led also to the possibility of its multiple uses. A biosensor based on a piezoelectric resonator with a longitudinal electric field was developed and successfully tested to detect hepatitis B virus (Zhou et al. 2002).

The possibility of using an acoustic biosensor based on lithium tantalate to quickly detect human immunodeficiency viruses (HIV) and differentiation between two different serotypes of HIV-1 and HIV-2 in complex matrices such as human blood has been demonstrated (Bisoffi 2013). This is extremely important for emergency assistance, which requires quick and reliable testing for the presence of blood-borne pathogens.

Piezoelectric resonators with a lateral electric field, in which there is no contact of the material under study with metal electrodes, are very promising for the study of biological objects (Fig. 6.3 a). These resonators are used to study the properties of various liquids, including biological ones. Such a resonator is a piezoelectric plate with two electrodes deposited on one of its side. The test suspension is in contact with the opposite side of the plate. It is known that a change in the viscosity and conductivity of a contacting liquid leads to a change in the characteristics of such a resonator (Zaitsev et al. 2015).

This allows the analysis of biological objects directly in the liquid phase without applying specific Abs to the surface of the resonator. On this basis, the possibility of detecting bacteriophages using specific microbial cells, antibodies, and phage mini-antibodies directly in the liquid phase has been shown (Zaitsev et al. 2012; Guliy et al. 2016a, b; Guliy et al. 2017a; Guliy et al. 2018; Guliy et al. 2019). It was found that the lower limit of detection of bacteriophages was $10^6$ phages/ml with an analysis time of $\sim$10 min. The degree of change in the characteristics of the resonator depends on the number of phage particles, which opens up prospects for conducting not only a qualitative but also quantitative analysis of bacteriophages. The obtained data showed the possibility of using the value of the real or imaginary part of the electrical impedance at a fixed frequency near the resonance as an analytical signal. Sensor capabilities have been demonstrated for bacteriophages belonging to different taxonomic groups.

For example, filamentous bacteriophage of class I M13K07 belongs to the Inoviridae family, and the bacteriophages $\Phi$AI-Sp59b and $\Phi$AI-SR65 correspond to the Podoviridae family. A criterion for confident registration of a specific interaction was developed, which consists in the fact that the change in the module of the electrical impedance of the sensor should be at least $\sim$5% when a certain amount of Abs is added to the suspension of bacteriophages. The advantage of a piezoelectric resonator with a lateral electric field is the possibility of its multiple use. This is because the resonator is made of a lithium niobate crystal, which is chemically resistant to almost all chemical compounds. In addition, the surface of the resonator, processed according to the 14th class of purity, does not allow any adsorption. Therefore, after washing the resonator, no trace of the suspension remains on its surface, and
Fig. 6.3  (a) The scheme of an acoustic sensor based on a piezoelectric resonator with a lateral electric field. (b) The general scheme of the cantilever sensor. (c) The scheme of a detecting system based on a microwave resonator: 1—segment of the rectangular waveguide; 2—plate of lithium niobate; 3—sensitive layer with immobilized cells; 4—coaxial waveguide adapter
confirmation of this is the complete restoration of all its characteristics after all experiments.

Obviously, for reliable determination of viral particles, one should select a specific receptor in each case. Currently, specific antibodies immobilized on carriers are most often used to detect viruses. Improvements in this area of research have focused on the development of platforms for immobilizing antibodies, such as micro- or nanochips, in which several agents can be identified in various ways. The development of virus detection technologies is focused on improving the sensitivity, cost-effectiveness, and reusability of the sensor.

Ideally, in order to elicit the most effective response to a virus, a “speed-type” biosensor network is needed to quickly receive an initial warning of the presence, spread, and virality of an infectious agent. To achieve this, it is desirable to use a portable biosensor with high sensitivity and accuracy, which can detect viruses in real-time. Continued research to improve probes and platforms should lead to the creation of effective biosensors that can be used in real samples. In this context, the method of electroacoustic analysis based on a piezoelectric resonator with a lateral electric field has shown the promise of its application for solving the problems of virus detection. Further standardization and automation of the electro-acoustic analysis method will expand the range of its application and use in microbiology, biotechnology, veterinary medicine, medicine, and phage therapy.

6.2.4 Cantilever Biosensors

Compact and autonomous sensors based on microcantilevers integrated into microfluidic chips have prospects for use as personalized diagnostic devices (Vasan et al. 2013; Kolesov et al. 2016). The main element in such a sensor is the cantilever, that is, high-quality mechanical resonator made of a piezoelectric rod of the rectangular cross-section. One end of the rod is mechanically fixed, and the other is mechanically free. Since the length of the rod is significantly greater than its shear dimensions, it is able to perform bending vibrations. When the microprobe moves along the surface of the sample, the spike tip rises and falls, outlining the surface microrelief, similar to the way a gramophone needle slides along a gramophone. At the protruding end of the cantilever (above the spike), there is a mirror area onto which the laser beam is incident and reflected. When the spike lowers and rises on surface irregularities, the reflected beam is deflected, and this deviation is detected by the photodetector. Photodetector data is used in a feedback system that provides a constant pressure force of the tip on the sample. Thus, the cantilever is a high-Q resonator, the resonant frequency of which depends on its effective mass and material stiffness. Figure 6.3 (b) as an example presents the general scheme of a cantilever sensor.

The dynamic mode of operation of cantilever sensors is based on a change in the resonant frequency of the cantilever during a specific interaction of analyte molecules with the receptor layer, which leads to an increase in the mass of the resonator.
To create a biological multifunctional sensor, you can use a system of cantilevers tuned to various microorganisms and viruses.

