



## Sensor for ampicillin based on a microwave electrodynamic resonator

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### ABSTRACT

The paper describes a new biological sensor which represents a resonator based on a segment of a rectangular waveguide of 8 GHz band with shear dimensions of  $28.5 \times 12.6 \text{ mm}^2$ . On one side, the resonator is bounded by a metallic short-circuited wall; on the other side, it is bounded by a lithium niobate plate with a porous polystyrene film. This film, applied by centrifugation and modified in high-frequency discharge plasma in argon, was used to immobilize cells of *Escherichia coli* K-12. This resonator was connected through a coaxial-waveguide adapter to the *S* parameter meter, by means of which the reflection coefficient  $S_{11}$  in the plane of the lithium niobate plate was measured. The addition of an aqueous solution of ampicillin at 4–50  $\mu\text{g/ml}$  to immobilized cells led to a significant change in the reflection coefficient of  $S_{11}$  from  $-10.15 \text{ dB}$  to  $-15.09 \text{ dB}$ . At the same time, the resonance frequency changed insignificantly within the range 8.06–8.068 GHz. The optimal time for modifying the polystyrene film for obtaining the required porosity and the optimal time for the immobilization of the bacterial cells were determined. The immobilized cells retained their activity for 4 months at a temperature of 4 °C. The study showed the promise of such a biosensor to determine  $\beta$ -lactam antibiotics in aqueous solutions by using ampicillin as an example. The limit of detection of the developed biosensor with respect to ampicillin was established (4  $\mu\text{g/ml}$ ).

### 1. Introduction

Antibiotics are widely used in medicine, veterinary practice, and food industry (conservation of foodstuffs and their treatment during transportation). An important problem is the control of the content of antibiotics in drugs and antibiotic detection in biological liquids of humans and animals, foodstuffs, and wastewater from pharmaceutical and other facilities.

At present, various methods are widely used to detect antibiotics and some of them are listed below.

Traditional microbiological methods, based on inhibiting the growth of microorganisms in the presence of antibiotics, are highly sensitive but require an analysis time of 2–3 h (Riediker et al., 2001; Methods for the determination of susceptibility of bacteria to antimicrobial agents, 1998). However, the wide specificity does not allow the identification of individual antibiotics; therefore, such methods are used, mainly for quality control.

Chromatographic methods are more suitable for the identification of antibiotics, because they are highly selective. However, these methods require significant time for analysis and are very expensive (Sorensen et al., 1997).

Enzymatic methods are characterized by an analysis time of about 20 min and are based on the specific effect of inhibiting the activity of certain enzymes in the presence of  $\beta$ -lactam antibiotics (Althaus et al., 2001). Immunoenzyme methods of analysis have found wide application for the determination of residual amounts of antibiotics, especially as routine screening methods (Gazzaz et al., 1992; Martlbauer et al., 1994).

For the determination of several types of penicillin, including ampicillin, amoxicillin, cloxacillin, dicloxacillin, and flucloxacillin sodium in the pharmaceutical dosage forms, the spectrophotometric method has been proposed (Amin, 2001). This method is based on measuring the change in the optical absorption ( $\lambda = 323\text{--}346 \text{ nm}$ ) owing to the reaction of penicillins with a solution of 1, 2, 4-triazole containing mercury chloride (II). It is applicable mostly for the determination of substances in drug form.

The spectrophotometric and spectrofluorimetric procedures for the determination of four types of penicillin (amoxicillin, bacampicillin, piperacillin, and sultamicillin) and ten cephalosporins in drugs are also known. They are based on the oxidation of antibiotics by cerium (IV) at 100 °C in a medium containing  $\text{H}_2\text{SO}_4$  and include the measurement of light absorption of cerium (IV) at  $\lambda = 317 \text{ nm}$  (Walily et al., 2000).

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A quantifiable effect of 10 antibiotics on biofilm inhibition measured by real-time cell analysis was demonstrated in Ferrer et al. (2016). This impedance assay was based on setting the gold micro-electrodes fused to the bottom surface of a microtiter plate. Longo et al. (2013) described a possibility of recording the effect of antibiotics on the viability of microbial cells (*Escherichia coli* and *Staphylococcus aureus*) by using a highly sensitive cantilever of the atomic force microscope.

The largest group of biosensors, used for the detection of antibiotics, is based on the use of immunochemical reactions of biological recognition. The most frequently applied immunosensors are based on electrochemical and optical effects. Although such immunosensors are very selective, the speed of analysis depends on the incubation time required to form an antigen/antibody complex. In addition, full regeneration of the sensor requires a long time interval (Fernandez et al., 2010, 2011).

A recent development in the sensing of antibiotic residues in milk is the application of molecularly imprinted polymer sensors (Lian et al., 2012; Liu et al., 2013; Yola et al., 2014). Molecular imprinting is a technique for the creation of synthetic materials containing specific receptor sites with a high affinity toward the target molecule.

For the determination of antibiotics, biosensors based on the use of aptamers are widely used (Ni et al., 2014; Song et al., 2012). Single-stranded DNA or RNA molecules, called aptamers, have a specific spatial structure and are able to recognize other molecules or to show catalytic activity. To obtain aptamers with specified properties, the technology of SELEX (systematic evolution of ligands by exponential enrichment) was proposed (Famulok and Mayer, 2011).

