Solutions for enhancement of sensitivity and metrological reliability of conductometric biosensor systems

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Abstract

The article presents the results of research aimed at the creation of highly sensitive, compact and inexpensive biosensor analyzers based on differential conductometry. The main factors, which reduce the sensitivity of the measuring channel and cause the measurement errors, are considered; the measurement methods to eliminate or minimize their effect are discussed. The results of theoretical and experimental studies of conductometric converters of biosensors and measuring circuits are presented. The technical realization of these methods is proposed; the important characteristics of the sensors and the developed devices are described, as well as the outcomes obtained at their approbation.

1. Introduction

The use of conductometric methods for determining the concentration of electrolytes in solutions has been known for a long time in biosensorics [1–4]. As shown in the literature, the conductometric biosensor systems have high potential for a wide use and continue demonstrating advantageous analytical performance compared to other electrochemical biosensors [5–7]. This is due to their high sensitivity, the possibility of simplifying and improving the manufacturability of the necessary equipment—both the electronic measuring channels and the biosensors themselves. However, the conductometric biosensor systems were not used in practice for a long time because of low selectivity at the varying background conductivity of the solution and the effect of uninformative parameters of the conductometric converters on the measurement results. The latter was successfully reduced, though, by the differential conductometric method using two miniaturized two-electrode transducers of interdigital topology deposited on a ceramic substrate by thin-film technology [8–10]. This pair of transducers is connected to the opposite arms of the AC bridge. One of them is working, and the second is reference. The output signal of the bridge in the form of a difference in currents or voltages in these arms is functionally related to the difference in impedances of a pair of transducers, which depends on the difference in the conductivity of the solution in them.

According to the literature, the commonly used differential conductometric systems are based on the above bridge circuit and standard equipment: the selective nanovoltmeter and generator of sinusoidal signals to supply the measuring circuit as shown in a block diagram in figure 1 [11–13]. The generator has additional outputs of the in-phase and quadrature reference voltages. The measuring unit contains a 4-arm alternating current bridge, two arms of which include a differential pair of conductometric transducers and two other—the identical resistors. Voltage of the imbalance signal is received from the diagonal of the bridge by means of operational amplifiers. The informative signal (its component is in phase with the generator signal) is selected by a synchronous detector of the selective nanovoltmeter [14, 15].

For biosensor fabrication, an active membrane (comprises a bioselective material) is deposited on one pair of electrodes (denoted as S1), whereas a passive membrane (contains a relatively inert biomaterial) is deposited on
another pair of electrodes (denoted as S2) as shown in figure 2. When an analyte is added to the buffer solution, the biochemical reaction between the analyte and the bioselective material in S1 happens, which produces new ions in the working membrane, changing, therefore, the solution conductivity. The output signal of the bridge increases due to an increase in the current across the working sensor S1. The value of its component, which is in-phase with the test signal of the generator, is informative and proportional to the amount of the analyte introduced.

In principle, the use of differential conductometric method allowed the creation of valid biosensor systems for biotechnology and medicine. However, their wide application is impeded by unstable errors of unknown origin, which consequently hindered their high sensitivity, normalized and stable signal conversion with sufficient suppression of external uninformative effects, and, thus, good reproducibility of the measurement results from different devices. Additionally, the requirements for portability, energy efficiency and low cost are typical for biosensor devices. Addressing these tasks simultaneously is a difficult problem. Based on these considerations, many experts supposed the conductometric method to be of little promise for development of the biosensor devices with metrological reliability despite their inherent positive properties. This apparently explains the lack of information about other developments in this area, in addition to those described in this work.

As it was determined at the initial stage of our work, the existing problems are associated with the complex dependence of the informative signal of the measuring circuit on (i) the method of conversion of the informative parameter and the structure of the bridge circuit used for this, (ii) the operating frequency, (iii) the composition,
concentration and temperature of the test solution, and (iv) the features of measurement techniques and preparation of sensors.

The goal of the current research was to comprehensively consider the causes (origin) of these problems and to develop approaches to solve them. This article summarizes the results of research and developments on the subject matter, which are still available to a limited number of specialists. A complex of the interrelated structural and algorithmic solutions, which ensure sensitivity and reproducibility of measurement results sufficient for practical needs, is discussed.

2. Stabilization of the sensitivity of conductometric channels with differential two-electrode sensors: identifying the root causes of the problem and searching for the solution

In general, the unstable sensitivity of conductometric systems with two-electrode sensors can be accounted for the complex electrochemical processes in a conductometric cell and complicated equivalent circuit of this type of transducers \[14, 16–19\]. Briefly, the current across the transducer is defined by the sum of impedances of its three regions: two near-electrode regions and the impedance between them, which includes the active resistance of solution in the interelectrode region and the reactive resistance of the interelectrode capacitance, connected to it in parallel. The near-electrode impedances are a parallel combination of the near-electrode capacitance with the charge transfer resistance and Warburg impedance, connected in series. For comparison purposes, a schematic of such equivalent circuit will be given in figure 7(a) and discussed in section 3. It is important to remember that the result of impedance measurement as a 2-dimensional vector quantity must be represented as a two-element equivalent circuit. At frequencies that are optimal for the implementation of a biosensor system, this circuit corresponds to a serial RC chain. The operating frequencies are chosen such that the prevailing parameters of the multi-element equivalent circuit when it is represented by a 2-element circuit are the near-electrode capacitance and the active resistance of the solution. The latter is an informative parameter. The rest (uninformative) parameters should make a minimum contribution to the measured values of the equivalent parameters of the RC chain.

