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pH AND TEMPERATURE MODELLING ON CONSTRUCTED BIOSENSOR WITH FUZZY LOGIC

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ABSTRACT
The influence of the pH and temperature over the output current of constructed tissue biosensor for the measurement of dopamine is treated in the paper. For modelling that influence is used the fuzzy logic system. pH and temperature have a significant influence over every enzyme reaction, because those reactions are sensitive to them. For the given temperature and pH the enzyme reaction has maximum rate and that is prefer for measurement because the output current is maximal. Experiments are time-consuming and very specific for that reason it is hard to receive a big amounts of measured data. Modelling the biosensor biosensor response with the fuzzy logic is able to cope with that small number of measured data with very good accuracy.
KEY WORDS: biosensor, mathematical modelling, transfer function, fuzzy logic.

1. INTRODUCTION

The quantitative determination of neurotransmitter dopamine in urine and plasma [1] is taking approximate to 4 hours. Biosensors are devices that propose fast and cheap way for measurement of some substrates - molecules upon which an enzyme acts. The neurotransmitter dopamine is one of them. The response time for the examinant biosensor is about 2-3 minutes [2]. Because there is used biological recognition element – active membrane from plant tissue (the tissue is from banana pulp) the pH and temperature have strongly influence over biosensor response. The pulp contains the enzyme polyphenol oxidize – PPO, and its primary substrate is dopamine. It is known that the rate of enzyme catalyzed reactions rises with the increase of temperature and has maximum, after that at some temperature the irreversible denaturation of enzyme goes on [3]. The influence of pH has the same effect – the biosensor response has a maximum at some pH. The optimum pH of the banana PPO is around pH=7. That optimum depends on the composition of the research medium and temperature. At the optimum the enzyme is stable and it is preferable to use that value for the measurements.

It is seen that is preferable to use biosensor at optimal pH and optimal temperature. From the experimental results it is seen that pH and temperature significantly affects the signal value, causing non-linearity (maximum) in the curve of output current. That mean if the measurements done in noncontroled conditions – pH and temperature, the output signal didn't have the exact meaning corresponding to measured concentration of substrate (the calibration curve will be other). For the modern devices it is typical to compensate the influences of different kinds and the temperature is one of them, but for the binderies and pH have very big influence. Modelling the value of output current versus pH and temperature will give the opportunity to do measurements in every condition and in that way to improve the accuracy of the measurements.

The neural networks are universal function approximators [4, 5] and with them we can approximate every function or experimental data. In [6] is modelling the influence of temperature over output of biosensor for determination of lactose. The used neural network provided the best results. In [7] artificial neural networks are applied as a new type of model to estimate the pH.

The CMAC-neural-network-based biosensor's input/output model [8] has overcome some of the known drawbacks of the neural networks – the proper number of neurons and hidden layers are not known in advance, the learning process often gets stuck in local minima when tries to learn complicated functions, the neural network could not generalize, if the training samples are insufficient. But CMAC needs sufficient number of experimental data for designing a large set of overlapping receptive fields. Using interpolated data is justified under the lack of data, because of difficulties associated with their experimentally deriving, but it decreases the main advantage of neural model – the high accuracy. For that we now use the fuzzy logic for modelling in [9] and it is seen that insufficient experimental data doesn't reduce the accuracy.

The paper proposes modelling the dependence of tissue ampere-metric biosensor output current versus pH and temperature by the means of fuzzy logic. The simulations are done in MATLAB environment. It is seen that the fuzzy approximation surface can be obtained in short time without computational difficulties, using small number of experimental data. The accuracy is sufficiently high, and the realization is easy.

2. MATERALS AND METHODS

A) The Biosensor

In the present work a tissue biosensor for measurement of dopamine concentration is investigated [2]. The active membrane uses a tissue from banana pulp (Fig.1).