Nanomaterials have been widely used to immobilize biomolecules and concentrate analytes for detection with good properties, including large surface area, good adsorption capacity, and high surface activity. In recent years, nanomaterials such as carbon nanomaterials, noble metal nanomaterials, polymers have been widely applied to the development of highly sensitive and selective immunosensors, which monitor the antigen-antibody reaction for the detection of tumor markers (Lai et al., 2018c). A novel molecular imprinted polymer electrochemical sensor was successfully fabricated for the sensitive detection of the carcinoma embryonic antigen (Lai et al., 2018a).

A highly selective acetylcholinesterase (AChE) biosensor based on the ultrasensitive nano-porous electrode with pseudo carbon paste and modified with chitosan and gold nanoparticles was successfully developed and used to detect carbaryl pesticides (Liu et al., 2016).

An electrochemical biosensor based on amine-functionalized graphene for the simultaneous detection of ascorbic acid, dopamine, and uric acid in human serum is discussed in Jing et al. (2018).

A new  $\alpha$ -fetoprotein-MIP (AFP-MIP) immunosensor based on a glass carbon electrode modified with polythionine and gold nanoparticles was successfully fabricated for the sensitive detection of  $\alpha$ -fetoprotein. With the help of electropolymerization, a “polydopamine-AFP” complex was obtained by applying AFP as the template and dopamine imprinted monomer. After elution, the specific cavities were able to absorb the target molecules (Lai et al., 2018b).

Major achievements in nanotechnology have been made owing to the use of zinc oxide (ZnO) nanomaterials. The ZnO nanostructures have been used in various areas, including optoelectronic activity, catalysis, biomedical sensing, and water treatment applications (Chaudhary and Umar, 2017).

Finally, there exist a few biosensors for detecting antibiotic residues in milk based on the application of the enzymatic activity of microorganisms (Ferrini et al., 2008; Das et al., 2014; Pellegrini et al., 2004). The systems for the monitoring of the  $\beta$ -Ls are based on similar principles as the microbiological inhibition tests (Chafer-Pericas et al., 2010; Kantiani et al., 2009), with the difference that the result of the biological recognition reaction is detected quantitatively or semi-quantitatively. Microbial biosensors based on the measurement of the inhibition of bacterial growth owing to the presence of antibiotics are

also being developed and widely used (Babington et al., 2012; Beltran et al., 2015).

However, pharmacological studies with biological media require the determination of low antibiotic concentrations ( $C_{\min} < 10 \mu\text{g/ml}$ ). Consequently, a more sensitive and rapid method is needed for these purposes. In this respect, methods of the electrophysical analysis, including the microwave electrodynamic method of microbial cell analysis, hold much promise for the determination of antibiotics in liquids. We have shown previously that one can use a sensor based on the microwave electrodynamic resonator to determine viral particles and the viability of microbial cells immobilized on the surface of a thin polystyrene (PS) film (Guliy et al., 2017). Thin polymer films, including PS films, are widely used in various fields of medicine, nano- and biotechnology, and sensory technologies (Tsui, 2008; Otero, 2000). The advantages of PS films include low weight, required level of strength, high moisture resistance, resistance to low temperatures, inertness to corrosive acids and alkalis, as well as the excellent electrical insulating and dielectric properties. However, the insufficient surface energy and adsorption capacity of PS films introduces some limitations in the field of their application. The plasma technology, leading to the reduction or increase in the surface tension, decreasing the metal parts and polymerization of thin films with specified properties, allows this problem to be solved (Chu et al., 2002; Jacobs et al., 2012). The results of studying the effect of plasma treatment on the morphology and surface adsorption properties of thin PS films are given in Smirnov et al. (2017).

There have been no reports of the use of the microwave electrodynamic methods to determine antibiotics interacting with microbial cells. This report is the first to examine the interaction of immobilized cells of *E. coli* strain K-12 with  $\beta$ -lactam antibiotics by using ampicillin as an example. We used a sensor including a polystyrene film with immobilized microbial cells and microwave electrodynamic resonator. The general experimental scheme is shown in Scheme 1.

