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BIOSENSORS

Simultaneous biosensing of biogenic amines: signal analysis and biosensor calibration

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Abstract. The application of biosensors in complex real-world samples is often complicated due to the combined effects and interferences of various compounds on the biosensor signal. However, thorough modelling and chemometric methods allow us to evaluate the impact of different analytes and calibrate these biosensors for the multiplexed analyses of the targeted compounds. A typical multianalyte mixture includes biogenic amines, produced during the putrefaction of proteins. Meanwhile, the detection of particular biogenic amines is a valuable tool for assessing the freshness and quality of a wide variety of protein-containing foods. In the current study, we analysed the signal of biosensors for the simultaneous detection of four major biogenic amines (cadaverine, putrescine, histamine, and spermidine) and proposed two different approaches for their multivariate calibration. The evaluation of the proposed models and the calculation of their characteristic coefficients were based on experimental data from over three hundred different mixtures with randomly varying substrate concentrations.

Keywords: biogenic amines, detection, biosensor, multiplex analysis, chemometrics.

1. INTRODUCTION

In response to the demand for rapid on-site analyses, the number of different biosensors and biosensor setups is growing fast. A biosensor is a device that combines specific bio-recognition with a physicochemical detector and generates a response related to the concentration of a targeted analyte (Thévenot et al. 2001). Commonly, the biosensor response calibration process requires a simple univariate regression (Martynko and Kirsanov 2020). However, when analysing samples with complex real-world matrices, the interpretation of results and biosensor calibration are much more complicated due to the combined effects and interferences from various compounds or groups of compounds.

The analysis of complex signals arising in group-selective biosensors, such as those measuring biogenic amines (BAs) or different sugars (Kivirand and Rinken 2009), or in enzyme inhibition-based biosensors (Luque de Castro and Herrera 2002), is complicated. Identifying and assessing single compounds, as well as calibrating biosensors, require thorough signal modelling and chemometric tools (Martynko and Kirsanov 2020). Chemometrics is also commonly used for the detection of multiple compounds with biosensor arrays or ‘bio-electronic tongues’ (del Valle 2010).

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Biogenic amines are natural basic nitrogenous compounds that are mainly formed through the decarboxylation of free amino acids during protein ageing or by the amination and transamination of aldehydes and ketones (Erdag et al. 2019; Li et al. 2018). In the food industry, the detection of particular BAs is a valuable tool for assessing the freshness and quality of a wide variety of protein-containing products, such as fish, meat, cheese, wine, and more (Gardini et al. 2016; Önal 2007; Papageorgiou et al. 2018; Vinci and Antonelli 2002; Yano et al. 1996). The most common BAs used to evaluate food quality are histamine, putrescine, and cadaverine (Papageorgiou et al. 2018). Other BAs often found in foodstuffs include spermine, spermidine, tyramine (Alonso-Lomillo et al. 2010), and trimethylamine (Mitsubayashi et al. 2004). Limits have been established only for the intake of histamine. However, the allowed maximum residue level (MRL) of histamine in food varies: the EU has established that the histamine level should be below 100 mg/kg, with a maximum of 200 mg/kg (European Commission 2005), while the U.S. Food and Drug Administration has set the histamine limit at 50 mg/kg (DeBeer et al. 2021). There are no regulations for other BAs, though there are indications that cadaverine and putrescine increase histamine toxicity by inhibiting the enzymes involved in histamine biodegradation (Niculescu et al. 2000).

Chromatographic techniques, such as high-performance liquid chromatography (HPLC), gas chromatography, and thin-layer chromatography, are used mostly for precise quantitative analysis of BAs (Neofotistos et al. 2019). The globally accepted standard method for the detection of BAs is liquid chromatography–mass spectrometry (LC–MS), allowing to achieve a limit of detection (LOD) within the range of 0.01 to 0.10 mg/kg in different matrices (Yoon et al. 2015). The LOD of HPLC analysis is two to three orders of magnitude below the allowed MRL, indicating that analytical methods with considerably higher limits can be used for routine food control. However, these methods require sample pre-treatment or derivatisation of complex samples before the final separation to remove compounds that may interfere with the measurements (Papageorgiou et al. 2018). As widely known, the precision of results and the analysis time are proportional quantities (Getzinger et al. 1994).