Figure 3. Scheme (a) and vector diagram (b) of a simple conductometric bridge.
The analyte injection to the cell results in the formation of additional active conductivity (ΔG), which is connected in parallel to the solution resistance and is an informative parameter. The voltage on the solution (outside the near-electrode regions) and the additional conductivity determine the increment of the current across the transducer (ΔI_s). Therefore, the use of bridge circuits with the current output signal is more promising.

The signal at such measuring circuit output (figure 3(a)) is the difference between the currents across the working and reference transducers, which is converted to voltage. Its informative part is the component, which is in-phase with the test signal. This component is selected by a synchronous detector (SD) controlled by the corresponding reference signal of the generator. Obviously, its value depends not only on the additional conductivity in the working sensor, but also on all parameters of the equivalent circuit of both the working and reference transducers, as well as on the operating frequency. A possible way to solve this problem to some degree is to increase the operating frequency, which reduces an influence of the resistance of the near-electrode capacitances and in general all the near-electrode impedances on the currents in the sensors. However, the influence of the interelectrode capacitance increases, the characteristics of the electronic channel transducers deteriorate, the equipment becomes more complex, the power consumption and the price of instrument increase. Therefore, the advantage of using higher operating frequency is limited. Our research has shown that the development of new measurement methods, structural and algorithmic solutions has a much greater effect.

In the work, the reasons of the significant dependence of the sensitivity of the conductometric measuring circuit on the parameters of the sensors and the operating frequency were considered using the simplest bridge circuit (figure 3(a)) and its vector diagram of currents and voltages (figure 3(b)) [20].

As previously shown, the optimal operating frequency for low-cost and portable conductometric biosensor systems ranges from 30 to 70 kHz [21]. Within this range, the frequency properties of sensors used in practice (S1 and S2) correspond quite well to a serial RC chain, and the tangents of their phase angles (tgφ) are within the range of 0.2–1. At this stage, we assume that the parameters of each transducer of the differential pair are identical. The diagram shown in figure 3(b) corresponds to the case of tgφ = 0.8. The Δ symbol denotes the vectors corresponding to an increase in the active conductivity (a decrease in R) by 10%. The diagram shows that the phase angle of the increment of the sensor current is about twice the phase angle of the sensor, and the informative signal (the projections of the ΔI_s vector on the Re axis) for this case (tgφ = 0.8) is very small. It is noteworthy that if tgφ = 1 then Re(ΔI_s) = 0. Earlier, the sensitivity of conductometric systems with differential sensors was studied in detail in simple bridge circuits [15, 20] that allowed the authors to obtain the analytical expressions of their sensitivity. In particular, the increment of the output current (ΔI_s) in the circuit in figure 3(a) can be described as follows.

Figure 4. The principle of rotation of the test voltage phase in the conductometric bridge circuit. (a)—vectors of the informative current and triangles of the voltage vectors on the sensors—full (U_A, U_P) and on the RC components of the impedances, indices 1—the initial state, and 2—the adjusted state; (b)—block diagram of the bridge.
Therefore, the decrease in the channel sensitivity within the entire range of possible values of phase angles of the working sensor is compensated by the capacitance of the working sensor. This imbalance can be reduced by adjusting the parameters of the pair of sensors. At a higher operating frequency, \( \tan \phi \) decreases and the sensitivity increases.

The method of measurement of local changes in the electrical conductivity of solutions, which was originally proposed in [20], allowed us to significantly reduce the range of sensitivity variations of a differential conductometric channel when \( \tan \phi \) of sensors varies. The bridge circuit of the device contains sensors \( R_{S1}, C_{S1} \) and \( R_{S2}, C_{S2} \), and also a \( \pi/2 \) phase shifter (e.g., an integrator with R and C standard elements, which performs conversion on modulus equal to 1 at the operating frequency), the scale converters \( K1, K2 \) (e.g., a DAC), an adder \( \Sigma \), and an inverter \( -1 \). The bridge is powered by the voltage \( U_G \) of the generator \( G \). The output signal \( U_{Out} \) of the device is obtained by converting the difference between the currents \( I_{S1}, I_{S2} \) across the sensors into a voltage in the \( I/U \) block. The test voltage \( U^* \) for sensors is formed as a sum of the generator voltage \( U_G \) and the adjustable (by \( K1 \)) voltage obtained by turning \( U_C \) on the phase angle \( \pi/2 \).