## 2. Materials and methods

### 2.1. Preparation and testing of microbial cells

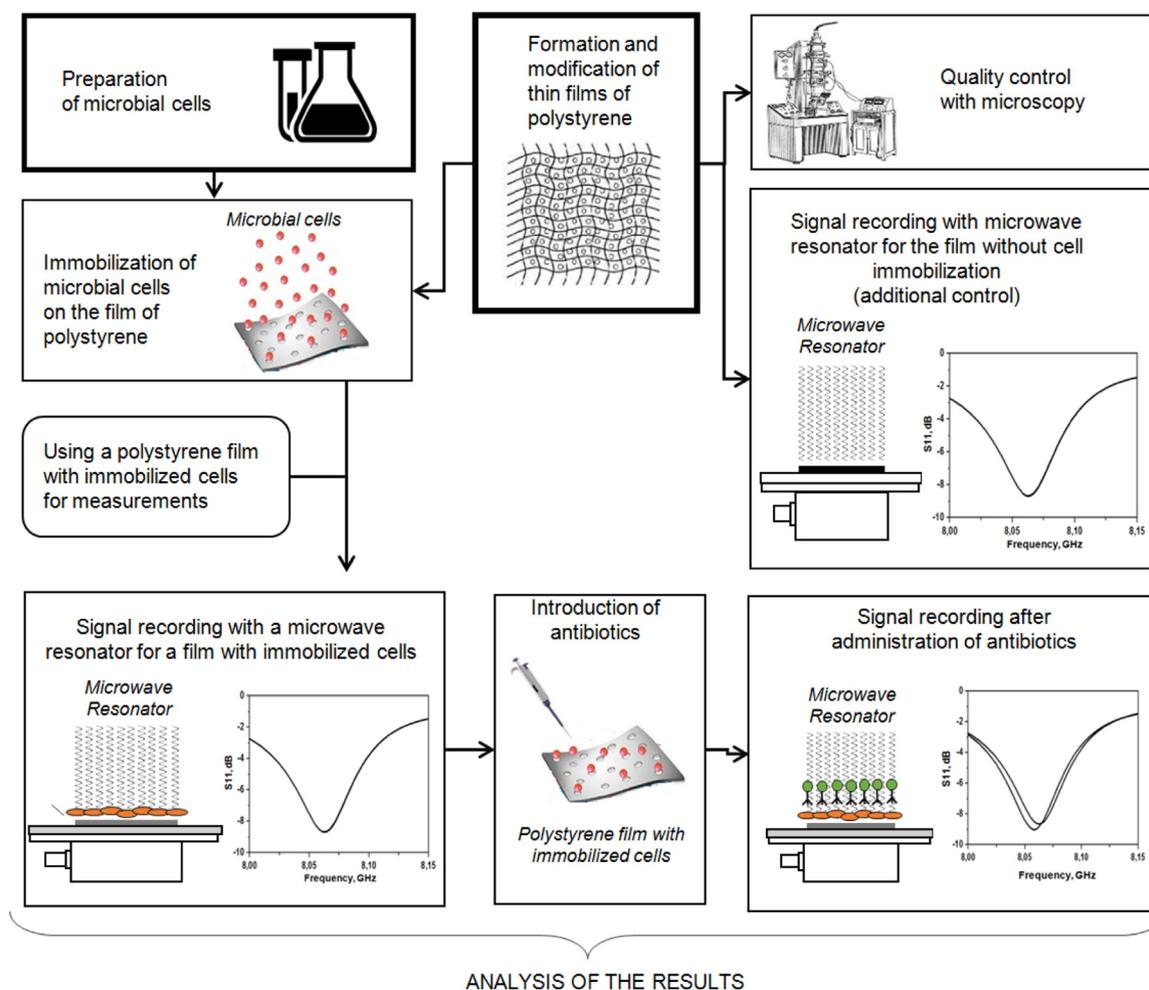
*Escherichia coli* strain K-12 was from the IBPPM RAS (Institute of Biochemistry and Physiology of Plants and Microorganisms of the Russian Academy of Sciences) Collection of Rhizosphere Microorganisms (<http://collection.ibppm.ru/>). Cultures were obtained by using a liquid LB nutrient medium composed as follows (g/l): NaCl, 5; yeast extract, 5; peptone, 10. The cells were shaken on a rotary shaker at 160 rpm and  $30 \pm 1^\circ\text{C}$  for 18 h. After the cells achieved a logarithmic phase of growth, they were rinsed with distilled water (conductivity of  $1.8 \mu\text{S/cm}$ ), separated by centrifugation at  $3354g$  for 5 min, suspended in distilled water, and used for immobilization.

The cell concentration was determined by measuring the optical absorption at 540 nm ( $D_{540}$ ) with the help of a Specol-221 spectrophotometer (Carl Zeiss Jena, Germany) in a cuvette (1-cm path length). The cell concentration was converted to that in terms of the dry cell wt ( $\text{g l}^{-1}$ ) by using preconstructed standard curves.

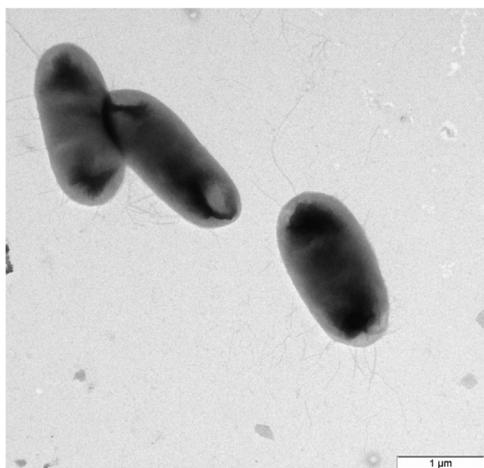
Transmission electron microscopy (TEM) identification of microbial cells was conducted with the help of a Libra 120 electron microscope (Carl Zeiss, Germany) at the Simbioz Center for the Collective Use of Research Equipment in the Field of Physical-Chemical Biology and Nanobiotechnology, IBPPM RAS. In this research, carried out by standard method (Borodina et al., 2018), the number of bacterial cells in the suspension under study was  $10^6$  cells/ml. A TEM image of *E. coli* K-12 cells is presented in Fig. 1.