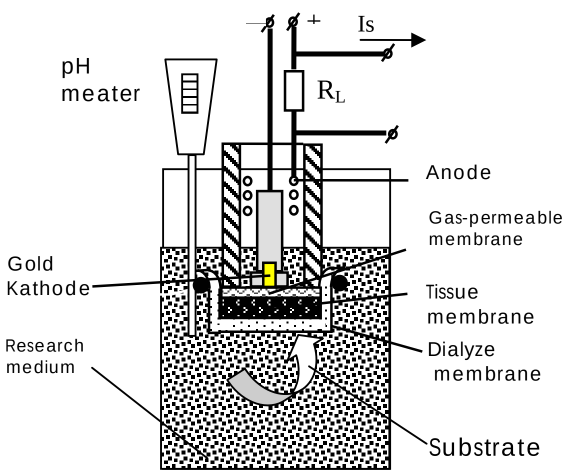


Figure 1. The biosensor and research medium

An oxygen electrode is used for medial transducer. The active membrane is situated over cathode between gas-permeable membranes and dialyze membrane. The biosensor has a contact with the substrate (dopamine) with concentration So. The substrate makes diffusion through dialyze membrane into tissue layer where it has biocatalyse conversation to melanin. The reaction results in a reduction of the oxygen concentration as it diffuses through the biocatalytic membrane to the cathode, this is detected by a reduction in the current between the electrodes. In hat way the output current is proportional to measured substrate dopamine. For carrying out the experiments the following treatment is used: measurement cell, biosensor for dopamine, nanoampermeter, X-Y plotter, homogenizing device (magnetic stirrer). Signal from the biosensor is measured with oxygenmeter. An oxygen probe is with flat face, the diameter of cathode is 1mm. The cathode is from pure gold (pureness 99.95%). The pH meter was calibrated with pH 4 and pH 7 standards and has an accuracy of ±0.01.For maintaining constant temperature a thermostat SPT-200 is used.

B) Modelling the Influence of pH and Temperature on Biosensor's Input-Output Relation

Fuzzy if-then rules and fuzzy inference system: A fuzzy system employing fuzzy if-then rules can model the qualitative aspects of human knowledge and reasoning without precise quantitative analyses. Consider a fuzzy (two inputs/one output) system, which is comprises of four principal components: fuzzifier, fuzzy rule base, fuzzy inference engine, and defuzzifier (Fig.2). Let $X_1, X_2, Y \subset R$ are universes of discourse of the variables x_1, x_2, and y, respectively. The *fuzzifier* performs a mapping from the observed crisp input spaces X_1 and X_2 to the fuzzy sets in these spaces. The fuzzy sets $X_1^i \in \mathrm{X}_1$ $(i = 1,2,...,l)$ and $X_2^j \in \mathrm{X}_2$ $(j = 1,2,...,m)$ are linguistic terms characterized by fuzzy membership functions $\mu_1^i(x_1)$ and $\mu_2^j(x_2)$, respectively. The two linguistic variables (for x_1 and x_2) with corresponding membership functions ($X_1^i, \mu_i(x_1)$, $X_1^{i+1}, \mu_{i+1}(x_1)$, $X_2^j, \mu_j(x_2)$, and $X_2^{j+1}, \mu_{j+1}(x_2)$) enter the fuzzy rule table. The *fuzzy rule base* consists of fuzzy if-then rules of Takagi and Sugeno's type [10]. The fuzzy rule set can be expressed in the following form:

$$\text{IF } x_1 \text{ is } X_1^i \text{ and } x_2 \text{ is } X_2^j \text{ THEN } y = Y_{i,j}, \tag{1}$$

where $i = 1,2,...,l$ and $j = 1,2,...,m$. Four fuzzy sets of the output signal are obtained from the fuzzy rule table: $Y_{i,j}$, $\mu_{i,j}(y)$; $Y_{i,j+1}$, $\mu_{i,j+1}(y)$; $Y_{i+1,j}$, $\mu_{i+1,j}(y)$; and $Y_{i+1,j+1}$, $\mu_{i+1,j+1}(y)$. ($Y_{i,j}$ is assumed to be the variable in the cell arranged in i-th row and j-th column of the rule table).

The *fuzzy inference engine* is a decision making logic which employs fuzzy rules from the fuzzy rule base to determine a mapping from the fuzzy sets in the input spaces X_1 and X_2 to the fuzzy sets in the output space Y. The firing strength of p,q-th rule ($\mu_{p,q}(y)$) is obtained as the T-norm of the membership values on the premise part (by using a multiplication operator):

$$\mu_{p,q}(y) = \mu_p(x_1)\mu_q(x_2), \tag{2}$$

where $p = i,\ i+1$, and $q = j,\ j+1$.

