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Acoustic sensor for detection and identification of microbial cells directly in the liquid phase

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***Abstract*—A sensor for the analysis of microbial cells based on a PZT ceramic resonator with a lateral electric field is presented. In this resonator, the shear component of the mechanical displacement, which does not lead to radiation loss at the contact with liquid, is prevailing. The frequency dependences of the real and imaginary parts of the electrical impedance of the resonator in the frequency range 50–300 kHz showed the presence of three resonances at frequencies of 68.7, 97.8 and 264 kHz. The possibility of the detection and identification of microbial cells by recording their specific interaction with antibodies, bacteriophages and mini-antibodies directly in suspension using this sensor has been shown.**

***Keywords*—detection of microbial cells, PZT resonator with lateral electric field, electrical impedance, bacteriophages, antibodies, mini-antibodies**

I. INTRODUCTION

The development of new methods for detecting microbial cells directly in a liquid (water, food liquids, etc.) is an urgent problem. The main requirements for such methods are speed, high sensitivity and accuracy [1]. Acoustic methods for the analysis of biological interactions quite satisfy these requirements [2–4]. Among a large number of acoustic biological sensors, sensors based on piezoelectric resonators with a lateral electric field are in a special position [5–11]. Such resonators are of particular interest for studying the properties of various liquids, since there is no contact of the material under study with metal electrodes. The presence of a surface free of electrodes allows the sensor to respond not only to the mechanical properties of the test sample (viscosity), but also to its electrical properties (conductivity).

Earlier, a team of authors developed acoustic biological and liquid sensors based on a resonator with a lateral electric field with a longitudinal acoustic wave based on a lithium niobate plate [8]. The promise of such sensors for detecting and identifying microbial cells directly in suspension was repeatedly shown. In these cases a detection limit of 10^4 cells/ml was successfully reached [9, 10]. Nevertheless, the problem of increasing the sensitivity of such sensors at the contact with a liquid is still urgent. This is due to the fact that the quality factor of the pointed resonator on the plate of lithium niobate in contact with the liquid turned out too low, since the excited longitudinal wave in such a resonator led to radiation loss in the liquid.

In the present work, the biological sensor including the resonator with a lateral electric field based on PZT ceramics was experimentally investigated. In this sensor the shear component of the mechanical displacement, which did not lead to radiation loss at the contact with liquid, was dominant. The possibility of detecting and identifying microbial cells by registering their specific interaction with antibodies, bacteriophages and mini-antibodies directly in suspension using this sensor was shown.

II. DESCRIPTION OF THE SENSOR AND METHOD OF THE EXPERIMENT

The sensor represented a resonator with a lateral electric field on the basis of PZT ceramic plate with thickness of 3.54

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mm and shear dimensions of 20×18 mm (Fig. 1). One side of plate was covered with an aluminum film with a gap in the center 4 mm wide, i.e. both halves of the coating served as electrodes. This resonator served as the bottom of a 4 ml liquid plexiglas container.

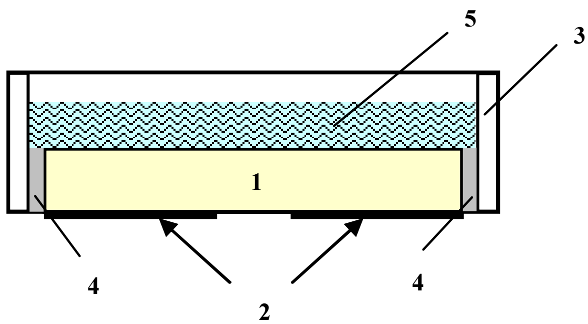

Fig. 1. The scheme of the sensor: 1 – the plate of the PZT ceramics, 2 – electrodes, 3 – the liquid container, 4 – the adhesive sealant, 5 – cell suspension under study.

For research, the resonator was connected to the LCR meter E4990A (Keysight Technologies), and the frequency dependences of the real and imaginary parts of the electrical impedance were measured in the frequency range 50–300 kHz. First, the characteristics of a sensor with an empty container were studied. Then the container was filled with distilled water and the sensor readings were repeated. At the next stage, distilled water with microbial cells was placed in the container and the sensor readings were again recorded. After this, a specific or nonspecific reagent (bacteriophages, antibodies or mini-antibodies) was added to the container with a cell suspension and the parameters of the sensor were measured. Measurements were carried out for different cell concentrations (10^3–10^8 cells/ml), as well as for different amounts of reagents added to the cell suspension.