The method comprises two stages. At the first stage, the active sensor \( S1 \) is connected by the \( Sw1 \) key. At this stage, the reference (passive) sensor \( S2 \) is turned off (\( Sw2 \)). The adjustment is performed on the values of quadrature component of the bridge output signal \( U_{Out} \). The phase of the voltage \( U^* \) is adjusted by changing \( K1 \) to the state, at which the current across the working (active) sensor \( S1 \) coincides in phase with \( U_G \). In this state, \( \text{Im}(I_{S1}) = 0 \), which is achieved under the condition:

\[
\frac{1}{\omega CR_1} = \frac{1}{\omega C_{S1} R_{S1}}
\]

The vector diagram (figure 4(a)) shows the voltage triangles on the active (A) and passive (P) sensors: the voltage on the sensor (\( U_A, U_P \)) and the voltage on the elements of its equivalent circuit (indices \( R, C \)). Index 1 corresponds to the initial state of the circuit, and index 2 — to the adjusted state. In the latter, the voltage drop on the capacitance of the working sensor is compensated by the \( U_{C1} \) component of the \( U_{A2} \) voltage (\( U^* \) in figure 4(b)), whereas the stable and normalized voltage \( U_{R2A2} \), which is equal to the generator voltage, is applied to the active resistance (resistance of the solution).

At the second stage, the reference sensor is connected by the \( Sw2 \) key to the inverted voltage \( U^* \). Its current is subtracted from the current of the working sensor at the input of the current-voltage converter. In practice, the parameters of the pair of sensors are always at least slightly different, so the bridge circuit will not be balanced completely. This imbalance can be reduced by adjusting \( U^* \) (with \( K2 \)) to obtain the minimum value of the output signal of the bridge. The values \( K1 \) and \( K2 \) of the transfer coefficients of the circuit elements numerically correspond to the value of \( \tan \phi \) of the working sensor and the ratio of the impedance moduli: the reference sensor to the working one. This information can be used to diagnose the differential sensor and to correct the channel conversion characteristic.

When the analyte is added to the cell, the output signal of the bridge circuit receives an increment \( \Delta I_A \), the vector of which forms an angle \( \psi_A \) with the Re axis, which is half the angle in the simple bridge in figure 3. Therefore, the decrease in the channel sensitivity within the entire range of possible values of phase angles of the
sensors turns to be much lower (practically does not exceed 30%) and is easily corrected during data processing. The increment in the output current of the bridge \(\Delta I_A\) and the changes in its sensitivity \(S\) relative to the informative signal, depending on the values of the phase angle of the working sensor, were estimated using the equations derived from equation (1) and presented as follows.

\[
\Delta I = U_G \frac{1}{1 - j \Phi} \frac{1}{\Delta G} ; \quad S = \text{Re} \left( \frac{\Delta I}{\Delta G} \right) \frac{1}{U_G} = \frac{1}{1 + \text{tg} \Phi^2}
\]  

Depending on the composition and concentration of the buffer solution, the design of the sensors and the material of the electrodes, the real equivalent circuit of conductometric transducers may differ less or more from the serial RC chain. In the latter case, the correction of the response of the measuring channel (its sensitivity) only by the \(\text{tg} \Phi\) values of the sensors will be incomplete.

The important requirement for practical application of biosensor systems is creation of a compact measuring device, which is suitable for serial production and automation, capable of self-tuning to the parameters of sensors and proper for diagnostics during measurements. Figure 5 shows the simplified functional diagram of a measuring channel of the portable conductometric biosensor system, which we developed on the basis of a unified measuring module described in [23]. Its measuring circuit is based on the principle discussed above and its basic elements are highlighted with a dashed line in figure 5. The rest of the circuit consists of blocks of the unified module from [23]. The K1 and K2 regulators (figure 4(b)) are implemented on integral DACs (12 bit K1 and 8 bit K2). K2 ranges from 0.7 to 1.4 to balance the bridge with non-identical sensors.

The developed biosensor system software allows quick, with one command, tuning of the instrument and determining the main diagnostic parameters of the measurement cell before each measurement. They are as follows: active resistance \(R_{SA}\) of the working sensor and its \(\text{tg} \Phi_A\), the \(R_{SP}\) to \(R_{SA}\) ratio, the difference between \(\text{tg} \Phi_A\) and \(\text{tg} \Phi_P\). Additionally, the response can be corrected during measurements with calibration.

This conductometric method combines the methods of balancing and direct conversion of the bridge output signal. The instability in sensitivity at direct conversion is reduced significantly by this combination, however, is not eliminated completely. From the above vector diagrams and equations, it can be seen that the \(\text{tg} \Phi\) of the sensor affects the vector amplitude of the response signal noticeably less than its projection on the Re axis. This can be used to create more accurate differential conductometric devices. The measurement methods, structures and algorithms of the devices based on the amplitude detection of the increment of the bridge imbalance signal are described in [24]. The means of precision balancing by active and reactive parameters, as well as determining the modulus of this signal by rebalancing the bridge using an additional control element are considered too there. These solutions can significantly improve the stability of the characteristics of the conductometric channel; however, it is inappropriate to use them in biosensor systems for widespread application because of their greater complexity.