The *defuzzifier* performs a back mapping of the output signal from the fuzzy sets to crisp points. So the overall output is computed as the weighted average of each rule's output:

$$y = \frac{\sum_{p,q} Y_{p,q}\mu_{p,q}(y)}{\sum_{p,q} \mu_{p,q}(y)}, \tag{3}$$

where $p = i,\ i+1$; $q = j,\ j+1$.

<u>*The task formulation*</u>*:* The output current of biosensor depends on pH and temperature and substrate concentration. The experimental data about that dependence is provided in the next section. The task is to approximate the experimental data by fuzzy logic. The fuzzy inference system, used as a function approximator, has two inputs pH and temperature *T* for the given substrate concentration S_0 (constant), and one output – the current I_S.

According to [10] the representation theorem states that any continuous nonlinear function can be approximated to any desired level of accuracy with a finite set of fuzzy variables, values, and rules. This theorem describes the representational power of fuzzy modelling, but it does not answer the questions, how many rules are needed and how they can be found, which are of course essential to real-world problems and solutions [11]. In a conventional fuzzy system, the number of rules is decided by an expert who is familiar with the system to be modelled. In the paper the number of membership functions assigned to each input variable is proposed to be equal to the number of corresponding measured values (Fig.4). So the fuzzy rule table can be filled in with all the experimental data for biosensor's output current (Fig.6).

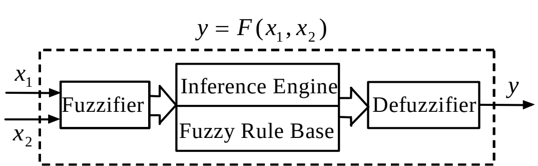
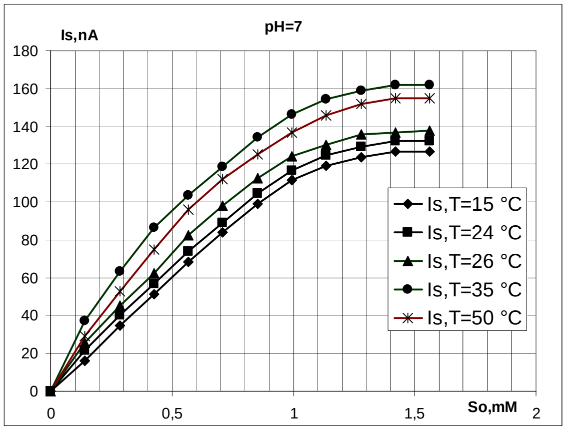


Figure 2. Basic configuration of fuzzy system

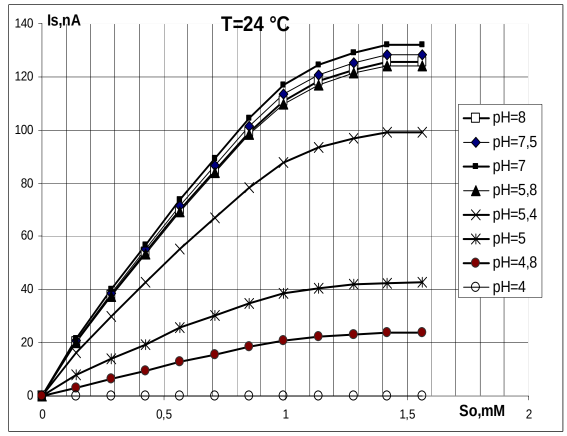
The validation of the proposed approximator is based on the relative error over a few new experimental data.

3. RESULTS AND DISCUSSIONS

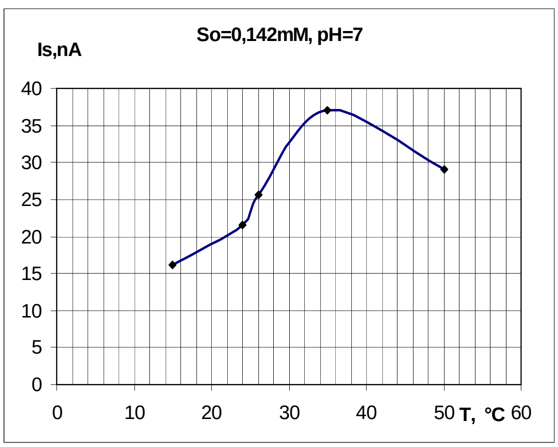
The experimental data used in the paper are the same as those available in [9] and some are new. They are derived under the following conditions. Calibration curves were carrying out for the steady state regime. The method of subsequent addition was used. Every addition was with volume 0.1ml and it is response to 0.142mM dopamine. Measurements were stooped when the saturation zone is reached, because the system was standing uninformative. Calibration curves received for the different temperatures (15, 24, 26, 35 and 50°C) and 12 steps of substrate additions and for the constant pH. Calibration curves received for the different pH (4 4,8 5 5,4 5,8 7 7,5 and 8) and for the constant temperature are received again for the same steps of substrate additions. The all results are shown in two figures Fig.3a and b – So [mM], Is [nA], T [°C], pH. At the Fig.3c and 3d is shown a vertical section of Fig.3a and Fig.3b for the given value of substrate concentration So=0,142 mM.