The principle of operation of the sensor was to record the changes in the real and imaginary parts of the electrical impedance when the conductivity of the cell suspension changed due to the biological interaction of microbial cells with specific reagents.

We used the following types of specific biological interactions: “E. coli XL-1 cells – the phage M13K07”, “A. brasilense Sp7 cells – polyclonal antibodies”, “A. brasilense Sp245 cells – mini-antibodies.

Additionally, control studies were conducted on the specificity of all of the above biological interactions using standard microbiological methods.

III. Results and Discussion

First, the parameters of the sensor with an empty container were measured. The presence of three resonances near the frequencies of 68.7, 97.8, and 264 kHz was found with the values of electromechanical coupling coefficient of 15%, 14%, and 5%, respectively (Fig. 2).

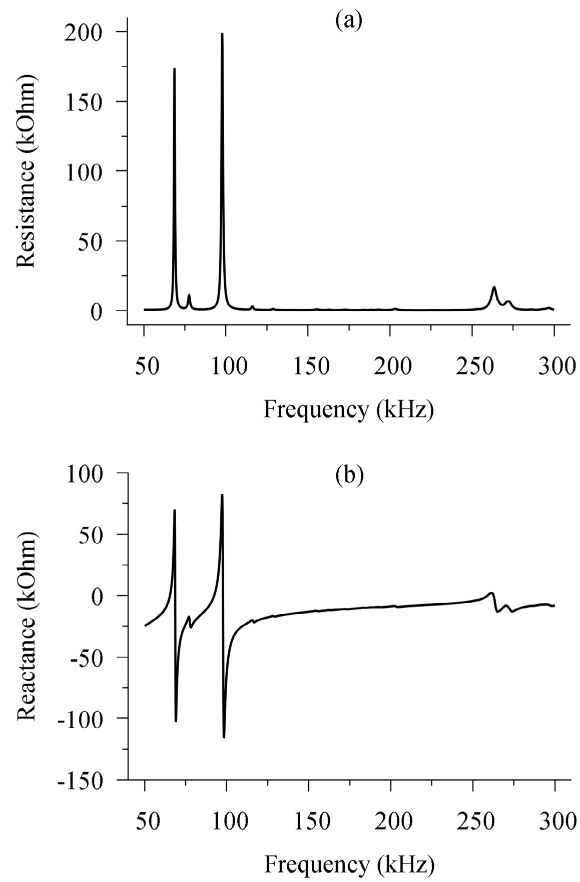

Fig. 2. The frequency dependencies of the real (a) and imaginary (b) parts of the electrical impedance of PZT resonator with empty liquid container.

Then distilled water was added to the container. It has been found that the quality factor of each resonance in contact with the liquid turned out to be significantly higher than one of the resonator with a longitudinal acoustic wave based on lithium niobate [8–11].

Then, microbial cells with a given concentration were added to the container. It was shown that the addition of microbial cells had practically no effect on the frequency dependences of the electrical impedance.

In the next step, a specific reagent was added to the cell suspension. The measurements were carried out for different amounts of reagent at a certain value of cell concentration. It has been shown that the addition of a specific reagent leads to a significant decrease in the real and imaginary parts of the electrical impedance.

In [11], it was shown that the real part of the electrical impedance of the resonator on the resonant frequency is more preferable for the use as an informative parameter for the measurement of the conductivity and viscosity of the liquid. Therefore, in this work, we also used this informative parameter.

It should be noted that the behavior of all the resonant peaks was the same. Therefore we will analyze the data on the example of one resonant peak near the frequency of 68.7 kHz.

Fig. 3 (a) shows, as an example, the frequency dependence of the real part of the electric impedance of the sensor for a suspension of A. brasilense Sp7 cells before (black curve) and after (pink curve) the addition of specific antibodies.

