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# Signal Analysis and Calibration of Biosensors for Biogenic Amines in the Mixtures of Several Substrates

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## 1. Introduction

In real life we should often conduct analyses, where several compounds with similar properties are simultaneously present in solutions. In traditional analytical chemistry, this problem is usually solved by the pre-treatment of probes, enabling to eliminate the interference effect of different compounds. This process requires skilled labour, resources and time and eliminates the possibility to carry out on-line analyses.

A promising option for the conduction of on-line analyses is the application of biosensors, which are considered to provide reliable results, at least concerning the issues of selectivity. As biosensors are based on a selective bio-recognition of assessable compounds, there are typically only a restricted number of molecules (besides the ones of the analyte) present in a probe, which can induce measurable signals. However, in cases we have several competing compounds which can generate identical measurable effects, the selectivity of a biosensor can be quite poor and the results illusory. This phenomenon occurs clearly in the studies with enzyme inhibition-based biosensors (Luque de Castro & Herrera, 2003), but can be also well observed with biosensors, based on enzymes having activity towards several substrates, e.g. biosensors measuring biogenic amines (Kivirand & Rinken, 2009), different sugars etc.

Biogenic amines (BAs) are natural nitrogenous compounds formed mainly in the process of decarboxylation and aging of free amino acids. The detection of these compounds is a valuable tool for assessing the freshness and quality of a wide variety of protein-containing products like fish, meat, cheese, wine etc. (Yano *et al*, 1996; Vinci & Antonelli, 2002; Önal, 2007). The most common biogenic amines, used for the indication of food quality are histamine, putrescine and cadaverine (Kivirand & Rinken, 2011). Other BAs, commonly determined in foodstuff are trimethylamine (Mitsubayashi *et al*, 2004), spermidine, spermine and tyramine (Alonso-Lomillo *et al*, 2010). At present, regulations have been established only for the intake of histamine, but no accordant limits are set for other BA-s, including putrescine and cadaverine, although several studies have indicated that putrescine and cadaverine could increase the toxicity of histamine by inhibiting the enzymes involved in histamine biodegradation (Niculescu *et al*, 2000). The allowed maximum residue level of histamine in food according to EEC regulations is 100 mg/kg (EEC, 2001); the international food safety organization FDA has established the histamine level to 50 mg/kg (FDA, 2001).

Biosensors for BAs comprise different amine - selective enzymes, like amine oxidase (previously copper-containing amine oxidase EC 1.4.3.6, in 2008 EC entry deleted and replaced by monoamine oxidase EC 1.4.3.21 and diamine oxidase EC 1.4.3.22), putrescine oxidase (EC 1.4.3.10), methylamine dehydrogenase (EC 1.4.99.3) and flavin-containing mono-oxygenase type-3 (EC 1.14.13.8) in combinations with a variety of signal transduction systems and are based on different signal rising mechanisms. No other bio-recognition systems beside enzymes are known to have been used in BA biosensors at present (Kivirand & Rinken, 2011).

The selectivity of the most widely used enzyme diamine oxidase is relatively poor. The available data about the substrate specificity of this enzyme towards different amines varies within a wide range for enzyme preparations from different sources, and even seems to be dependent on the applied experimental method. The best substrates for diamine oxidase from the seedlings of different papilionaceuos (*Pisum sativum*, *Lathyrus sativus*, *Lens culinaris*, *Vicia faba*) are cadaverine (1,5-diaminopentane) and putrescine (1,4-diaminobutane); other BAs have much lower affinities. The relative specific activity of pea seedlings' diamine oxidase (PSAO) is found to be 100 % towards putrescine, 111 % towards cadaverine, 56 % towards agmatine and spermidine, 44 % towards 1,6-diaminohexane, 30 % towards histamine, 8 % towards spermine and no activity has been found towards 1,3-diaminopropane (Kenten & Mann, 1952). Characterizing the relative specificity of this enzyme with the help of a oxygen sensor, it was found to be 100 % towards cadaverine, 86.2 % towards putrescine, 42.6 % towards 1,6-diaminohexane, 37.7 % towards 1,7-diaminoheptane, 11.6 % towards dopamine and 9.8 % towards histamine (Kivirand & Rinken, 2007). It has also been reported that PSAO has at least 3 times higher specificity towards histamine than other plant amine oxidases (Medda *et al*, 1995). According to literature, PSAO has very low activity towards 1,3-diaminopropane (Matsuda & Suzuki, 1977) and 1,2-diaminoethane (Kivirand & Rinken, 2007).

The above - presented data concerns studies, where only one substrate has been present in solution. In real probes, there are several BAs, produced in the process of putrefaction of proteins, simultaneously present and the inhibition phenomenon by competing substrates (amines) has to be considered, since the enzyme accepts different BAs as alternatives. The competing substrates generate analogous signals, measured with a reaction signal transduction system. In the case of oxidases, the enzyme actually requires a second substrate (oxygen) or a substance, which can act as an electron acceptor in the oxidation reaction and makes the reaction possible.

In BA biosensors the most commonly used signal transducers are various electrochemical sensors. According to the electron transfer mechanism utilized (with or without an additional mediator), electrochemical biosensors are classified into three generations (Eggins, 1996;Freire *et al*, 2003). An overview of the studied to date BA biosensors, all based on an amine-selective enzyme and some electrochemical signal transduction system, is given in (Kivirand & Rinken, 2011). A big problem for most BA biosensors is that it is not possible to differentiate between different BAs. As the ratio of BAs in a probe is resulting from the amino-acidic consistence of proteins, the results of BA analyses with biosensors are sometimes vague and reflect the combination of the levels of several BAs.