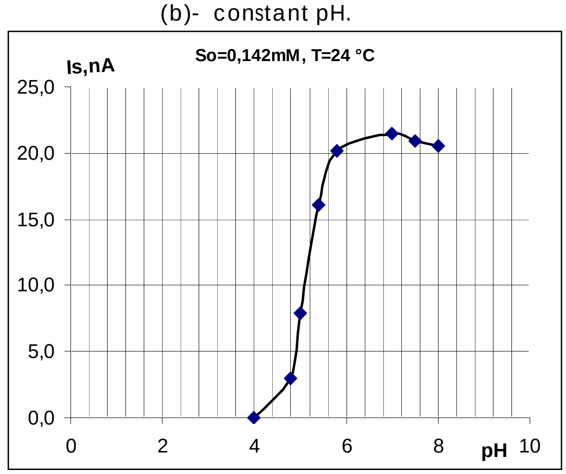
(a) - constant temperature.



(b)- constant pH.



(c) – constant So and pH.



(d) - constant So and T.

Figure 3. Experimental data: (a) influence of temperature; (b) influence of ph; (c) influence of t for the given so and ph; (d) influence of ph for the given so and t on dopamine tissue biosensor.

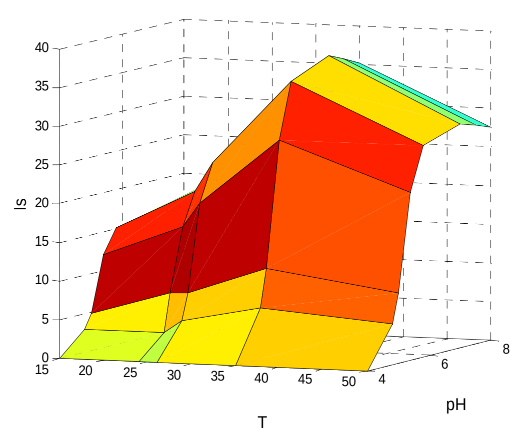


Figure 4. Experimental data: 3D biosensor's input-output presentation, for the substrate concentration So=0,142mM.

One can see that the temperature and pH induces non-linear behaviour of the output current *Is* for one fixed value of substrate concentration. The plot of output current vs. both pH and temperature for the given concentration So =0,142mM is shown in Fig.4. That case we will modelling, for the other value of So the view will be other, because the output current depends of pH and T simultaneously.

The pH and temperature (for the given substrate concentration) are the two input signals of the fuzzy approximator, i.e., $x_1 \leftarrow T$ and $x_2 \leftarrow pH$. The number of membership functions assigned to each input variable is equal to the number of corresponding measured values, i.e., *T*= $l = 5$ and *pH*= $m = 8$. The triangular form of membership functions (Fig.5) and T-norm (using the multiplication operator) of the membership values on the premise part were chosen.

The fuzzy rule table contained all the measurements of the biosensor's output current ($y \leftarrow I_S$), which are $\mathrm{Y}_{i,j} : l \times m = 40$. After removing the repeating values, the output current was presented by 34 different values (Fig.5). For the sake of convenience the values $Y_{i,j}$ in the fuzzy rule table are presented with 34-level gray scale squares (Fig.6), corresponding to the values of $\mathrm{I_s = I_s(pH, T)}$ (Fig.4). The fuzzy approximation of the biosensor's input-output relation is shown in Fig.7, where, for the simulation, discrete steps 0.25 and $1^0\mathrm{C}$ were used along pH and temperature T, respectively.