3. Guarantee of metrological reliability of conductometric biosensor systems

Improvement and stabilization of the sensitivity of conductometric channel are a prerequisite for expanding the functionality of biosensor systems and ensuring reproducibility and comparability of the measurement results obtained at different times, under different measurement conditions and with different devices. However, as the studies have shown, this is sufficient to ensure the reliability of the results (without special restrictions to the measurement conditions) only at small values of \(\text{tg} \Phi_A\) and \(\text{tg} \Phi_P\) of a differential pair of conductometric transducers (less than 0.3) and at the minor difference between them (no more than 5%). Among the biosensors used, only the transducers with platinum electrodes meet this requirement. However, platinum transducers are expensive and, thus, unacceptable for a wide application. Taking into account the technical and economic realities, transducers with gold, nickel and stainless steel electrodes are more appropriate. At acceptable frequencies, the above parameters for these transducers can be several times higher. Therefore, several series of differential pairs of these types of transducers were studied with regard to the frequency characteristics of the parameters of two-element serial equivalent circuits \((R_S, C_S, \text{tg} \Phi)\) and their changes during operation in the frequency range of 1–100 kHz [21, 25]. At this stage, our goal was to estimate the real ranges of changes in the basic electrical parameters of differential biosensors.

For this purpose, we studied the conversion characteristics of the above differential conductometric channel (figure 5) in the range of possible differences between the parameters of the working and reference transducers [26]. The results showed that the sensitivity of the working transducer was constant. Finally, we estimated the levels of errors from the common-mode interferences caused by changes in the background conductivity of the buffer solution when the analyte is added to the measurement cell and changes in temperature [27].

In particular, the transducers were studied using the P5083 universal AC bridge with the test signals less than 0.1 V. For measurements, the transducers were immersed in 5 mM phosphate buffer solution.
Figure 6. Frequency characteristics of RC parameters of a differential pair of nickel transducers (a) and their changes during operation with periodic cleaning of electrodes (b).
Figure 7. Full equivalent circuit of a conductometric sensor (a), its equivalent two-element scheme (b), and the vector diagrams of balancing the bridge with the results of influence of the common-mode interference when the parameters of a pair of sensors are not identical (c, d).
Figure 8. The advanced compensation bridge circuit.

(KH₂PO₄-Na₂HPO₄, pH 7.4). The typical frequency dependences of the parameters (Rs, Cs and $\tan \phi$) of a differential pair of transducers with nickel electrodes are shown in figure 6(a), where the transducers are marked as ‘left’ and ‘right’ pairs. The analysis of these dependences shows that the real equivalent circuit of the transducer is rather close to the two-element serial RC chain, but still it has noticeable additional elements. Figure 6(b) shows the typical changes in parameters (Rs, Cs and $\tan \phi$) of the equivalent RC circuit of a pair of transducers with nickel electrodes after their first use. In total, the figure 6(b) demonstrates a series of 9 measurement results. As indicated on the horizontal axis of the graph, the transducers were placed between measurements and kept for some time in different media (working buffer solution and distilled water). In the experiment, the electrode surfaces were periodically cleaned with ethanol; the transducer parameters measured immediately after such cleaning are marked as ‘after treatment’ (the points #1–3, and #7–9). Additionally, the transducers were investigated after incubation for 1 and 2 h in distilled water (the points #4 and #5), as well as after storage in dry form (the point #6). The obtained data show changes in the transducer parameters in the working buffer over different time period except for the measurements in distilled water. The points #3 and #7 prove the possibility of restoring the original parameters of the transducers. Thus, the data in figure 6(b) allowed us to evaluate the changes in parameters of the differential pair of transducers under the conditions that simulate the operation of real biosensors and, in particular, when replacing their biomembranes. These data were also the starting point for determining the limits of regulation of the parameters of the bridge circuit during its balancing.

As seen in figure 6(a), at the frequencies over 30 kHz, the parameters of a pair of transducers at the beginning of their use differ, as a rule, by no more than 3%, which corresponds to the technologically determined accuracy of the electrode fabrication. However, at the further operation a significant and unequal decrease in the near-electrode capacitances of the transducers is observed. This triggers an essential (up to tens of percent) differences in values of their $\tan \phi$ and Rs because of the growing influence of the charge transfer resistance.

Our studies have shown that periodic cleaning of the electrodes surfaces makes it possible to restore the sufficient values of the near-electrode capacitances. This indicates that the main cause of the instability of the parameters of the equivalent circuit of transducers is the formation of a dielectric (oxide) layer or other contaminants, which reduce the near-electrode capacitance over a significant area of the electrode surface. Therefore, it is critical to have the electrode surface cleaned before immobilization of the biosensor membranes. As our experience with biosensors shows, the deposition of biomembranes on the electrode surface contributes to ensuring good electrical parameters of the transducers for a long time.