### 2.2. Antibiotics

We used  $\beta$ -lactams, which belong to a large group of antibiotics. The activity of  $\beta$ -lactams is mainly determined by their ability to interact with the cell surface and change the barrier properties of the



**Scheme 1.** The general scheme of the experiments.



**Fig. 1.** TEM microbial cells *E. coli* K-12 ( $\times 10,000$ ).

cytoplasmic membrane (Antibiotic Resistance Protocols et al., 2010). Because it is active against several gram-negative bacteria, we chose *E. coli* strain K-12 for research in this work. Our previous study has shown that this strain is sensitive to ampicillin (Guliy et al., 2005).

For the study of the possibility of detection of the antibiotics by using the described sensor, we used ampicillin purchased from Sigma (USA). We prepared aqueous solutions of ampicillin with the following concentrations: 4, 10, 20, and 50  $\mu\text{g/ml}$ . These concentrations were

chosen on the basis of our earlier studies (Guliy et al., 2005).

### 2.3. Formation and modification of thin films of polystyrene

Thin films of polystyrene (PS) were obtained by centrifugation. Two types of the plates were used as the substrates. The plates of the first type, made of single-crystal silicon, had shear dimensions of  $10 \times 10 \text{ mm}^2$  and a thickness of 0.5 mm. They were used to study the effect of the plasma processing time on the efficiency of immobilization of bacterial cells on a modified surface. The plates of the second type were made of lithium niobate and were used for the study of the interaction of bacterial cells with antibiotics. PS was dissolved in carbon tetrachloride ( $\text{CCl}_4$ , 96% by weight). The mixture was applied in a sealed chamber in an atmosphere of saturated solvent vapors. The thickness of the resulting films, estimated from its cleavage by scanning electron microscopy (SEM), was  $150 \pm 10 \text{ nm}$ . The thin-film surface was modified in an Orion-40T vacuum chamber (VTC, South Korea). The substrates with a deposited PS film were located in the erosion zone of the magnetron target. A high-frequency discharge (13.56 MHz) was ignited in an argon atmosphere and plasma filled the chamber at a flow rate of 100 ml/min. The discharge power and the pressure in the chamber were 100 W and  $10^{-3} \text{ mbar}$ , respectively. Processing was performed during 10, 20, and 30 s.

### 2.4. Immobilization of microbial cells

Before the cells were immobilized, the absorbance of their suspension was checked. For cell adsorption, a substrate with a modified PS

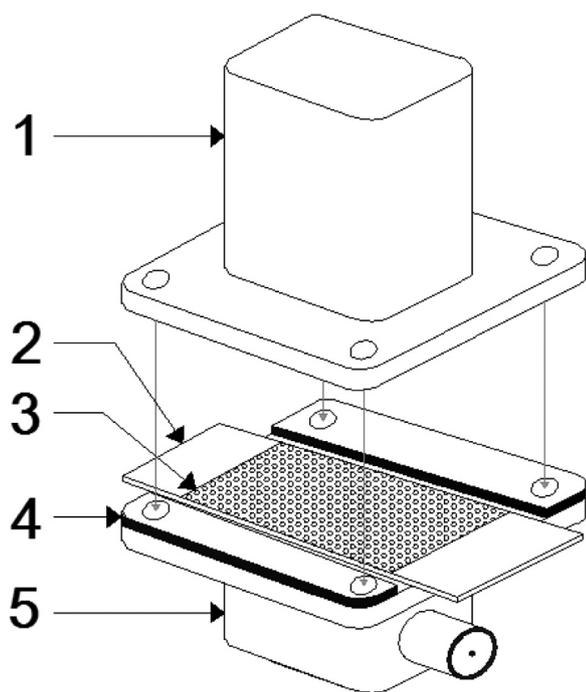


Fig. 2. The scheme of the detection system including the section of the waveguide (1), the lithium niobate plate (2), the sensitive layer (3), the guides for the exact orientation of the lithium niobate plate (4), and the waveguide-to-coaxial adapter (5).

film was placed in a cell-containing suspension. The film was incubated under aeration at a low speed of rotation at 35 °C for about 20 min. The percentage of adsorbed cells was found as the difference between their concentrations in the supernatant liquid before and after the incubation with the carrier (percentage of the cells immobilized on the carrier). After the cells were immobilized, the polystyrene surface was examined by atomic force microscopy (AFM) by using an Ntegra Spectra system (NT-MDT, Russia). The surface images were obtained in the semi-contact mode by using an NSG-10 Si probe. The scanned area was  $5 \times 5 \mu\text{m}^2$  ( $25 \mu\text{m}^2$ ). The images were processed with Gwyddion software. The electron microscopy allowed a close look at the modified surface of the film, which was represented by chainlike micro domain structures.