The studies with biosensors are usually based on the steady state response of the measuring system, where the system generates the maximum response. Most authors claim that with this method of data acquisition, the sensitivity of biosensor systems towards certain amines is not interfered by other biogenic amines, present in the sample. For example, Carsol *et al*.

studied a pool of different amines instead of a single amine substrate with amine oxidase based biosensors and detected no interactions of different amines (Carsol & Mascini, 1999). Albrecht-Ruiz et al. used diamine oxidase based colorimetric method for histamine detection and found that the absorbances of putrescine, cadaverine and histamine are additive, as the measured absorbances were less than 10 % smaller than their expected values. According to the presented data, the absorbances were smaller in all cases, where putrescine and/or cadaverine were present (Albrecht-Ruiz, 1999). Simultaneous analyses of the total BA content in fish probes with diamine oxidase based biosensor and ion-chromatography (conductivity detection) showed, that the obtained results with both methods were similar in cases when the BA contents were low. When BA concentrations began to rise during the storage of fish samples, differences between the results, obtained with diamine oxidase biosensor and ion-chromatography, began to increase (Carelli *et al*, 2007). There exists also a report about enzyme-based BA biosensor array, using an artificial neural network for the pattern recognition (Lange & Wittmann, 2002).

In the present study we analyze the output currents of BA biosensors, based on pea seedlings diamine oxidase and an electrochemical oxygen sensor to find the potential impact of different biogenic amines into the biosensor response and propose several models for the calibration of these biosensors in case of simultaneous presence of these amines in solutions. The biosensor response has been characterized by the maximum signal change parameter of the reaction, calculated from the transient phase data (Rinken, 2003).

## 2. Materials and methods

### 2.1 Materials and experimental procedures

Diamine oxidase was isolated from *Pisum sativum* seedlings (PSAO, EC 1.4.3.22), purified as described earlier (Kivirand & Rinken, 2007) and used in soluble form (PSAO activity in the enzyme extract was 5.43 IU/mg solid; 7.55 mg/mL). All other reagents used in the study were of analytical grade.

PSAO catalyzes the oxidation of amines by dissolved oxygen:



The change of dissolved oxygen concentration in reaction medium in the course of reaction was followed with a simple Clark - type oxygen sensor. All kinetic measurements were carried out under continuous stirring in a closed and thermostated glass cell in air-saturated 0.1 M phosphate buffer (pH 7.0) at 25° C. The reaction was started by injection of 100 µl PSAO solution into reaction medium, which was containing amine(s) and the sensor output signal was registered at 1 sec intervals (final PSAO concentration 0.108 IU/ml). Each experimental curve consisted of minimum 800-1600 data points, allowing the calculation of the biosensor response parameters according to the dynamic biosensor model (Rinken & Tenno, 2001). For these calculations, SigmaPlot® 9.0 (SPSS Software, USA) and GraphPad Prism® 5.0 (GraphPad Software, San Diego, USA) software were used.

### 2.2 The basic principles of the applied dynamic biosensor model

The dynamic model for biosensors is designed to take into account the kinetics of enzyme reactions with ping-pong mechanism, the diffusion of substrates and the inertia of the

diffusion – limited sensors (or the whole bio-sensing system). It enables the calculation of steady state parameters from the biosensor transient response with errors less than 3 % and with no need for additional determination of the system's geometrical, diffusion or partition parameters (Rinken & Tenno, 2001). According to this model, the normalized biosensor output current  $I(t)/I_0$  (corresponding to the normalized dissolved oxygen concentration  $c_{O_2}(t)/c_{O_2}(0)$ , as  $I$  depend linearly on  $c_{O_2}$ ) is expressed as a 3-parameter function of time  $t$ :

$$\frac{I(t)}{I_0} = \frac{c_{O_2}(t)}{c_{O_2}(0)} = A \exp(-Bt) + (1 - A) - 2A \sum_{n=1}^{\infty} (-1)^n \frac{\tau_s}{n^2} \left[ \exp(-Bt) - \exp\left(-n^2 \frac{t}{\tau_s}\right) \right] \quad (2)$$

where  $I(t)$  is the biosensor output current and  $c_{O_2}(t)$  the corresponding dissolved oxygen concentration at time moment  $t$ ;  $I_0$  is the output current and  $c_{O_2}(0)$  the corresponding dissolved oxygen concentration at the start of the reaction. Parameters  $A$  and  $B$  are complex parameters characterizing the ongoing chemical reaction, both depending hyperbolically on substrate concentration. Parameter  $A$  corresponds to the maximum possible biosensor signal change in case time  $t \rightarrow \infty$  (normalized signal change at steady – state) and parameter  $B$  is the kinetic parameter (the initial maximal slope of the enzyme – catalyzed process curve);  $\tau_s$  is the time constant of the internal processes of oxygen transducer taken together and characterizes the inertia of the transducer's (system's) response (Rinken & Tenno, 2001). Parameters  $A$ ,  $B$  and  $\tau_s$  are independent on each other. The most suitable parameter for the characterization of results obtained with different biosensors and the calibration of these biosensors, is the maximum signal change parameter  $A$ , as the kinetic parameter  $B$  includes an intercept (diffusion constant of oxygen) resulting from its definition and is more sensitive to experimental noise (Rinken, 2003). The application of the dynamic model enables to calculate the biosensor response parameters quickly from the transient signal, minimizing the influence of side processes, going on in the system ( $H_2O_2$  degradation, oxygen absorption through the liquid – air surface etc.) and to avoid the uncertainty of determining the steady state.