The influence of changes in the near-electrode capacitances of the differential sensor on the response of the output current of the bridge ($\Delta I_3$) under changes in the specific conductivity of the solution is considered using figure 7. Here, the near-electrode capacitance is divided into two parts: Cn and the chain with the reduced capacitances Cn1 and Cn2, which represent clean and contaminated areas of the electrodes, respectively.

Together, these capacitances shunt the active resistances of charge transfer $R_n$ and of Warburg impedance $Z_W$ that upon the conversion into the serial equivalent circuit increase the Rs values with respect to the resistance of the buffer solution ($R_p$) by the value $R_n$ (see figure 7(b)). Because of the presence of $R_n$, the voltage drop on the solution $U_p$ decreases compared to $U_n$, which changes the amplitude of the current’s increment $\Delta I_3$ (the channel response to the informative change in the buffer solution conductivity $\Delta \sigma_p$ in the working sensor) and hence the channel sensitivity. The influence of $R_n$ can be determined from the sensors’ diagnostics. A decrease in the total value of $R_n$ increases these distortions.

The values of the near-electrode capacitance, the parallel resistance of charge transfer, the solution resistance, and, to a lesser extent, the interelectrode capacitance $C_e$ within the operating frequency range are of practical importance for the conversion characteristics of conductometric biosensors. With the correct choice of operating frequency, the Warburg impedance can be neglected. The rest of the components of the multielement circuit form the values of the two impedance parameters as a vector in a 2-dimensional coordinate system with the Re and Im axes. The real and imaginary components of this impedance are located along these
axes and displayed as $R_S$ and $C_S$ in the equivalent 2-element circuit (figure 7(b)); the values of $R_S$ and $C_S$ depend on the frequency of the test signal. Identical changes in these components do not disturb the balance of the bridge in the differential circuit; their effect on the conversion function is small and is corrected when the microcontroller processes the data.

More annoying fact is the difference between the changes in $C_n$ in the working and reference sensors, which leads to the significant differences between their $\tan \phi_A$ and $\tan \phi_B$ and, consequently, of their sensitivity according to the equation (3) [26]. This causes a false response when the equal changes in the conductivity of the buffer solution in the sensors of the differential pair (as a result of the presence of common-mode interference) take

Figure 9. The developed devices as part of the single channel (a) and multi-channel (b) conductometric biosensor systems and the electronic measuring module of the portable biosensor analyzer (c). The components of the measuring systems: 1—electronic measuring module, 2—sensor holder, 3—measurement cell filled with the working buffer solution, 4—magnetic stirrer, 5—user interface of the software that provides control of the device, the data visualization and processing.
place [23]. Figure 7(c) shows the triangles of the vectors of voltages on the elements of their equivalent circuits, the currents in the sensors after balancing the bridge circuit, the vectors of increments of these currents and their projections on the Re axis under influence of the common-mode interference. However, when we used the devices built according to the circuits in figure 4(b) and 5, it was found that the amplitudes and phases of the current increments differed, which resulted in an additive error of measurements $\Delta (\Delta AP)$. An effective way to reduce this error is to decrease the values of sensor’s $\tan \varphi$ below 0.3. However, as mentioned earlier, this comes with a number of problems.

4. Created equipment

A structure of the measuring circuit of the improved differential conductometric system with automatic balancing and diagnostics of sensor parameters is shown in figure 8. The circuit in figure 8 differs from the circuit in figure 5 by the replacement of the inverter in the branch of the reference sensor with the second inverting adder, which adds an additional component with the sign ‘+’ or ‘−’, which is quadrature to voltage $U_{d}$, to the voltage $U^+$ (figure 4(b)). The amount of the addition is regulated using a second digital-to-analog converter (K2 in figure 4(b) and DAC2 in figure 5) with a constant resistance $R_c$ and adjustable resistance $R_v$, which are part of the adder. The zero level of the additive is set in the middle of the regulation range of this DAC by resistor $R_3$. The amplitude of the addition corresponds to the change $\Delta K_2$ of the control code K2 relative to the midpoint.

At the first stage of measurements, the functioning of the device in figure 8 corresponds to such in figures 4(b) and 5. At the second stage, the specified additive compensates the difference in voltage drops on the capacitances of the equivalent circuits of active and passive sensors. The state of the bridge balance is registered as zero value of the quadrature component of the current $I_1$. With this method of the bridge balancing, the voltages on $R_{SA}$ and $R_{SP}$ are identical and equal to $U_c$, for any RC parameters of the converters. This ensures the equality of the amplitudes of the current increments in the active and passive branches of the bridge under influence of the common-mode interference. Finally, this provides more complete subtraction of the current increments at the output of the circuit.