In the past few years, the development of biosensors for detecting viruses in the environment has become popular. For example, Timurdogan et al. (2011) described the use of a cantilever biosensor to detect hepatitis A and C viruses in bovine serum. In this case, antibodies specific for hepatitis A and C viruses were immobilized on cantilevers with different resonant frequencies. The detection limit was shown to be 0.1 ng/ml (1.6 pM). Gupta (2004) has shown that the sensitivity of a cantilever to a change in its mass can be quite sufficient for the detection of a single vaccinia virus if a specific sensory layer is not used.

Gorelkin et al. (2015) reported the possibility of detecting duck virus A in a static mode using a cantilever modified with a synthetic glycopolymer containing sialic acid residues. The authors believe that the polymer layer on the surface of the cantilever creates a matrix that increases surface tension due to additional interaction with viral particles. The detection limit in this system was $10^6$ virions/ml. The resonant-mode piezoelectric cantilever sensor was used to detect hepatitis C virus helicase with a concentration of 100 pg/ml (Hwang et al. 2007). Helicase is an enzyme responsible for the deployment of viral RNA, and it is specific for this virus. RNA aptamers representing short nucleotide sequences capable of specifically binding an antigen (protein) also were used as a receptor. They can be easily synthesized and are more shelf-stable than antibodies.

Thus, cantilever biosensors provide a promising platform for creating highly sensitive and selective sensor devices. However, under operation in liquids, the cantilever sensor operating in dynamic mode has low sensitivity due to a decrease in the quality factor of bending vibrations. As for sensors operating in static mode, they are highly susceptible to changes in external influences: changes in the flow rate of the analyzed liquid, changes in temperature, etc.

### 6.2.5 Microwave Resonator for Detecting Bacteriophages

Bacteriophages are an excellent model in developing methods for detecting viruses. Some types of bacteriophages have a wide spectrum of lytic activity and infect only certain strains of one bacterial species, while others are characterized by multiple virulences. Due to receptors located on the cell surface, bacteriophages are recognized and attached only to specific bacterial cells. This principle can be applied to the detection of bacteriophages using biosensor methods. Studies in the field of biosensors have shown that it is advisable to use microbial cells as a biologically sensitive element in sensors. Microorganisms immobilized in various ways on carriers, in combination with an electrophysical sensor, can represent simple, sensitive, high-speed biosensors.

The study by Guliy et al. (2017b) showed the possibility of determining bacteriophages using a detection system based on a microwave resonator operating in the
frequency range 5–8.5 GHz. The circuit of such a resonator is shown in Fig. 6.3 (c). The electrodynamic resonator is a segment of a rectangular microwave waveguide, which is bounded by a short-circuit metal plate on one side. A plate of lithium niobate with a porous polystyrene film containing immobilized cells is set on the other side. When a small amount of a suspension containing bacteriophage specific to immobilized cells was applied to the polystyrene plate, the reflection coefficient of the wave from the resonator changed significantly. The detection limit of such a sensor was $10^6$ phages/ml, with an analysis time of about 10 min.

6.3 Conclusion and Future Perspectives

Viral infections still pose a danger to humanity, which stimulates the development of new rapid methods for their detection. There is no doubt that the medical manifestations of the disease, confirmed by biochemical, microbiological, and animal tests, remain the gold standard in clinical diagnostic laboratories. However, the number of methods to obtain information on viral danger is steadily growing. At that, the main requirements for new methods, in addition to high sensitivity, are the ability to analyze a large number of samples in a short time, as well as in “field conditions.”

The use of biosensors for the signal indication of viruses will prevent the spread of viral infection due to fast and timely pro-anti epidemic measures. In general, the biosensor methods for determining viruses can be developing in two directions: with the immobilization of the components of the analysis and without their immobilization. Each of these areas has its own advantages and disadvantages. The main point for all sensors is that the sensors allow to clearly distinguishing between situations when viruses interact with specific reagents from control experiments when this interaction does not occur. The biosensors considered, being highly sensitive to virus detection, allow working with different taxonomic groups. In addition, biosensors allow you to measure in real-time, conduct analysis without the use of markers, and reuse one chip for analysis. They also have high-performance stability and reproducibility of results. Low cost, small sample requirements, and the possibility of miniaturization justify their growing development.

An analysis of the scientific literature on the research and development of technologies in this direction shows the significant potential of acoustic biosensor systems for detecting viruses. Despite the fact that acoustic sensors are used to detect microbial cells, their use for detecting viruses is described very poorly. Acoustic sensors can analyze viruses directly in a liquid without immobilizing analysis components on the surface. This advantage allows for avoiding the procedure for optimizing the process of immobilization and selection of the sensor surface. The acoustic assay constitutes a specific and sensitive alternative to other methods for virus detection. The indisputable advantages of acoustic sensors are simple sample preparation, sensitivity, efficiency, and the possibility of multiple uses. Further
standardization and automation of the electro-acoustic analysis method will expand the range of its application for virus detection.

It should be emphasized that all developed biosensor methods are universal and can be adapted to detect viruses belonging to different groups. In the future, with the development of methods for the specific treatment of viral diseases, test systems developed on the basis of biosensor diagnostics will undoubtedly find application in clinical practice for making a diagnosis and prescribing adequate treatment. Thus, the development of sensor technology for the analysis of viral particles can be used in microbiology, biotechnology, veterinary medicine, medicine, and phage therapy.

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