### 2.5. Detecting system and detection of antibiotics

The scheme of the biological sensor based on a microwave electrodynamic resonator is presented in Fig. 2. The main element was a rectangular waveguide with a length of 74 mm and a cross section of  $28.5 \times 12.6 \text{ mm}^2$  (1). One of its ends was electrically shorted with a soldered copper plate and the second one ended by a standard flange with guide rails in the form of copper polished plates 0.8 mm thick (4) for precise positioning of the lithium niobate plate (2) with a permittivity of 35–40. This plate completely filled the cross section of the waveguide. Thus, a resonance arose on the segment of the waveguide bounded on one side by a copper plate, and on the other side by the lithium niobate plate with a sensitive layer. The sensitive layer was a thin film of polystyrene (3), which was evenly applied to the surface of the lithium niobate plate and modified in the high-frequency (HF) discharge of argon. This device was connected to the input of the meter of the S-parameters E5071C (Agilent, USA) with the help of a coaxial-waveguide adapter (5) and a coaxial cable. The reflection coefficient

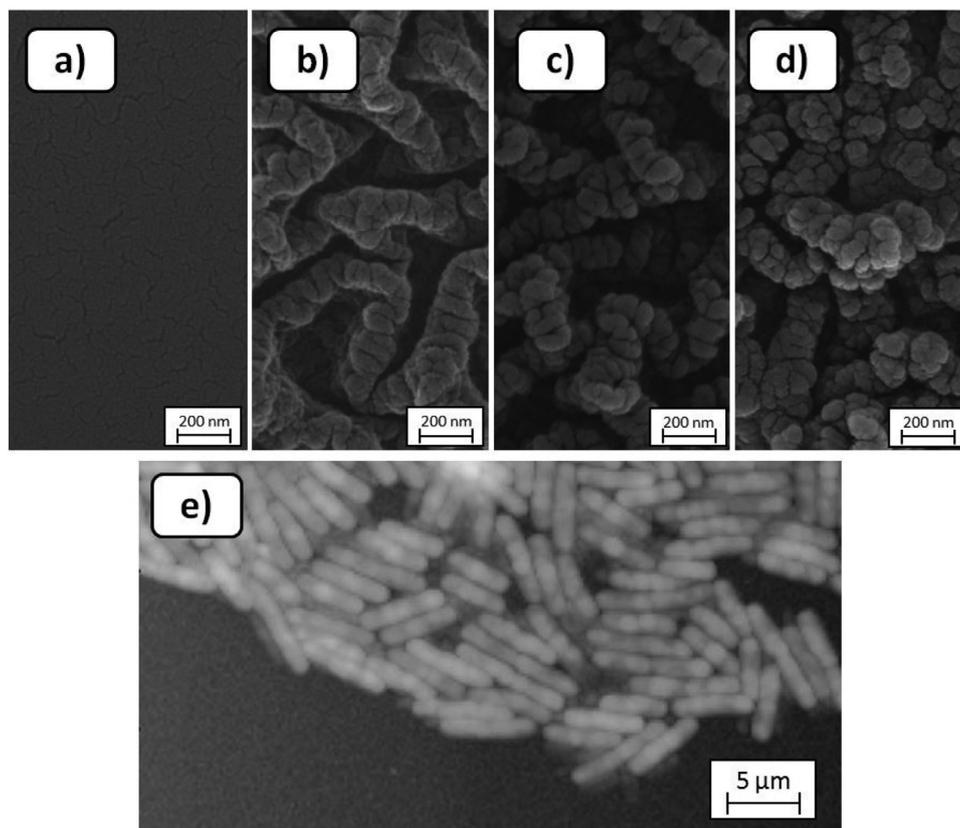


Fig. 3. The SEM image of the surface of a thin film of the polystyrene before (a) and after the modification in plasma for 10 s (b), 20 s (c), 30 s (d) and the SPM image of the film containing the immobilized microbial cells (e).

$S_{11}$  was measured in the range 5–8.5 GHz.

Detection of the antibiotics consisted in the measurement of the reflection coefficient  $S_{11}$  of the sensor with immobilized cells before and after addition of the antibiotics to the cells. The absence of a difference between the values of the reflection coefficient was an evidence of cell resistance to the antibiotics.

All experiments were carried out at least five times. The relative error of the measurements was  $\sim 2\%$ . This means that for several experiments with the same effect of the antibiotics on immobilized microbial cells, the scatter of the recorded values was  $\pm 2\%$ .

### 3. Results and discussion

#### 3.1. Study of polystyrene films

Fig. 3 shows SEM images of the surface of a thin film of polystyrene before (a) and after the modification in the plasma for 10 s (b), 20 s (c), and 30 s (d). One can see that with increase in the treatment time, the number of pores increased. These data allowed us to choose the optimal time of film treatment in plasma (30 s). It should be noted that the treatment in argon resulted in a decrease in the wettability of the surface by water. Therefore, after the creation of open pores on the surface, the process was continued in nitrogen plasma, which increased the wettability of the surface owing to the grafting of the nitrogen groups (Idage and Badrinarayanan, 1998).

#### 3.2. Immobilization of microbial cells

After the preparation of the PS films modified in the plasma of the high-frequency magnetron discharge, the conditions for the immobilization of microbial cells were optimized. This optimization included the selection of the optimal microbial loading, temperature, the pH of the solution, and the time of immobilization. As a result, immobilization of the cells on the carrier in all experiments was done under optimized conditions.