### 2.3 Correlation analysis of the biosensor data

The biosensor data (the values of maximum signal change parameters) was obtained over a longer period from experiments, carried out with different diamine oxidase – based biosensors in solutions, where one, two or three different biogenic amines (cadaverine, putrescine and/or histamine), which concentrations varied from 0 to 2 mmol/L, were present. For data analysis with different models we used the results of overall 112 measurements. The multivariate concentration – biosensor signal correlation analyses were carried out using DataFit 9.0 software (Oakdale Engineering, USA).

## 3. Discussion

### 3.1 Inhibition of diamine oxidase by a competing substrate

The selectivity towards different amines of diamine oxidase from pea seedlings (PSAO, EC 1.4.3.22), used in our studies, was characterized with the normalized maximum signal change parameter  $A$ , calculated from the decrease of oxygen concentration due to the oxidation of a particular substrate. The dependences of this parameter  $A$  on the concentrations of 1,5-diaminopentane (cadaverine), 1,4-diaminobutane (putrescine) and histamine in single substrate solutions are shown on Fig. 1 (a-c).



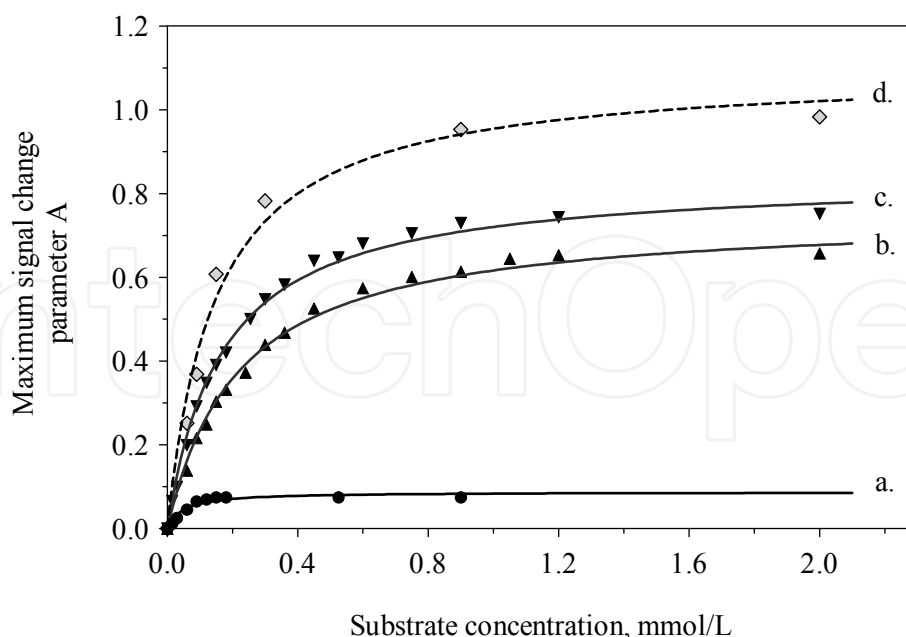


Fig. 1. The maximum signal change parameter  $A$  for (a) histamine; (b) putrescine; (c) cadaverine and (d) cadaverine and putrescine equimolar mixtures. Measurements were carried out in 0.1 M phosphate buffer (pH 7.00) at 25°C, [PSAO] = 0.108 IU/mL

As expected, all these curves were hyperbolas (Rinken, 2003). The maximum values of these hyperbolic curves indicate the PSAO activity towards particular amines and were used for the characterization of PSAO selectivity. Among the studied three substrates, PSAO showed the highest specific activity towards cadaverine – the normalized maximal signal decrease was 76% of the initial signal. Taking the PSAO specific activity towards cadaverine as 100%, the relative activity of PSAO towards other common BAs was 86.2% for putrescine and 9.8% for histamine. These results showed that concerning BAs, PSAO was highly selective towards cadaverine and putrescine and its activity towards histamine was approximately 10 times smaller. Applying a different method, spectrophotometrical studies, a much higher (27%) selectivity of PSAO towards histamine in comparison with cadaverine has been reported (Medda *et al*, 1995).

The selectivity of PSAO determines the relative speed of oxidation of different amines and has to be taken into consideration if PSAO is exploited for bio-recognition for analytical purposes in solutions, which simultaneously contain several biogenic amines, which oxidation is catalyzed by PSAO.

To study the inhibition of PSAO by a competing substrate, we followed the biosensor signal in the mixtures of two different amines. In equimolar solutions of cadaverine and putrescine, the resulting signal was considerably higher than the signals of cadaverine and putrescine by themselves, but lower than the sum of the signals of single substrates at similar concentrations (Fig.1 c-d). Comparing the parameter  $A$  values, obtained from solutions, containing only cadaverine or putrescine and from their different mixtures, it turned out that, as an average, the values of parameter  $A$  for mixtures were  $1.14 \pm 0.02$  times smaller than the summarized parameter  $A$  values for single substrates (Fig.2).