The output signal $\Delta I_1$ is determined by the difference between the currents in the passive and active sensors according to the equation obtained in [27]:

$$I_p - I_\lambda = U_c \left[ \frac{1 - j(K_1 + \Delta K_2)}{1 - j\tan \varphi} \right] \frac{1}{R_{SP}} = \frac{1 - j\tan \varphi}{1 - j\tan \varphi} \frac{1}{R_{SA}} \right]$$  

(4)

After the main ($K_1$) and additional ($\Delta K_2$) adjustments, the output signal of the bridge can be presented as:

$$\Delta I_1 = \frac{1}{R_{SP}} - \frac{1}{R_{SA}} \right]$$  

(5)

The in-phase component of the current $\Delta I_1$ on the output of the bridge is not compensated completely if $R_{SA} \neq R_{SP}$. However, this remainder does not affect the value of the informative increment and can be digitally subtracted by the microcontroller during data processing. In such device, the influence of non-equality of the sensor’s RC parameters on degree of suppression of the errors, originated from changes in the background electrical conductivity of the buffer solution, is lower by half. The same principles were used for construction of the multisensor conductometric systems described in [28].

The vector diagrams of bridge balancing taking into account the results of influence of the common-mode interference are shown in figure 7(d). The balancing steps are indicated by the numbers with an arrow. In this model, which is close to the frequently used sensors, $R_{SA} = R_{SP} = 1 \text{ kOhm}$, $C_{SA} = 5.5 \text{ nF}$, $C_{SP} = 1.2 \times C_{SA} = 6.8 \text{ nF}$, $\tan \varphi_1 = 0.8$ and $\tan \varphi_2 = 0.66$. The amplitudes of increment of the sensors’ currents $\Delta I_{1b}$, $\Delta I_{2b}$ were taken as 3 arbitrary units (% of the sensor current). After balancing the voltages on the capacitances (stage 2), the moduli of $\Delta I_{1a}$ and $\Delta I_{2a}$ were the same and their informative components were 2.4 and 2.6, respectively. The magnitude of the error from interference was 0.15 arbitrary units (7% under equality of the useful signal and interference). The above sensor parameters and their differences corresponded to those possible in real biosensor systems. Therefore, the effect of rejection of the common-mode interference became sufficient for many practical tasks. At the same time, there are measurement conditions, under which the common-mode interference can be many times greater than the useful signal. For this case, the measurement method has been developed with bringing the bridge circuit to a quasi-equilibrium state, in which its output signal after balancing differed from zero, but the increment of this signal under the equal effect on the sensors of the differential pair was practically absent [29, 30]. This method was implemented in two variations: (1) by the additional step of the bridge balancing using a block that regulates the voltage on the reference sensor, changing its modulus or phase [29], and (2) using a bridge circuit with the balanced modulus and phase [30]. After such
Table 1. Main functional and metrological characteristics of the single channel and multi-channel conductometric biosensor analyzers.

| Parameter                                      | Value                  |
|------------------------------------------------|------------------------|
| The main measured parameter:                  | the change in the difference $\Delta G$ of the output active conductivities of two conductometric sensors |
| Diagnostic parameters of a pair of sensors:   | the active resistance $R_{Ax}$ and the tangent of the phase angle $tg_j\psi_{Ax}$ of the working sensor; the ratio of parameters of the passive and working sensors: $R_{pA}/R_{Ax} \cdot tg_j(\psi_{pA} - \psi_{Ax})$ |
| Resolution by $\Delta G$:                      | 0.02 $\mu$S            |
| The voltage and its frequency on the sensor:  | 35–70 kHz; 10–15 mV    |
| The error $\Delta G$ from the nonlinearity of the conversion at $tg_j\psi_{Ax} \leq 0.5$: | 3%–5%                  |
| Relative measurement error $R_{G}$:           | 1.5%                   |
| Absolute measurement error $tg_j\psi$:        | 0.025                  |
| Recommended range of $R_{Ax}$ and $R_{pA}$:   | 0.5–5 k$\Omega$        |
| Random error:                                  | 0.02 $\mu$S            |

Figure 10. Calibration curves for arginine determination obtained for the conductometric bi-enzyme biosensor using the stationary differential conductometric biosensor system (curve 1) and the novel conductometric biosensor system (see figure 9) presented in the article (curve 2). The measurements were carried out in 5 mM phosphate buffer solution, pH 6.2.

Figure 11. Linear range approximation for the arginine biosensor studied with the stationary differential conductometric biosensor system (curve 1) and the novel conductometric biosensor system (figure 9) presented in the article (curve 2). The measurements were carried out in 5 mM phosphate buffer solution, pH 6.2.
additional bridge balancing, the current vector in the reference sensor is rotated in one way or another by the angle \( \phi_f - \phi_s \) (step 3). Upon the changes in the background conductivity of the solution, the vectors of current increments (\( \Delta I_{LS} \) and \( \Delta I_{RP} \)) in the sensors become collinear with opposite phase angles (for ease of perception of diagrams in figure 7, the vectors of voltages and currents in the branches of the bridge are shown in one complex half-plane \( \Im / \Re \)).