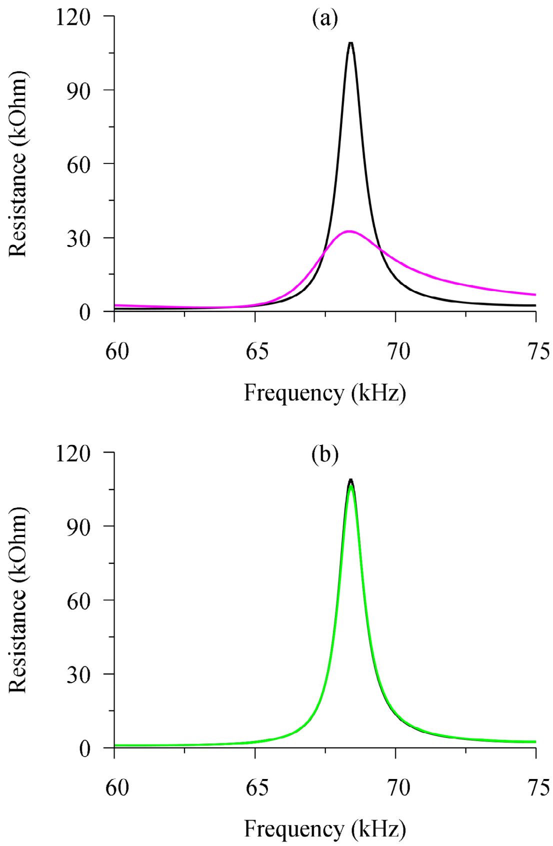

Fig. 3. The frequency dependencies of the real part of the electrical impedance of PZT resonator: (a) – container with A. brasilense Sp7 cells before (black curve) and after (pink curve) adding the specific antibodies; (b) – container with E. coli XL-1 cells before (black curve) and after (green curve) adding the nonspecific antibodies.

One can see that the value of the real part of the electrical impedance decreased significantly after the addition of specific antibodies. The change in the parameters of the sensor occurred due to an increase in the conductivity of the cell suspension due to the specific biological interaction [8–10].

As control experiments, the interactions of a cell suspension with non-specific reagents were investigated. Nonspecific interactions did not lead to a change in the parameters of the sensor. Fig. 3 (b) shows, as an example, the frequency dependences of the real part of the electric impedance of the sensor for a suspension of E. coli XL-1 cells before (black curve) and after (green curve) the addition of non-specific antibodies. It can be seen that in this case no changes in the sensor parameters are observed.

The process of interaction of microbial cells with specific and non-specific reagents is clearly visible from Fig. 4, which presents the time dependences of the maximum value of the real part of the resonator electrical impedance (R_{max}) for the specific interaction "A. brasilense Sp7 cells – specific antibodies" (red curve) and the nonspecific interaction "E. coli XL-1 – nonspecific antibodies" (green curve) for the resonance peak near 68.7 kHz.

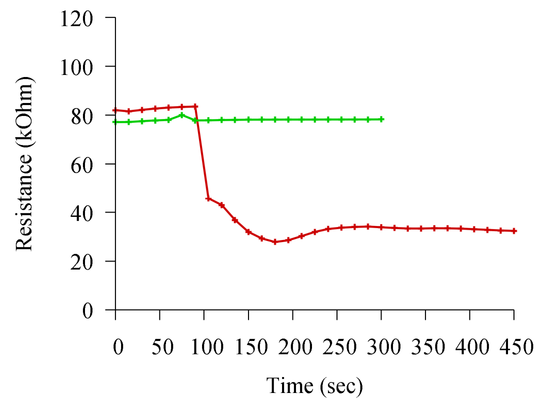

Fig. 4. The time dependences of the maximum of real part of the electrical impedance of PZT resonator during a specific interaction "A. brasilense Sp7 cells – specific antibodies" (red line) and nonspecific interaction "E. coli XL-1 cells – nonspecific antibodies" (green line) for the resonance peak near 68.7 kHz.

At the initial time, a cell suspension was introduced into the container, and sensor readings were taken at intervals of 12 seconds. It is seen that there were no changes in the value of R_{max}. Then (at 100th second), a specific/nonspecific reagent was introduced into the container with the cell suspension and the sensor readings were again recorded. It is seen that in the case of a specific interaction (red curve), the value of R_{max} decreases sharply, and then the saturation process takes place.

In the case of nonspecific interaction (green curve), no changes in the sensor parameters occurred. The analysis time in all cases did not exceed 8 minutes.