The analysis of the values of parameter  $A$  in mixtures at different substrates' concentration rates showed that neither cadaverine nor putrescine had a 100 % impact into the parameter

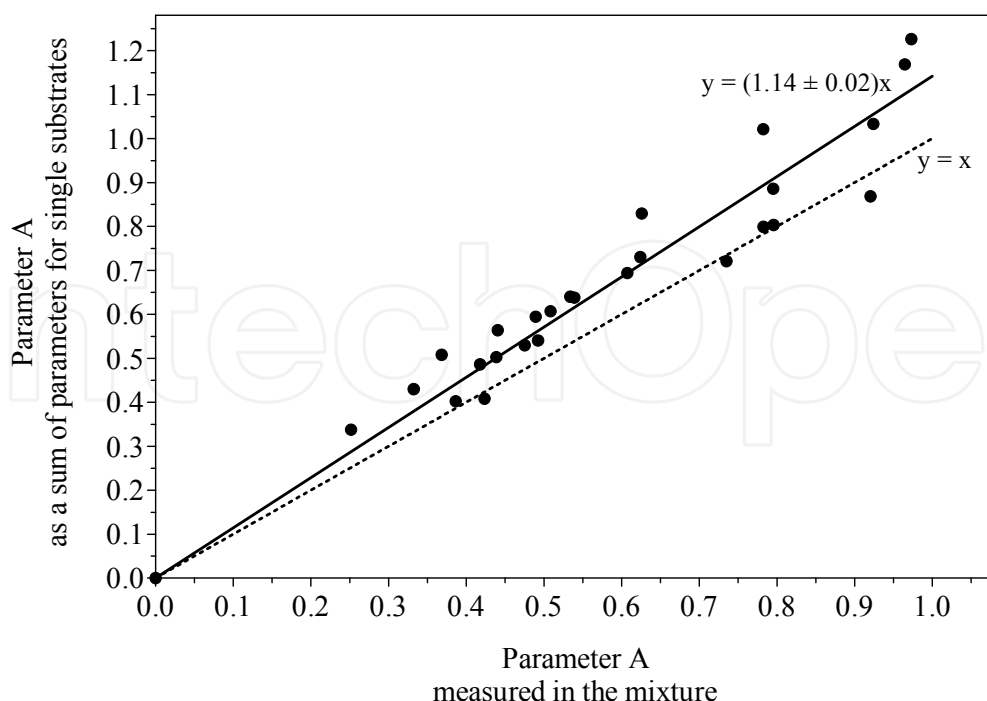


Fig. 2. The curve of the signal change parameter  $A$  as the sum of cadaverine and putrescine signals vs. the same parameter, measured in the mixture of cadaverine and putrescine, at similar substrate concentrations

$A$  value. Both these substrates had an impact between 60 and 85 % of the maximum effect at a certain concentration and the impact was smaller at concentrations below their particular  $K_M$  value. It is interesting to notice that the effect of one of these substrates was not dependent on the concentration of the other, as for example the putrescine effect in the resulting value of parameter  $A$  in the mixture was not dependent on the cadaverine concentration.

The biosensor response was also studied in mixtures, which besides cadaverine and/or putrescine contained histamine. In case the solution contained cadaverine and/or histamine, even at low cadaverine concentrations it was not possible to detect any histamine effect on the maximum signal change parameter  $A$  of the biosensor response (Fig. 3).

The calculated values of the parameter  $A$  were constant at fixed cadaverine concentrations, even if the histamine concentrations in the mixture varied from 0.01 – 3.0 mmol/L, the latter being over 20 times above histamine  $K_M$  value and exceeding several times the histamine maximum residue level in foods allowed by EEC regulations (100 mg/kg, corresponding to 0.9 mmol/L). Similar “screening” effect of histamine was also found in the mixtures of putrescine/histamine and cadaverine/putrescine/histamine.

These studies indicate that applying a PSAO based biosensor for the detection of histamine or the content of total amines, the concentrations of some amines are underestimated in case there are several BAs simultaneously present in the sample. This “screening” phenomenon and the dependence of the output signal on the rate and relative concentrations of different biogenic amines in the sample can lead to the underestimation of the content of biogenic amines in food, especially in cases when some particular biogenic amines become dominant in the course of putrefaction, like putrescine and cadaverine in decomposing of white fish.