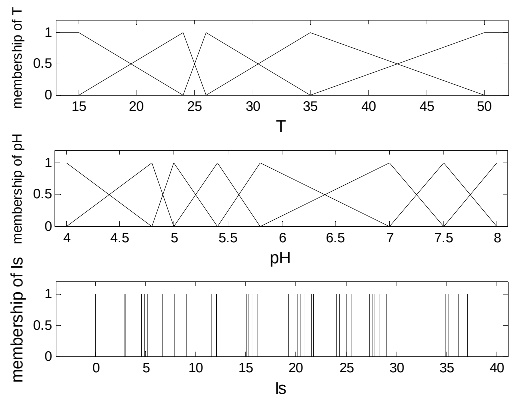


Figure 5. Membership functions

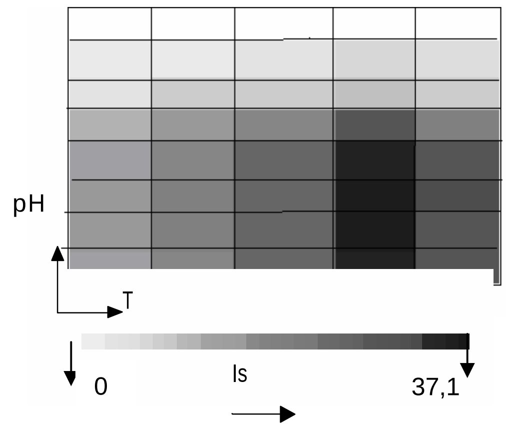


Figure 6. Fuzzy rule table

The generalization of the fuzzy system was tested on three experimental data unused in training process. The results are listed in Table I, where the relative error of each of the three experiments is calculated as $\varepsilon = \frac{|I_S^{approx} - I_S^e|}{I_S^e} 100, \ \%$, ($I_S^e$ and I_S^{approx} are the output current determined experimentally and by means of fuzzy approximation. The average relative error over the three test samples is $\bar{\varepsilon}_3^{\mathrm{FUZZY}} = 2.06\,\%$.

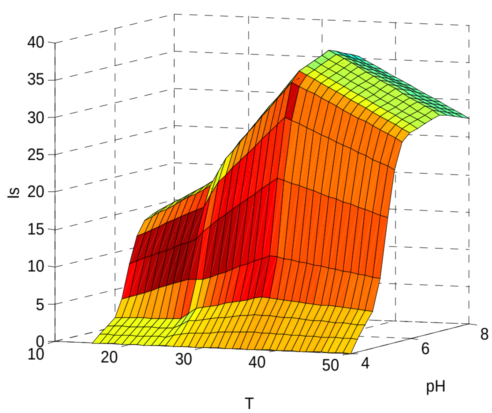


Figure 7. Fuzzy approximation surface

TABLE 1. Results from validation test

No.	Test Data			Fuzzy	
	T	pH	I_S	I_S	ε
	[^{0}C]	-	[nA]	[nA]	[%]
1	18	5	6	5.90	1.67
2	18	7	17.5	16.98	2.97
3	25	8	22	22.34	1.54

From Table I and Fig.7 one can see that the performance of the fuzzy approximator is good.

The interpolated data predetermine the type of approximation surface and usually decrease the main advantage of neural model – the high accuracy. Although neural networks with backpropagation algorithm can approximate each function with sufficient high accuracy, practically, it is not so easy to determine the proper number of hidden layers and the number of neurons per each layer. Training is time-consuming, it requires millions of iterations. Due to the gradient method there is a tendency the learning rule to become trapped in local minima. When the training samples are insufficient the neural network could not generalize.

Fuzzy systems are more favourable in that their behaviour can be explained based on fuzzy rules and thus their performance can be adjusted by tuning the rules. In our case the partitioning of universe of discourse of input signals and the rules in fuzzy rule base strictly depend on the experimental data. The fuzzy approximator does not need interpolated data and its use is justified under the lack of data, because of difficulties associated with their experimentally deriving.

3. CONCLUSION

Modelling the dependence of tissue biosensor output current versus pH and temperature by means of fuzzy logic is proposed in the paper. The fuzzy model works well under small number of experimental data. pH and temperature are of the big meaning for the proper passing of the enzyme reaction and for the correct measured of substrate concentration in any non controlled conditions. The value of pH and T are often under control but the calibration curves may be done in some other conditions, but not that are preferred for the measurements. In that reason it is well done to make the computer compensation of T and pH for whatever working conditions of measurements. The modelling allowed all that.

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