Measurements were performed for cells with a concentration of 10^3, 10^4, 10^6 and 10^8 cells/ml. A different amount of specific reagents was used in the experiments.

Fig. 5 shows, as an example, the dependences of R_{max} on the number of specific reagents added to microbial cells at a concentration of 10^3 cells/ml.

Fig. 5 (a) shows data for a suspension of E. coli XL-1 cells before (orange curve) and after (blue curve) the addition of a specific bacteriophage M13K07 in an amount of 0.2–20 phages/ml. Fig. 5 (b) presents the data for a suspension of A. brasilense Sp7 cells before (orange curve) and after (blue curve) the addition of specific antibodies in an amount of 0.5–3 antibodies/ml. Fig. 5 (c) shows data for a suspension of A. brasilense Sp245 cells before (orange curve) and after (blue curve) the addition of specific mini-antibodies in an amount of 0.5–3 mini-antibodies/ml.

It is seen that in all cases of specific biological interactions, a significant decrease in R_{max} (by 40–80 kOhm) occurs.

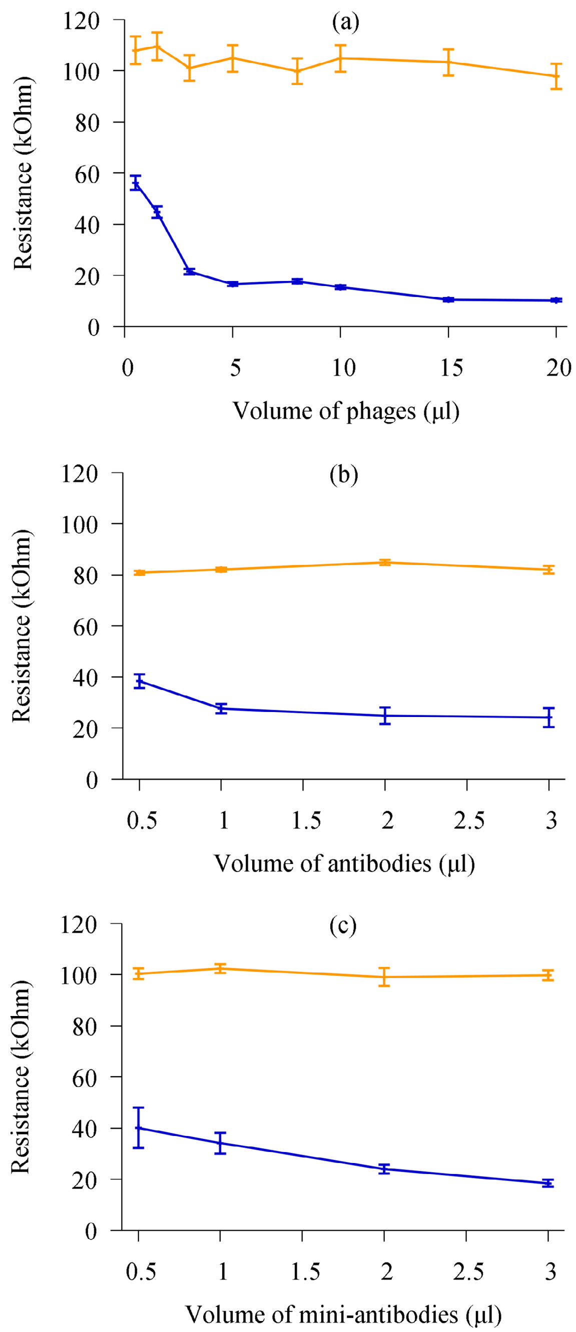

Fig. 5. Dependences of the maximum value of real part of electrical impedance of the resonator on the number of specific reagents added to microbial cells: (a) – E. coli XL-1 + phage M13K07; (b) – A. brasilense Sp7 + antibodies; (c) – A. brasilense Sp245 + mini-antibodies.

It was shown that significant changes in the real part of the electrical impedance of the sensor during specific biological interactions were observed even at low cell concentrations (10^3 cells/ml) and a minimal amount of reagents.

When microbial cells interacted with nonspecific reagents, no changes in the electrical impedance of the sensor were observed.

Studies have shown the promise of a sensor based on the RZT resonator with a lateral electric field for detection and identification of microbial cells in the liquid phase.

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