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Investigation of Repeatability and Error Instability Analysis of Tissue Biosensor

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ABSTRACT

Co-substrate sensitive amperometric system for the measurement of dopamine has been constructed. The repeatability of constructed biosensor has been investigated. A formulae for the total instability has been obtained. The instability due to inner factors has led to instrumental error of instability of the system. The analysis of the error from the instability has been carried out. As an active membrane immobilized banana tissue on dederone screen has been used. The measurements are conducted in the normal working conditions. A uniform scale has been chosen.

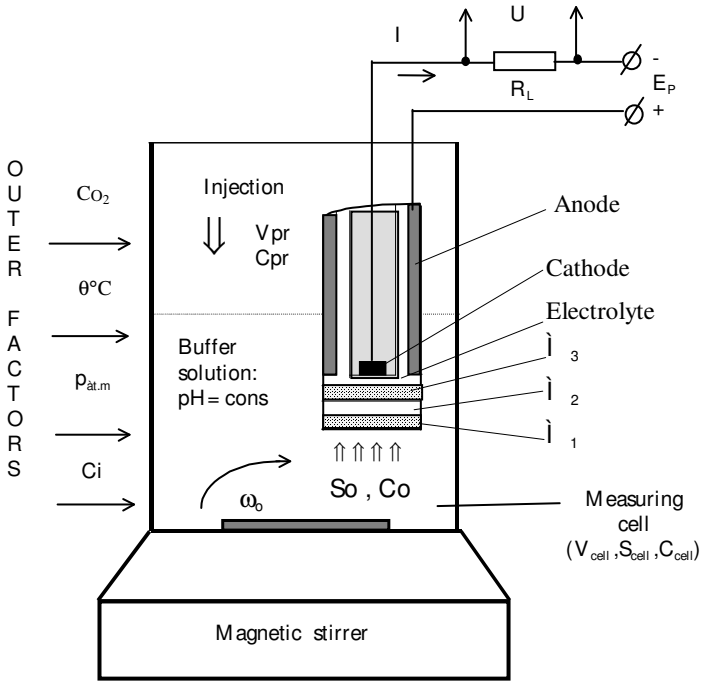
Keywords: Biosensor, tissue, repeatability, errors

1. INTRODUCTION

The research of the new and various tissue biosensor systems (BSS) leads to their usage in the different devices and instruments for control and analysis of the concentration of different substances. The measurement objects are amino acids [1,2], amines, alcohols and phenols [3,4], gases [5], pesticides [6] and others. When the biosensor systems are used as a technical device it is necessary that they have repeatability of their results. Therefore they have to have stability of the readings in certain working conditions. Working conditions in which the system works in optimal regime are usually determined. In the present paper a tissue biosensor for the measurement of the neurotransmitter dopamine has been investigated. The system is co-substrate sensitive. Its transfer function is received in [7].

2. ERROR INSTABILITY ANALYSIS

The setting of the experiment is presented at Figure 1.

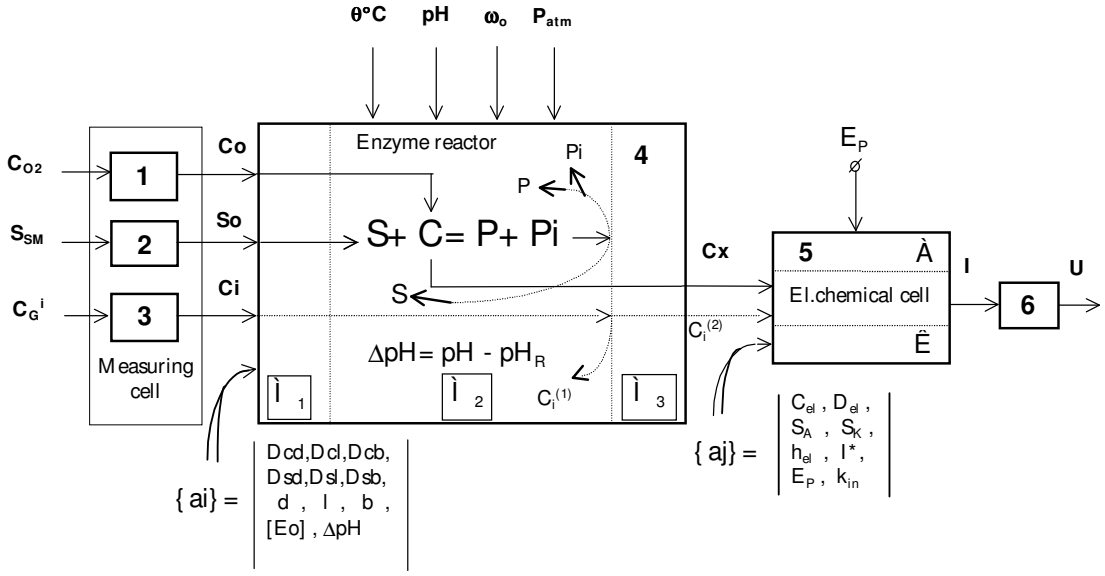


Фиг.1. Setting of the experiment.