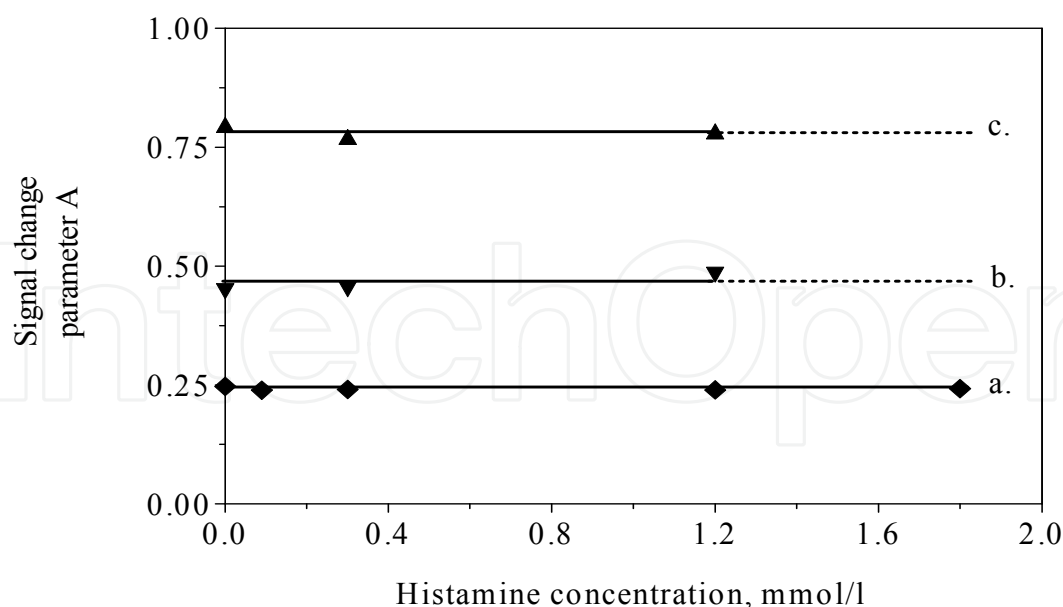


Fig. 3. The calculated maximum signal change (parameter A) in the mixtures of cadaverine and histamine at different concentration rates: histamine concentration is shown on x-axis and cadaverine concentration is (a) 0.15 mmol/L; (b) 0.30 mmol/L; (c) 0.60 mmol/L. Measurements were carried out in 0.1 M phosphate buffer (pH 7.00) at 25°C, [PSAO] = 0.108 IU/mL

The mechanism of PSAO - catalyzed oxidation of different amines follows a similar ping-pong pattern (Yamasaki *et al*, 1970) and the enzyme accepts different amines as alternatives to each other. For the characterization of the individual impact of the studied BAs into the biosensor output signal, which is a lump sum of several parallel reactions, we applied multivariate correlation analysis.

### 3.2 Multivariate correlation analysis

As different substrates generate analogous signals, a proper model should characterize the impact of these substrates into the resulting signal, enabling the application of the signal parameters for the calibration of a biosensor for several substrates. This task of calibration inescapably requires signal measurements in solutions with varying concentration ratios of BAs, which can be achieved by addition of a certain amount of definite amine to the probe and so producing a series of BA solutions with variable concentrations. A good model in combination with a vital number of measurements (equal to the number of coefficients in a model) in different solutions forms a solid base for the calibration of biosensors, which selectivity is relatively poor, e.g. PSAO-based biosensors for biogenic amines. Similar models for biosensor calibration can be applied for other low selectivity biosensors, like in the case of inhibition - based biosensors, where the catalytic action of an enzyme is modified not only by the presence of given species, but also by very different compounds like metal cations, various inorganic and organic species etc (Luque de Castro & Herrera, 2003).

#### 3.2.1 Hyperbolic model

In single substrate solutions, the biosensor maximum signal change parameter A depends on the substrate concentration hyperbolically (Rinken & Tenno, 2001):



$$A = \frac{k_{cat}^* [E]_{total} c_S^{bulk}}{k_{diff}^{O_2} K_{O_2} K_S + (k_{cat}^* [E]_{total} + k_{diff}^{O_2} K_{O_2}) c_S^{bulk}} \quad (3)$$

In Eq.(3),  $k_{cat}^*$  denotes the apparent catalytic constant of the enzyme-catalyzed reaction,  $[E]_{total}$  is the overall concentration of the enzyme in biosensor,  $k_{diff}^{O_2}$  is the apparent diffusion constant of oxygen,  $K_{O_2}$  is the dissociation constant for the enzyme-oxygen complex,  $K_S$  is dissociation constant for the enzyme-substrate complex and  $c_S^{bulk}$  is substrate concentration in solution.

As the reaction mechanism for competing BAs is similar, we used the sum of three hyperbolas, each describing the impact of an individual substrate. After transformation, the dependence of parameter  $A$  on substrate concentration (Eq.3) can be presented as a function with 2 coefficients (Rinken, 2003), which is the minimal number of coefficients to determine one normalized hyperbola, which maximum value equals to 1:

$$A = \frac{m c_S^{bulk}}{K_S + (m + 1) c_S^{bulk}} \quad (4)$$

In Eq. (4), the meaning of  $K_S$  is as defined above and the parameter  $m$  is a combination of 3 different physical constants and the total amount of enzyme  $[E]_{total}$ :

$$m = \frac{k_{cat}^* [E]_{total}}{k_{diff}^{O_2} K_{O_2}} \quad (5)$$

The resulting biosensor signal's maximum change parameter  $A$  in the mixture of 3 substrates can be expressed as a function of 3 variables (the number of variables corresponds to the number of competing substrates in solution) and 6 coefficients as following:

$$A = \frac{m_1 x}{K_1 + (m_1 + 1)x} + \frac{m_2 y}{K_2 + (m_2 + 1)y} + \frac{m_3 z}{K_3 + (m_3 + 1)z} \quad (6),$$

where  $x$ ,  $y$  and  $z$  are the variables denoting the concentrations of cadaverine, putrescine and histamine accordingly;  $m$  and  $K$  are appropriate coefficients. Applying Eq. 6 as a model for the biosensor parameter  $A$ , we got a good correlation with the experimental results with standard deviation  $\sigma = 0.097$  and correlation coefficient  $R = 0.93$ . The basic problem with this approximation was the great absolute values of coefficients  $m_3$  and  $K_3$  characterizing the effect of histamine, which were up to  $10^{20}$  times higher than the coefficients  $m_1$  and  $K_1$  for cadaverine and  $m_2$  and  $K_2$  for putrescine (Table 1, model 1). This may lead to the distortion of the assumption that the reaction mechanism is similar for all substrates and amplification of experimental noise.