Figure 9 shows the developed single channel (figure 9(a)) and multi-channel (figure 9(b)) differential conductometric analyzers and the electronic measuring module of the portable biosensor analyzer (figure 9(c)), which is suitable for both autonomous and system use. Their main functional and metrological characteristics are given in table 1. The multi-channel conductometric analyzer allows performing measurements with one to four biosensors, which, therefore, enables simultaneous detection of different analytes under the same measurement conditions.

All types of the developed conductometric systems consist of the electronic measuring module, the sensor holder, the measurement cell filled with the working buffer solution, the magnetic stirrer and the software, which allows the data visualization and processing.

For the created equipment, we carried out the experimental assessment of the achieved level of the additive errors at small and maximum permissible differences in the RC parameters of the sensors (up to 25%). The measurements were carried out with a normalized level of the main factor, which causes the error—the change in the background electrical conductivity of the solution during measurements. This level corresponded to 100% of the normalized informative effect, which we accepted as equal to 1% variation of the local change in the electrical conductivity of the solution in the working sensor. The data of such estimates for the system in figure 1 and the devices created in the course of the current study were obtained using the electrical equivalent of the differential biosensor and are given in [23, 26, 27]. The comparison of data of such estimates was presented in [27]. As mentioned in the article introduction, the expensive and large stationary conductometric biosensor systems, consisting of the complex equipment, were generally used prior to this work [9–13, 31–33]. Their characteristics roughly correspond to those of the developed here biosensor analyzer given in table 1. However, the presented devices significantly surpass them in terms of price, weight and dimensions, the degree of measurement automation and the user handiness. Due to availability of the sensor diagnostics and bridge circuit balancing, the accuracy and metrological reliability of the results obtained with the newly created devices are higher. It is important that the degree of suppression of the errors from changes in the background electrical conductivity of the buffer solution has been doubled in the developed devices [27]. Because of the differences in measurement conditions and research tasks, such characteristics of different conductometric systems can be compared only statistically or when solving the same specific tasks. The developed here devices were applied in the biosensor measurements for detection of a wide range of analytes as demonstrated in [34–38].

Lately the developed devices were used to make measurements with the conductometric enzyme biosensor for determination of arginine. The biorecognition element of such biosensor was based on arginase and urease, which provided the sequential cleavage of arginine to the final product, the highly mobile ammonium species (equations (6) and (7)). To prepare the biorecognition element, the enzymes were co-immobilized in one layer on the sensitive surface of one pair of electrodes using a cross-linking technique with glutaraldehyde as a bi-functional agent. The principle of the biosensor was based on detection of the conductivity change caused by generation of \( \text{NH}_4^+ \) in the second enzymatic reaction that takes place in the bioselective membrane of the biosensor according to equation (7).

\[
\text{Arginase(E.C.3.5.3.1)} \quad \text{L-arginine} \rightarrow \text{L-ornithine} + \text{urea} \quad (6)
\]

\[
\text{Urease(E.C.3.5.1.5)} \quad \text{Urea} + 2\text{H}_2\text{O} + \text{H}^+ \rightarrow 2\text{NH}_4^+ + \text{HCO}_3^- \quad (7)
\]

For preparation of the biosensors, the gold thin-film transducers were used (figure 2). Each conductometric transducer consisted of two pairs of interdigitated electrodes; the sensitive surface area of each pair of electrodes was about 2.9 mm². The electrodes were fabricated by vapor deposition of gold onto a non-conducting pyroceramic substrate (5 × 30 mm). A 50 nm thick intermediate chromium layer was used to improve the adhesion of gold to the substrate. The digit width and interdigital distance were 10 μm, and their length was about 1.5 mm. Such geometry ensured complete and stable transduction of the biochemical reaction effect from the bioselective membrane to the sensitive surface of the sensor. One pair of electrodes covered with the bi-enzyme membrane served as a working sensor; another pair covered with the conditionally non-reactive BSA layer served as a reference sensor.

The biosensor measurements were carried out in a glass cell filled with 5 mM phosphate buffer solution, pH 6.2 (volume 3.4 ml) under vigorous magnetic stirring. A steady-state response of the biosensor (\( \Delta G \)) was plotted as a function of the arginine concentration in the measurement cell. To study the dynamic range of the developed biosensor, the biosensor’s signals to arginine in the concentration range of 0 to 20 mM in the
measurement cell were obtained. The corresponding dependences are presented in figure 10, where the typical calibration curve of the biosensor obtained using the stationary differential conductometric biosensor system corresponds to curve 1, and the calibration curve obtained using the latest modification of the single channel conductometric biosensor system build based on the advanced compensation bridge circuit (see figure 8) corresponds to curve 2. According to the obtained dependences, the saturating concentration of arginine within the dynamic range of the biosensor in both cases reached 20 mM of L-arginine.