It consists of three membranes: M_1 - is dialyze, M_2 - is the active and M_3 - is the gas-permeable membrane, and an electrochemical cell of the basic transducer in which an anode, cathode and electrolyte are situated.

The electrodes are supplied by an outer polarizing voltage E_P. When the current I is passed through the loading resistance R_L an output voltage U is received. The object of measurement is the concentration of substrate in the measuring cell So=Scell. So by taking an assay with a volume Vpr and concentration Cpr from the measuring substance sample is formed which is injected in the buffer solution. The buffer has a volume Vcell and ensures exactly the predetermined pH which is chosen to be the optimal for the running enzyme reaction. With the help of the magnetic stirrer the buffer solution is stirred permanently to provide enough dissolved oxygen Co and also and convection of substrate towards the active membrane.

In the membrane M_2 whish is like enzyme reactor, the measuring substrate is converted into product. Oxygen which is necessary for the running of the enzyme catalyzing reaction ensured from the air. The rest of the oxygen is passing through the gas-permeable membrane and by depolarizing the cathode is resulted an output current I.
For the short-time instability of the constructing BSS the following sourse can be pointed out: variation of condition of the standard buffer, variation of the parameters of elements of the biosensor, variation of the givinen concentration from the sample, variation of the settled concentration of the oxygen at the beginning of the measurement.



Фиг.2. Structure scheme of the influence factors .

More deep analysis of the causes for the instability of the bioanalyzer is given in Figure 2. Here with block 1 is denoted the transformation of the gas phase of the oxygen Co_2 to the liquid phase Co. With block 2 is denoted the dilution of the concentration on the sample Ssm to the measuring one So. With block 3 is denoted the conversing of the gas components C_G in to the same concentrations but in the liquid phase Ci.
In the second membrane from the membrane set is running enzyme catalyzing reaction from the type S+C→P+Pi. Here with Pi is denoted the lateral products from the conversion of substrate S into the product P. They change the acidity of the medium to the value pH_R. In the active membrane the optimal pH is given and for these reason it is changed with ΔpH.
Because the system is co-substrate sensitive through the membrane M_3, the residual concentration of oxygen Cx and a part of obstructive substances $Ci^{(2)}$ only is passing. The rest reagents with concentration $Ci^{(1)}$, product P and substrate S are returned to the active membrane.
The electrochemical cell 5 is supplied by outer polarizing voltage Ep. The output current I is transformed to outer voltage U by the help of block 6.
The outer influence factors are: temperature θ°C, pH, stirring rate ω_o and atmospheric pressure p_{atm}. They have significant influence over the processes into the measuring cell. For the leading researches all this parameters are within the limits of the normal working conditions.

The design parameters are divided into two groups. The first one $\{a_i\}$, includes all diffusions coefficients Dij, the thickness of membranes b,l,d, variation of ΔpH and concentration of enzyme [Eo]. The second group $\{a_j\}$ include the parameters of the electrochemical cell : concentration of electrolyte Cel and corresponding diffusion coefficients Del, the thickness of the electrolyte layer h_{el}, anode area S_A and cathode area S_K, the initial value of current I*, the size of the polarizing voltage Ep and the rate of the electrochemical reaction Kin. Researches are carried out in normal working condition, for that we estimates only the influence of the inner factors. An uniform scale is selected. The measurement is provided in steady state. We use the transfer function [8] to estimate the influence:

$$I = nFA\frac{[Co]}{\frac{h_{el}}{Del}+\frac{b}{Dcb}+\frac{l}{Dcl}+\frac{d}{Dcd}+\frac{1}{Kin}} - nFA\mu\frac{\left(1-\frac{1}{H(\Phi)}\right)[So]}{\frac{h_{el}}{Del}+\frac{b}{Dcb}+\frac{l}{Dcl}+\frac{d}{Dcd}+\frac{1}{Kin}}, \qquad (1)$$