To avoid this obstacle, we transformed Eq. 6 so, that the values of coefficients characterizing the impact of different substrates were in the same order, although we had to add 3 additional coefficients:

$$A = \frac{a_1 x}{b_1 + (a_1 + c_1)x} + \frac{a_2 y}{b_2 + (a_2 + c_2)y} + \frac{a_3 z}{b_3 + (a_3 + c_3)z} \quad (7)$$

In Eq. (7) coefficients  $a_1$ - $a_3$ ,  $b_1$ - $b_3$  and  $c_1$ - $c_3$  have the following physical meanings:

$$a = k_{cat}^*[E]_{total}$$

(8)

$$b = k_{diff}^{O_2}K_{O_2}K_S$$

(9)

$$c = k_{diff}^{O_2}K_{O_2}$$

(10)

Resulting from Eqs. 9 & 10, the quotient of coefficients b and c equals to  $b/c = K_S$ . Applying Eq. 7 as a model for the biosensor parameter A, we got similar fit as with Eq. 6, but the values of the equation coefficients, characterizing different substrates were in the same order (Table 1, model 2).

	Model	n	Coefficient values	σ	R <sup>2</sup>
1.	$A = \frac{m_1x}{K_1 + (m_1 + 1)x} + \frac{m_2y}{K_2 + (m_2 + 1)y} + \frac{m_3z}{K_3 + (m_3 + 1)z}$	6	m <sub>1</sub> = 6.05 K <sub>1</sub> =1.48 m <sub>2</sub> =1.65 K <sub>2</sub> =0.48 m <sub>3</sub> =5.12·10 <sup>18</sup> K <sub>3</sub> =6.26·10 <sup>20</sup>	0.097	0.871
2.	$A = \frac{a_1x}{b_1 + (a_1 + c_1)x} + \frac{a_2y}{b_2 + (a_2 + c_2)y} + \frac{a_3z}{b_3 + (a_3 + c_3)z}$	9	a <sub>1</sub> =1.57 b <sub>1</sub> =0.39 c <sub>1</sub> =0.26 a <sub>2</sub> =1.42 b <sub>2</sub> =0.41 c <sub>2</sub> =0.86 a <sub>3</sub> =0.26 b <sub>3</sub> =2.06 c <sub>3</sub> =10.73		

Table 1. The number and values of the calculated coefficients, the value of standard deviation  $\sigma$  and square of the correlation coefficient  $R^2$  for studied hyperbolic models of the BA biosensor

The correlation of the calculated and experimental values of parameter A is graphically shown on Fig. 4, where the ideal correlation is shown with a solid line. It can be seen that the calculated with hyperbolic model values of parameter A correlate normally with the experimental data and there are no systematic drifts, except in case of very low reaction effects.

The main disadvantage of this hyperbolic model is the rather high number of coefficients, which is 9 (6); 3 (2) coefficients for each substrate. So for the calibration of the BA biosensor towards 3 substrates, it is necessary to carry out measurements at least 9 (6) different BA concentration ratios: with the original sample and 8 (5) additional solutions, where a definite amount of one or more substrates has been added. This procedure is time-consuming and may also lead to notable experimental noise, although it enables the calibration of biosensors in mixtures of several substrates.

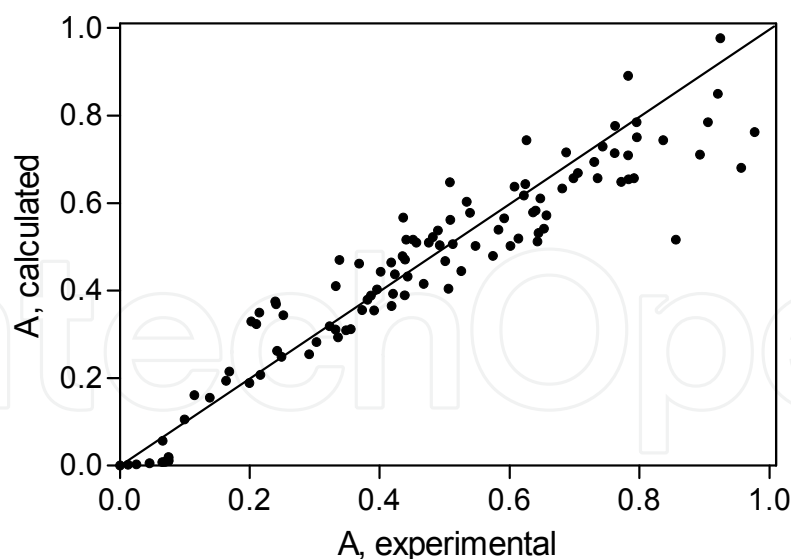


Fig. 4. Correlation of the values of maximum signal change parameter  $A$ , calculated with the help of the hyperbolic model and from experimental data. Line  $x=y$  marks the ideal correlation.