A rapid alternative for the detection of BAs is biosensor technology. BA biosensors commonly comprise different amine-selective enzymes, such as amine oxidases (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), or flavin-containing mono-oxygenase type-3 (EC 1.14.13.8) in combination with a variety of signal transduction systems, including electrochemical and optical ones. Most biosensors presented in the literature use enzymes as biorecognition systems (Kivirand and Rinken 2011; Papageorgiou et al. 2018). In recent years, some authors have proposed molecularly imprinted polymers (MIPs) as alternatives to be used as synthetic recognition elements in histamine analysis in sensor systems (Bongaers et al. 2010; Horemans et al. 2010; Mattsson et al. 2018). Nevertheless, most of these methods are time-consuming and have yet to be applied to real samples. Additionally, in most cases, the sample matrix effect is a major concern.

The most commonly used enzyme for the biorecognition of BAs is diamine oxidase from pea (*Pisum sativum*) seedlings (PSAO, EC 1.4.3.22). This enzyme catalyses the oxidative deamination of BAs according to the following overall chemical equation (Di Paolo et al. 2011; McGuirl and Dooley 1999; Prabhakar and Siegbahn 2001):

$$R - CH_2NH_3^+ + O_2 + H_2O \rightarrow NH_4^+ + R - CHO + H_2O_2. \quad (1)$$

The activity of PSAO towards different BAs varies widely (Halász et al. 1994; Medda et al. 1995; Pietrangeli et al. 2003; Kivirand and Rinken 2011) and is highest towards symmetric diamines – cadaverine (pentane-1,5-diamine) and putrescine (butane-1,4-diamine). PSAO also exhibits substantial activity towards spermidine (N-(3-Aminopropyl)-1,4-butanediamine) (Bóka et al. 2012; Kivirand and Rinken 2009; Wimmerová and Macholán 1999), although spermidine acts as an inhibiting substrate towards PSAO and can cause problems when samples analysed are older than one week (Kivirand et al. 2016). The activity of PSAO towards the most common BA, histamine (2-(1*H*-Imidazol-4-yl)ethanamine), is much lower (Bóka et al. 2012; Kivirand and Rinken 2009; Pospiskova et al. 2013; Wimmerová and Macholán 1999). A condensed overview of the substrate specificity of PSAO is presented in Table 1.

Table 1. The specific activity of PSAO towards different substrates

Cadaverine %	Putrescine %	Spermidine %	Histamine %	Method of detection	Reference
100	80	24	11	Electrochemical sensor: enzyme-modified graphite electrode	Bóka et al. 2012
100	96	17	9	Electrochemical sensor: enzyme-modified carbon paste/graphite powder electrode	Wimmerová and Macholán 1999
100	126	56	17	Fibreoptical sensor: enzyme modified SEPABEADS® EC-HA 403	Pospiskova et al. 2013
100	117	19	13	Fibreoptical sensor: enzyme-modified magnetic chitosan microparticles	Pospiskova et al. 2013
100	86	n/a	10	Oxygen sensor	Kivirand and Rinken 2009

n/a – not applicable

Biosensor analyses, including enzyme-based BA sensors, commonly rely on measuring the system's steady-state response, meaning that the signal remains within an agreed error limit for some time (Baker and Gough 1996). Data on the effect of different BAs on the BA biosensor signal are controversial. Some authors declare that by using this data acquisition method, the biosensor system's sensitivity to particular amines is not interfered with by other BAs in the sample (Albrecht-Ruiz et al. 1999; Carsol and Mascini 1999). The validity of this claim is limited to cases where the enzyme activity is high enough to catalyse the oxidation of all BAs present during the measurement time. For example, Carsol and Mascini (1999) studied a pool of different amines instead of a single amine substrate with amine oxidase-based biosensors and detected no interactions among different amines. Albrecht-Ruiz et al. (1999) used a diamine oxidase-based colorimetric method for histamine detection. They found that the signals of putrescine, cadaverine, and histamine were additive, as the measured absorbances were less than 10% smaller than their expected values. However, the absorbances were smaller in all cases where the most active substrates, putrescine and/or cadaverine, were present. Simultaneous analyses of the total BA content in fish probes with diamine oxidase-based biosensors and ion chromatography showed similar results when the BA contents were low (Carelli et al. 2007).