As seen in figure 10, the sensitivity of the biosensor analysis carried out with the novel conductometric biosensor system was significantly higher compared to the sensitivity of analysis with the traditional (stationary) differential conductometric biosensor system. The improvement in the sensitivity of detection was especially pronounced at low concentrations of arginine; for instance, the biosensor responses to 0.08 mM L-arginine measured with the stationary conductometric biosensor system and the conductometric biosensor system presented in the article were 0.1335 ± 0.00809 μS and 4.76333 ± 0.23094 μS correspondingly (the difference was more than 35 times). The increased sensitivity of the biosensor analysis was an important achievement at this stage of the work as it enables further determination of arginine with the lower limit of detection, which is particularly important for the analysis of real samples of different origin and with the different dilution factors. The linear concentration range of the studied biosensors was found approximately the same for both types of the biosensor systems: 0–5.4 mM and 0–5.2 mM L-arginine for the stationary conductometric biosensor system and the biosensor system presented in the article correspondingly (see figure 11).

The fact that the dynamic range and the linear range of the biosensor were the same regardless of the measuring system used was determined by the quantity, biological activity and method of immobilization of the enzymes in the biosensor structure as well as the conditions of measurements (mostly parameters of the working buffer solution).

5. Summary: problems, ways and means of their solution and the results obtained

At the initial stage of the described studies, it was revealed that when using simple bridge circuits (figures 1 and 3(a)) with differential conductometric transducers (figure 2), a strong dependence of the measurement results on the operating frequency is observed. There is also instability of results when changing measurement conditions: replacement of transducers and buffer solution, etc.

The reason for this problem was established—a strong dependence of the response of such bridge circuits on the value of the tangent of the phase angle (tgφ) of the impedance of conductometric converters.

The way to eliminate this problem was proposed—to use a bridge circuit with compensation of the voltage drop on the capacitive component of the impedance of the converters. Such bridge circuit should be balanced on the component of the output signal, which is quadrature to the supply voltage of the bridge.

An electronic measuring channel was developed for a biosensor system with a bridge measuring circuit, which is automatically balanced by the microcontroller of the device by the quadrature component and by the modulus of the output signal before measurements (figure 5). In this process, the main parameters of the working transducer and their relationship with the parameters of the reference transducer are determined in order to diagnose the suitability of the biosensor. In such channel, differential biosensors with tgφ ≤ 1 can be used with a difference in RC parameters of up to 20%. Sensitivity changes are reduced to ±20%. Channel sensitivity calibration can be performed with an accuracy of ±5%. An experimental series of biosensor systems based on this channel has received practical application [34–38].

Further studies showed that during the operation of biosensors, the measurement differentiability deteriorates: a change in the background electrical conductivity of the buffer solution causes a false response of the bridge circuit. In those cases, when the addition of the analyte to the buffer solution changes its background electrical conductivity, a significant additive error occurs. The main reason for that, as it turned out, was the difference in the decrease in the near-electrode capacitance of converters. Changes in the RC parameters of pairs of working and reference converters were investigated, their limits were established, and a method to restore the initial values was found (the electrode cleaning with ethanol).

To reduce the effect of non-equality of the capacitances of the converters, it was proposed to perform additional balancing of the bridge circuit to compensate the difference between those components of the currents in the working and reference converters, which are quadrature to the supply voltage of the bridge. Such balancing replaces the balancing of the bridge on the modulus of the output signal, which improves the differentiability when the active resistances of transducers are not identical, which can also occur.

To implement the improved balancing method, a new bridge circuit of the measuring channel was developed, the operating principle of which is explained using figure 8. This circuit is applied in the devices shown in figure 9. General characteristics of the electronic channels of differential conductometric biosensor systems developed according to the schemes in figures 4, 5 and 8, which are obtained using electrical equivalents.
of biosensors, are summarized in table 1. Such biosensor systems differ from each other (as well as in relation to the devices in figures 1 and 3(a)) in expanded possibilities of obtaining reliable results when used in a measuring system with real biosensors in wide ranges of measurement conditions and characteristics of conductometric transducers. Additionally, they differ in their stability under influence of uninformative factors. These advantages have been confirmed experimentally using electrical equivalents [26, 27] and in the measurements with the real biosensors.

6. Conclusion

The complex of theoretical and experimental studies allowed us to establish the physical nature and quantitative parameters of the influence of uninformative factors associated with the processes in the sensors and with the properties of the used measuring circuits on the conversion characteristics. The obtained results allowed developing the measurement methods, which comprised the procedure of the bridge circuit balancing. This provided enhancement and stabilization of sensitivity of the measuring devices, as well as the possibility to correct their conversion characteristics according to the results of diagnostics of the sensor’s parameters. The results of studies of parameters of conductometric transducers used in differential conductometric biosensors, the methods of stabilization of their values, and the influence of their non-equality on the additive errors of the biosensor systems with different methods of transformation of the informative signals allowed developing new measurement algorithms and to significantly reduce such errors. The overall result of the presented work is the creation of the biosensor analyzers with demonstrated metrological reliability that ensure good reproducibility of the measurement results regardless of the measurement conditions.

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