where :
n - the number of electrons taking part in the electrochemical reaction; F - the Faraday's constant; A - the indicator electrode surface; [Co] - the start concentration of oxygen; [So] - the measurement concentration; $\Phi^2 = (Vm/Ks)/(l^2/Dsl)$, so called Tile's module; $H(\Phi) = ch\Phi + \Phi.(sh\Phi).d/l$; Dcd,Dcl, Dcb - diffusion coefficients for co-substrate; Dsd, Dsl - diffusion coefficients for substrate; μ = Dsl/Dcl; b,l,d - the thickness of gas-permeable membrane , active membrane and dialyze membrane; Ks - Michaeli's constant; Vm-maximal value of the enzyme reaction velocity; Kin - the coefficient's of the indicatory reaction;

Here is added also the ratio h_{el}/Del which is characterizing the influence of electrochemical part. From these expression it can be seen that a cause for the inner instability of the bioanalyzer are these 18 parameters which is describe the output current I. If we denote these parameters with X_1……X_{18}, the total instability of the output current ΔI can be estimate with the method of the full differential [9] and there follows:

$$\Delta I = \sum_{i=1}^{18}\frac{\partial I}{\partial X_i}\Delta X_i \ . \qquad (2)$$

The partial derivatives on the outer current to each parameter is in fact the coefficients of influence on the every one elements of the BSS.
But in practicel the dominant influence have nine parameters : the determined value of the initial concentration on oxygen [Co], given measurement concentration [So], the thickness on active l and dialyze d membrane, the thickness on electrolyte layer h_{el}, diffusion coefficient of substrate in active membrane Dsl, diffusion coefficient of co-substrate in active membrane Dcl and dialyze membrane Dcd, and concentration of enzyme [Eo].
As we take in mind that the plant tissue is used for the active membrane, where enzyme is naturally immobilysed we can assume that the influence of [Eo] will be negligible. Because of that it is not taken in mind. An so after using the method of the full differential with the pointed out conditions the following expression for the ΔI is received :

$$\Delta I = \Delta I^* - \Delta Is \qquad (3)$$

$$\Delta I^* = K\left[\frac{\Delta[Co]}{Rc} - \frac{[Co]}{Rc^2}\left(\frac{\Delta l.Dcl - l.\Delta Dcl}{Dcl^2} + \frac{\Delta d.Dcd - d.\Delta Dcd}{Dcd^2} + \frac{\Delta h_{el}}{Del}\right)\right] \qquad (4)$$

$$\Delta Is = K\left\{(\Delta Dsl - \mu\Delta Dcl)\frac{[So]}{Dcl.Rc} + \mu\left[\frac{\Delta[So]}{Rc} + \frac{[So]}{Rc^2}\left(\frac{\Delta l.Dcl - l.\Delta Dcl}{Dcl^2} + \frac{\Delta d.Dcd - d.\Delta Dcd}{Dcd^2} + \frac{\Delta h_{el}}{Del}\right)\right]\right\}, \qquad (5)$$

where : K=nFA;

$$Rc = \frac{h_{el}}{Del} + \frac{b}{Dcb} + \frac{l}{Dcl} + \frac{d}{Dcd} + \frac{1}{Kin}$$

is in fact the diffusion resistance of the membrane set.

Now we can find the formulae for the relative instability error $\gamma_{ins} = \Delta I/I$, the signs minus are changed to plus [10]:

$$\gamma_{ins} = \frac{\Delta[Co] + \frac{[Co]}{Rc}(\Delta Rc) + (\Delta Dsl + \mu Dcl)\frac{[So]}{Dcl} + \mu\left[\Delta[So] + \frac{[So]}{Rc}(\Delta Rc)\right]}{[Co] - \mu[So]}, \quad (6)$$

where:

$$\Delta Rc = \frac{\Delta l.Dcl - l.\Delta Dcl}{Dcl^2} + \frac{\Delta d.Dcd - d.\Delta Dcd}{Dcd^2} + \frac{\Delta h_{el}}{Del}. \quad (7)$$

From the received formulae (6) for the relative instability error it is seen how each of pointed out 9 parameters influence over deviation of output current.

The deviation in settling down the determined value of the initial concentration of oxygen [Co] is influenced directly over the error. This influence is additive and can be easy compensated with the zero regulating. But it is better to wait for enough long time and the system itself takes the steady state initial concentration of oxygen value.