### 3.2.2 Quantitative Concentration – Signal Relationship (QCSR)

We also studied the application of some more formal models with a potentially smaller number of variables to characterize the biosensor signal parameter  $A$ . The QCSR model for the characterization of the biosensor maximum signal parameter  $A$  included different addends as “descriptors” of reactions, going on in the biosensor and taking into account the individual concentrations of BAs and the interference phenomena (inhibition by a competing substrate) of these compounds. The interference was described with 3 different types of “descriptors”, such as the products of duplicate substrate concentrations along with the quadrates and exponents of each single BA concentration:

$$A = ax + by + cz + dxy + fxz + gyz + hx^2 + jy^2 + kz^2 + le^x + me^y + ne^z + p \quad (11).$$

In Eq. 11,  $x$ ,  $y$  and  $z$  are the variables denoting the concentrations of cadaverine, putrescine and histamine accordingly; coefficients  $a, b, \dots, n$  denote the impact of each “descriptor” and  $p$  is the constant term.

The correlation studies were performed with 5 models composed of different number of “descriptors”, symmetrical towards all three studied substrates. The simplest model with 4 variables comprised the concentration terms of the three BAs and the constant term ( $A = ax + by + cz + p$ ). As expected, this simple model showed systematic deviations from the experimental data and the correlation was rather poor ( $R^2=0.437$ ), as it didn’t include the interference of the substrates (Fig. 5, blue dots). The value of standard deviation was 0.198 (Table 2, model 3).

Complementing the model with the addends of the products of duplicate substrate concentrations ( $A = ax + by + cz + dxy + fxz + gyz + p$ ), the number of “descriptors” increased to 7 and the correlation improved ( $R^2=0.547$ ), but not sufficiently to be applicable for practical purposes (Fig. 5, green dots). Model  $A = ax + by + cz + hx^2 + jy^2 + kz^2 + p$ , also comprising of 7 “descriptors” resulted in a bit better correlation ( $R^2=0.777$ ) (Fig. 5, lilac dots). Combining the 2 abovementioned models together, the number of “descriptors” increased to 10 resulting in the square correlation coefficient  $R^2=0.784$  (Fig. 5, black dots). As

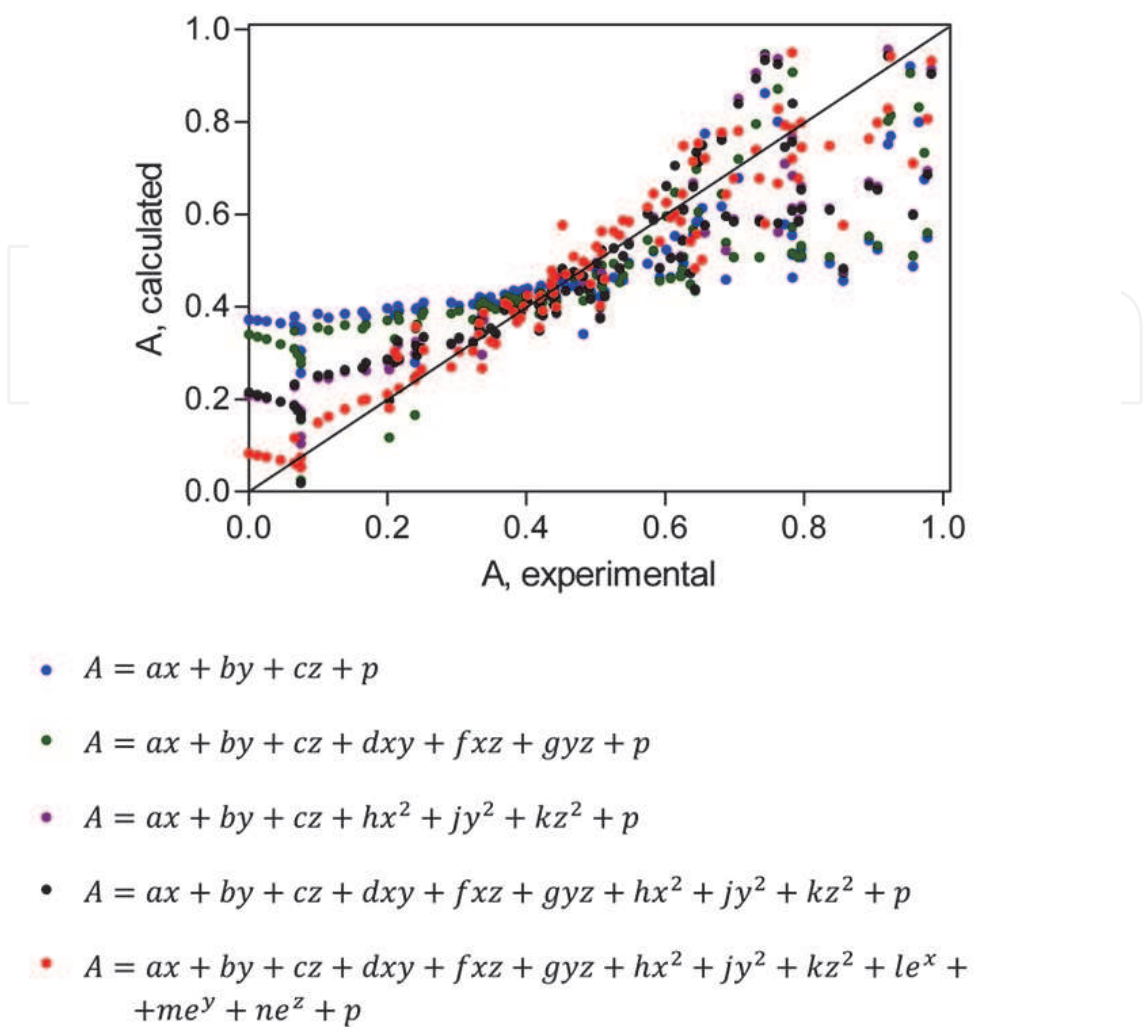


Fig. 5. Correlation of the values of maximum signal change parameter *A*, calculated with the help of different QCSR models and from experimental data. Line *x*=*y* marks the ideal correlation.

the correlation was not sufficiently improved with the addition of the products of duplicate substrate concentrations, these “descriptors” do not describe the interference of substrates in solution satisfactorily and need not be considered in models, applied for the calibration of BA biosensors.