The cells were immobilized in a suspension with a conductivity of 1.8–2.0  $\mu\text{S}/\text{cm}$ . The number of adsorbed cells was estimated at 27 °C as the difference between the cell concentrations in the supernatant liquid before and after incubation. Whereas this procedure does not rule out errors in the determination of the sorption magnitudes, the reproducibility of the results was attested by their statistical analysis. For the determination of the optimal time of immobilization, we performed immobilization for 10, 20, and 30 min. Fig. 3(e) shows a SPM (scanning probe microscopy) image of the film after cell immobilization for 20 min. It can be seen that the film has immobilized cells on its surface.

#### 3.3. Biological sensor

Next, the possibility was studied of determining ampicillin by using a biological sensor based on a microwave electrodynamic resonator. A schematic representation of the detection system is shown in Fig. 2. With the aid of a waveguide-to-coaxial adapter, the microwave electrodynamic resonator was connected to the meter of the S parameters E5071C. The dependence of the reflection coefficient  $S_{11}$  on the frequency in the range 5–8.5 GHz was measured. This dependence is shown in Fig. 4 for a modified PS film that does not contain immobilized microbial cells. One can see that in the indicated range, three minima were observed that corresponded to resonant frequencies of 5.67, 6.69, and 8.07 GHz. This means that the resonator resounded at three frequencies. Theoretical analysis showed that for these resonances one, two and three half wavelengths were laid in the waveguide along the length of 74 mm of the resonator, respectively. The third resonance had the lowest reflection coefficient (–8.6 dB in power or 0.37 in amplitude). In this case, the standing wave ratio (SWR), which was  $\approx 2$ , turned out to be the smallest of all three resonances. This means that the change in the electrical boundary conditions in the plane of the

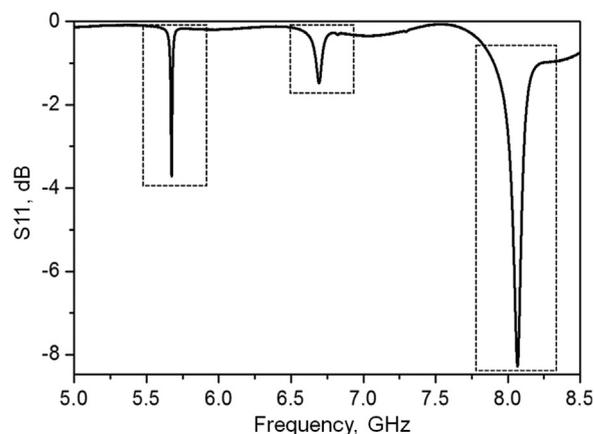


Fig. 4. The frequency dependence of the reflection coefficient  $S_{11}$  for a resonator with the polystyrene film modified in plasma without the microbial cells.

lithium niobate plate in this case should have a stronger effect on the resonator parameters, as compared to other resonances. Therefore, further study of the effect of the immobilized bacterial cells on the PS film and their biological interactions on the resonator parameters was carried out precisely for this resonance.

#### 3.4. Choice of immobilization time

After 10 and 20 min of immobilization of the bacterial cells on the modified polystyrene film, the values of the parameter  $S_{11}$  near the third resonance peak were –7.18 dB and –8.66 dB, respectively (Fig. 5). At the same time, the resonant frequency remained practically unchanged. Further increase in the immobilization time (up to 30 min) did not lead to any changes. Fig. 5 also shows, for comparison, the frequency dependence of the parameter  $S_{11}$  near the third resonant peak for a modified polystyrene film without immobilized cells.

These results allowed us to choose the optimal immobilization time (20 min). The number of cells in the initial suspension (microbial load) was about 1.32 g dry cell weight/l. The use of a higher cell concentration was impractical, because we observed a decrease in the number of adsorbed cells, and correspondingly, an increase in the

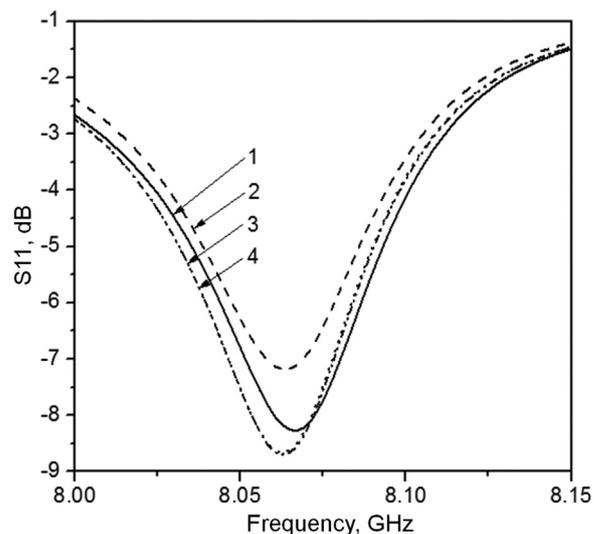


Fig. 5. The frequency dependence of the reflection coefficient  $S_{11}$  for a resonator with the modified polystyrene film (1) and the plasma-treated polystyrene film containing the microbial cells after the immobilization during 10 (2), 20 (3), and 30 (4) min.