The deviation in settling down the given value of the measured concentration of substrate [So] is influenced too. But here the influence is decreased with the ratio μ to the two diffusion coefficients Dsl/Dcl, which for the research system is lower than 1 and it is equal to 0,12. The main reason is due to the inaccuracy of the used measuring flask and digital pipette. It is very important the operators skills, who have to prepared the solution of substrate and injecting it into the buffer in the same way, so it doesn't increase the error given in the passport.

The influence of the deviation on the thickness on active l and dialyze d membrane is double – in the ΔI* and in ΔIs. Although these deviations are with different signs they have to sum up, we explain why above. For the same reason all minuses in the formulae for the relative error have to be changed in to plusses. The reason for the deviation of the thickness on the membranes is the pressure which is rendered from the fixing ring for the keeping the active membrane over the top of the oxygen electrode. For the same reason is also the variation of the thickness on the electrolyte layer h_{el}, which again change the value on the diffusion resistance Rc.

Diffusion coefficient of substrate Dsl is influenced from the uniform distribution on tissue to the dederone screen and from the pollution when preparing the membrane. It is increased with the same value in the square. The same is valid for the diffusion coefficients of co-substrate Dcl and Dcd.

Because the enzyme is naturally immobilized in the banana tissue, then the influence of enzyme concentration [Eo] variation will be insignificant and we assume that it hasn't great influence over the total instability of the biosensor.

3. EXPERIMENTAL RESULTS

A number of experiments Have been carried out. 5 times repeating for receiving the transfer function of one designed biosensor system are performed. The BSS was checked for the uniform scale with the method of subsequent additions. Every one scale was received with 7 points, which are chosen that way, so that to be in the linear area of the transfer function.

The biosensor is prepared by the folowing way. From the purchased from the near shop bananas a thin layer is cut and it is immobilised over the dederone set. Then we measure the mass of active membrane – screen with tissue- it was 61mg. After that we put it over the gas-permeable membrane on the forehead on the oxygen electrode. The dialyze membrane was put over it. The thickness of the dederone screen was 70μm.

The thickness of the gas-permeable membrane was b = 10μm, and thickness of the dialyze membrane was d = 25μm. The experiments were made in 15 ml phosphate buffer solution with pH = 6, which is close to the pH optimum for the polyphenoloxidase [11]. The solution was permanently stirred with velocity 700r/min, the temperature was 20 ±0,5°C. The diameter of the golden oxygen cathode (purity 99,95) is 1mm. The oxygen meter was APK 101 (accuracy 1). Measuring range was 0-15mgO_2/l. It was calibrated at 20°C. After putting

the active membrane APK shows 4,13mgO_2/l. The capacity of injection supplement was 100µl (preciseness - 1%) with 4mg/ml dopamine - it is equal to 0,141mM dopamine . The number of additions were 7. Every time we waited about two minutes for the steady state to settle and then we took the reading.
After that the buffer was thrown away out and the new one was prepared. The BSS was put in the new solution and we waited until the system reversed to the first conditions. And all that was repeated 5 times to receive 5 scales.
The results are given at the Figure 3. On the abscissa the measurement concentration of dopamine are put and the concentration of oxygen in ppm was put on the ordinate but it is centred to the 0 of the scale.