	Model	n	Coefficient values	σ	R <sup>2</sup>
3.	$A = ax + by + cz + p$	4	a=0.41 b=0.20 c=-0.13 p=0.37	0.198	0.437
4.	$A = ax + by + cz + dxy + fxz + gyz + p$	7	a=0.51 b=0.34 c=-0.35 d=-0.24	0.180	0.547

			f=0.95 g=2.66 p=0.34		
5.	$A = ax + by + cz + hx^2 + jy^2 + kz^2 + p$	7	a=1.26 b=0.86 c=-0.25 h=-0.54 j=-0.34 k=0.15 p=0.21	0.127	0.777
6.	$A = ax + by + cz + dxy + fxz + gyz + hx^2 + jy^2 + kz^2 + p$	10	a=1.22 b=0.84 c=-0.33 d=-0.01 f=0.41 g=2.48 h=-0.52 j=-0.33 k=0.13 p=0.21	0.126	0.784
7.	$A = ax + by + cz + dxy + fxz + gyz + hx^2 + jy^2 + kz^2 + le^x + me^y + ne^z + p$	13	a=0.40 b=0.34 c=1.97 d=-0.13 f=0.27 g=-0.35 h=-2.98 j=-2.16 k=1.87 l=1.86 m=1.35 n=-2.26 p=-0.87	0.079	0.917
8.	$A = ax + by + cz + hx^2 + jy^2 + kz^2 + le^x + me^y + ne^z + p$	10	a=0.49 b=0.42 c=1.24 h=-2.87 j=-1.99 k=1.28 l=1.73 m=1.18 n=-1.48 p=-1.35	0.086	0.901

Table 2. The number of “descriptors” n, the values of the calculated coefficients, the values of standard deviations  $\sigma$  and squares of the correlation coefficients  $R^2$  for studied models of the BA biosensor



Addition of the exponential “descriptors” to the model improved the correlation and the fit of the model with the experimental data was similar to that of the hyperbolic model (Eq. 6). We used two different models, including the exponential terms, with overall 13 or 10 “descriptors” (Table 2, models 7 & 8). Similarly to the earlier results (Table 2, models 5 & 6), the products of duplicate substrate concentrations didn’t improve considerably the correlation and could be omitted. The QCSR model, including 10 “descriptors” (Table 2, model 8) resulted in  $R^2=0.901$ , which value is similar to that obtained from hyperbolic model ( $R^2= 0.871$ ; Table 1 model 2).

According to data on Figs. 4 & 5 and Tables 1 & 2, the smallest divergence of the calculated values from the experimental ones were achieved with the application of the hyperbolic models (models 1 & 2) and the more complicated QCSR models (models 7 & 8), although among the QCSR models one should prefer the one including smaller number of “descriptors”. All these models could theoretically be used for the calibration and measurements with BA biosensors in the presence of different amines simultaneously.

#### 4. Conclusions

The application of diamine oxidase based biosensors is a good option for the rapid determination of food quality, although in the case of simultaneous presence of several biogenic amines, the sensor signal is influenced by the rate of concentrations of different amines, formed during the process of protein putrefaction. In the presence of cadaverine and putrescine, the effect of histamine on the biosensor response is totally screened and the interaction of cadaverine and putrescine partially eliminates their own impact into the signal, causing the decrease of the resulting signal output, which is not an additive sum of the signals of single substrates. This screening effect can be successfully characterized with different multivariate models of biosensor signal. These models enable the calibration of biosensors towards several substrates in mixtures of different biogenic amines, which generate analogous biosensor response. Similar methodology can be applied in other systems, where the selectivity of a biosensor is low and the traditional ways of calibration do not enable to determine the concentrations of individual compounds in mixtures.

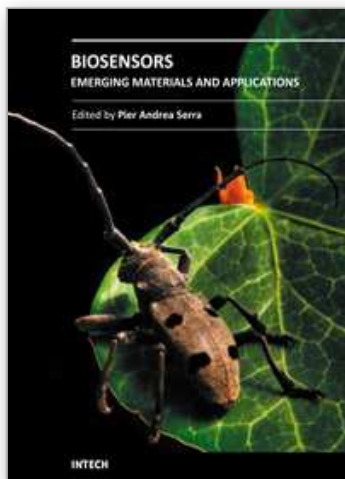
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A biosensor is a detecting device that combines a transducer with a biologically sensitive and selective component. Biosensors can measure compounds present in the environment, chemical processes, food and human body at low cost if compared with traditional analytical techniques. This book covers a wide range of aspects and issues related to biosensor technology, bringing together researchers from 19 different countries. The book consists of 27 chapters written by 106 authors and divided in three sections: Biosensors Technology and Materials, Biosensors for Health and Biosensors for Environment and Biosecurity.

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