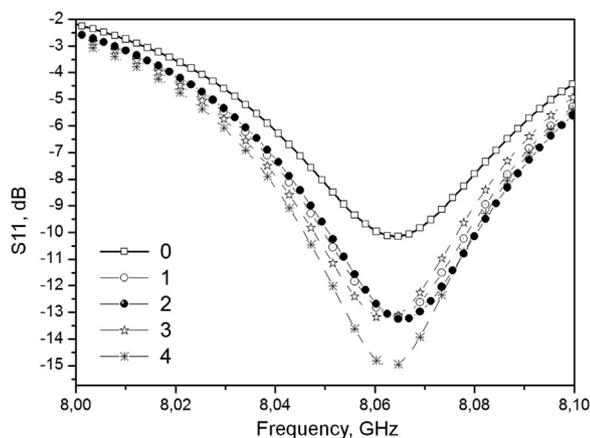


Fig. 6. The frequency dependencies of the reflection coefficient  $S_{11}$  for the resonator with the immobilized cells before (0) and after the addition of the ampicillin in the amount of 4  $\mu\text{g/ml}$  (1), 10  $\mu\text{g/ml}$  (2), 25  $\mu\text{g/ml}$  (3), and 50  $\mu\text{g/ml}$  (4).

number of cells in the free state.

### 3.5. Influence of ampicillin on the sensor's parameters

Different amounts of ampicillin (4, 10, 20, and 50  $\mu\text{g/ml}$ ) were added to the immobilized *E. coli* K-12 cells, and the frequency dependence of the parameter  $S_{11}$  near the third resonant peak was measured for each case (Fig. 6). One can see from this figure that increasing the amount of ampicillin from 4 to 20  $\mu\text{g/ml}$  changed in the minimum value of the parameter  $S_{11}$  from  $-10.15$  dB to  $-13.24$  dB. For 50  $\mu\text{g/ml}$ , the minimum reflection coefficient  $S_{11}$  was equal to  $-15.09$  dB. In all cases, the resonant frequency varied in the small range 8.06–8.068 GHz.

In our experiments, we used the interaction of immobilized cells of *E. coli* K-12 with ampicillin, a  $\beta$ -lactam antibiotic. It is known (Antibiotic Resistance Protocols et al., 2010) that this interaction is bactericidal; i.e., the antibiotic completely destroys the cells physically. In this case, the effect of ampicillin leads to a disruption of the cytoplasmic membrane of the cell and a release of the intracellular components into the extracellular space. This, in turn, leads to an increase in the conductivity of the capillary aqueous medium in which the cells are located. Thus, a polystyrene film that completely fills the waveguide cross section increases its conductivity and the degree of shunting the electric field of the electromagnetic wave propagating in the waveguide. As a result, the coupling coefficient of the resonator with the waveguide decreases, which leads to a change in the reflection coefficient of the electromagnetic wave from the entrance to the resonator, i.e., to change the parameter  $S_{11}$ . Thus, if, after the addition of an antibiotic, the  $S$ -parameter meter records a change in the reflection coefficient  $S_{11}$ , this means that the immobilized *E. coli* K-12 cells are not resistant to this antibiotic.

Thus, one can see that the effect of ampicillin leads to a significant change in the minimum value of the reflection coefficient  $S_{11}$  near the resonant frequency. This means that with the help of a sensor based on the microwave electrodynamic resonator, one can estimate the sensitivity of immobilized microbial cells to ampicillin.

### 3.6. Influence of time of exposure to ampicillin on the sensor's parameters

We next examined the dynamics of the change in the physical parameters of a film of polystyrene with the immobilized *E. coli* K-12 cells owing to the influence of ampicillin for different times of influence: 10, 15, 20, 25, and 30 min. The experiment allowed us to determine the optimal time of cell exposure to the antibiotic, which was found to be 15 min Fig. 7 shows the results of a change in the microbial

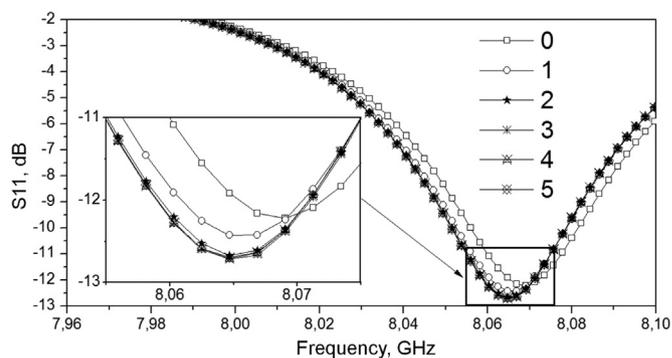


Fig. 7. The frequency dependencies of the reflection coefficient  $S_{11}$  for the resonator with the immobilized cells before (0) and after the interaction with the ampicillin (50  $\mu\text{g/ml}$ ) during 10 (1), 15 (2), 20 (3), 25 (4), and 30 (5) minutes.

biocatalyst when ampicillin was added at 50  $\mu\text{g/ml}$ . The graph shows that after 10 min of interaction, the resonant frequency decreased from 8.07 to 8.067 GHz, and the minimum value of the parameter  $S_{11}$  changed from  $-12.2$  to  $-12.45$  dB. After 15 min of interaction with the antibiotic, the resonant frequency remained unchanged, and the  $S_{11}$  value was  $-12.74$  dB. A further increase in the interaction time did not lead to a change in the observed frequency dependence of the parameter  $S_{11}$ . These experiments were carried out several times and gave the same results.