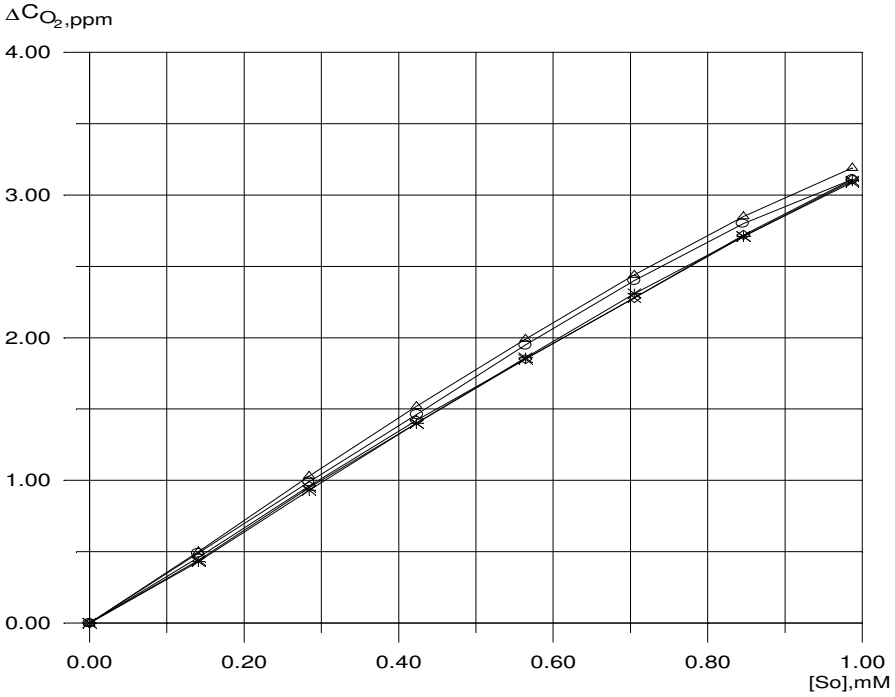


Fig.3. Five uniform scales for the BSS .

4. RESULTS PROCESSING

We have investigated the repeatability of each scale for constructed BSS. We have 7 points for the x_i (i=1…7) and 5 observation y_{ij} (j=1..m) for each points from the scale we have done too.[12,13]. The estimation of the variance of each y_i will be :

$$s_i^2 = \frac{1}{m-1}\sum_j \left(y_{ij} - \overline{y_i}\right)^2 \qquad (8)$$

The uniformity of the variances we check with the Fisher's criteria :

$$F = \frac{s_{i\max}^2}{s_{i\min}^2}$$

where s_{imax} is the maximum value of the calculated variances and s_{imin} is the minimum value. The degrees of freedom are ν_1 =m-1,ν_2=m-1 and for the level of significant α = 0,025 , Fcr (4;4;0,975) = 9,60. In table 1 are given the values of variances for the BSS.

Table 1

	n_i	1	2	3	4	5	6	7	F
BSS 1	s_i	0.030	0.038	0.050	0.065	0.074	0.064	0.040	6.08

It is seen that the variances for the BSS are uniform and we can speak of a repeatability of each scale.

Now we calculate the regression line for the BSS with the least squares method. Correlation coefficient r^2 is 0,997 , 95% confidence interval for the mean of a = -0,034 to 0,187 and for b = 2,97 to 3,33. After that we calculate the maximal relative error for constructed BSS (for the 5 rezults for each point of the scale) and this line. The results are given at Figure 4. The received maximal value is 4,4 %.

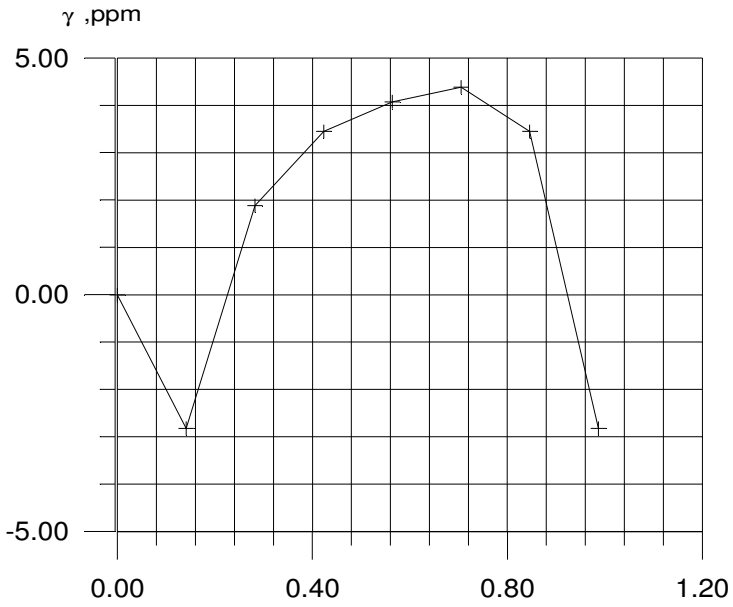


Fig.4. Maximal relative repeatability errors

5. CONCLUSION

The following conclusions can be made by conducted researches for the short-time instability and repeatability of tissue biosensor system with tissue from banana for measuring of dopamine. 18 parameters can be pointed like a reason for the system instability, 9 of them have more significant influence and the absolute and relative error on output current is received for them. From the experimental measurements for the constructed biosensor system it is seen that there has a repeatability for the receiving of uniform scales. The maximal relative instability error is about 4,4%. That means that the constructing biosensor system can be usied for the technical measurements at the given normal working conditions.

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