Overall, the experiments have shown that the limit of detection of the developed biosensor with respect to ampicillin is 4–5  $\mu\text{g/ml}$ , which corresponds to the requirements of the European Community for the determination of MRL of the penicillins (Council Regulation EEC no, 2377/90, 1990; Commission Regulation EU No. 37/, 2010). We hope that this limit can be significantly reduced by increasing the  $Q$ -factor of the resonator and optimizing its coupling to the waveguide.

At present, most biosensing methods focus on the detection of single antibiotic groups, but there are also studies dealing with the simultaneous determination of different groups of antibiotics. Nevertheless, in practice, when choosing antibiotics, researchers are guided by the principle of their belonging to a certain group. We conducted studies with ampicillin, which is a representative of the  $\beta$ -lactam antibiotics. The  $\beta$ -lactam antibiotics belong to the most numerous group of antibiotics. The volume of their sales in the EU is 25 % of the total volume and takes 2nd place after the tetracyclines (European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption, 2017).

### 3.7. Optimal conditions for storage of immobilized cells

Immobilized cells retain their proliferative function both immediately after immobilization and after use. In this case, the kinetic characteristics of the growth (specific growth rate and doubling time) of immobilized cells do not significantly differ from those of free cells (Beshay et al., 2011). Another important characteristic of immobilized cells, which distinguishes them from free cells, is the long-term functional activity. We examined the change in the activity of the microbial sensor during storage at 4  $^{\circ}\text{C}$ . The effect of the developed sensor with immobilized cells on ampicillin (4  $\mu\text{g/ml}$ ) was checked as described above every 2 weeks. It was found that the sensor retained its activity at 4  $^{\circ}\text{C}$  for 4 months of storage. The results are shown in Table 1.

A possible reason for the long-term preservation of microbial biocatalyst activity is the capillary condensation of the water vapor in the pores of samples of polystyrene coatings during storage. It is known that the adsorption of cells depends on the area of the available surface, which consists of macropores exceeding the size of a microbial cell (Sinityn, 1994). The polystyrene samples treated in glow discharge plasma had pores that can be considered as cylindrical capillaries with a

**Table 1**  
Activity of microbial cells immobilized on polystyrene films with respect to the ampicillin.

Microbial cells	Time of carrier processing	Activity checked in relation to specific bacteriophages							
		After immobilization	1 month	2 months	3 months	4 months	5 months	6 months	
<i>E. coli</i> K-12	0 s	+	+	+	–	–	–	–	–
	10 s	+	+	+	+	–	–	–	–
	20 s	+	+	+	+	+	–	–	–
	30 s	+	+	+	+	+	+	–	–

given radius. Thus, by lowering the storage temperature of the microbial biocatalyst, it is possible to set conditions for the capillary condensation of the moisture in the pores of the polystyrene sample, which possibly provides a nutrient medium for the long-term viability of biological objects immobilized on the sample (bacteria). An important characteristic of the microbial cells immobilized on the surface of the polymer carriers is the preservation of their activity for 4 months. It can be assumed that the carrier can act as a protective barrier for immobilized cells, preventing the negative effects of the metabolites on cells. The research described in this article showed the prospects of using thin films of polystyrene as an immobilizing agent.

#### 4. Conclusion

A new biological sensor, which represents a microwave electrodynamic resonator based on a segment of a rectangular waveguide of 8 GHz band with shear dimensions of  $28 \times 12 \text{ mm}^2$  is described. On one side, the resonator is bounded by a metallic short-circuited wall; on the other side, it is bonded by a lithium niobate plate with a porous polystyrene film. This film, applied by centrifugation and modified in high-frequency discharge plasma in argon, was used to immobilize cells of *E. coli* K-12. This resonator was connected through a coaxial-waveguide adapter to the  $S$  parameter meter, by means of which the reflection coefficient  $S_{11}$  in the plane of the lithium niobate plate was measured. The addition of an aqueous solution of ampicillin at 4–50  $\mu\text{g/ml}$  to immobilized cells leads to a significant change in the reflection coefficient of  $S_{11}$  from  $-10.15 \text{ dB}$  to  $-15.09 \text{ dB}$ . It has been shown that the cells immobilized in the porous polystyrene film retain their activity for 4 months at a temperature of  $4^\circ\text{C}$ .

Thus, the investigations have shown that the biological sensor based on the microwave electrodynamic resonator allows determination of the ampicillin in less than 15 min in a liquid volume of  $50 \mu\text{l}$  with minimum threshold of  $4 \mu\text{g/ml}$ . We hope that this limit can be significantly reduced by increasing the  $Q$  – factor of the resonator and optimizing its coupling to the waveguide. This work will be carried out in future.

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#### Conflict of interest

There is no conflict of interest about this article

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