Nevertheless, when the concentrations began to increase during the storage of fish samples, differences between the results started to rise (Carelli et al. 2007). The effect of higher BA concentrations on the specific activity of PSAO oxidase towards single BAs has been demonstrated by Sánchez-Pérez et al. (2022). We have also found the non-additivity of cadaverine, putrescine, and histamine signals in the case of their simultaneous presence in the sample analysed with a BA biosensor (Rinken et al. 2011).

It is currently impossible to distinguish the signals of different BAs in their naturally occurring mixtures, such as those produced during the putrefaction of proteins. These complex mixtures contain all major BAs, acting as competing substrates and generating similar signals. To complicate things further, spermidine acts as an inhibiting substrate towards PSAO (Kivirand et al. 2016), and its presence can decrease the apparent concentrations of all other BAs. The effect of spermidine is dynamic and changes in time depending on measurement conditions and protocols used for signal detection. Multi-enzyme sensor arrays have been proposed for the simultaneous detection of three BAs (histamine, tyramine, and putrescine) by pattern recognition

with an artificial neural network (Lange and Wittmann 2002). However, this approach requires sample pre-treatment (extraction and neutralisation) and is unsuitable for on-site analyses.

In the present study, we analyse the signal of BA biosensors, which are based on PSAO and an amperometric Clark-type oxygen sensor, and propose two different models for their calibration in BA mixtures. The proposed physicochemical approach considers ongoing processes, the catalytic activity of PSAO towards different amines, and the inhibiting effect of spermidine on PSAO, while the formal model is based on the use of formal 'descriptors' of the BA concentrations. The models are evaluated, and their characteristic parameters for the applied biosensing system are calculated using experimental data from different mixtures of four BAs: cadaverine, putrescine, histamine, and spermidine.

2. EXPERIMENTAL PART

2.1. Materials and experimental procedures

An extract from pea seedlings rich in diamine oxidase (PSAO, EC 1.4.3.22, PSAO-specific activity 41 U/mL) was used in soluble form as described earlier (Kivirand and Rinken 2007). The extract was stored at –20 °C and used within four hours after melting at 4 °C. The standards of spermidine, cadaverine, putrescine, and histamine were purchased from Sigma-Aldrich (Germany). All reagents used in the study were of analytical grade. All solutions were prepared with ultrapure deionised water (18.2 MΩ/cm).

The decrease of dissolved oxygen concentration due to the oxidation of BAs (Eq. (1)) was monitored with a robust Clark-type oxygen sensor (Elke Sensor, Estonia) connected to a computer via an A/D converter and using a custom Oxysens software (Fig. 1). The kinetic measurements were performed under constant stirring in a closed glass cell in air-saturated BA solutions in 0.1 M phosphate buffer (pH 7.0) at 25 °C. The reaction was started by injecting 100 µL of PSAO solution (with PSAO-specific activity always adjusted to 41 U/mL) into the reaction medium, containing a single amine or a mixture of different amines with concentrations of BAs varying from 0 to 2 mmol/L.

The sensor output was registered at 1-sec intervals. Each measurement consisted of at least 800 data points. The biosensor steady-state signal was calculated using the dynamic biosensor model that considers the kinetics of ping-pong mechanism enzyme-catalysed reactions, the substrates' diffusion, and the inertia of the diffusion-limited sensors (Rinken and Tenno 2001). According to this model, the normalised biosensor

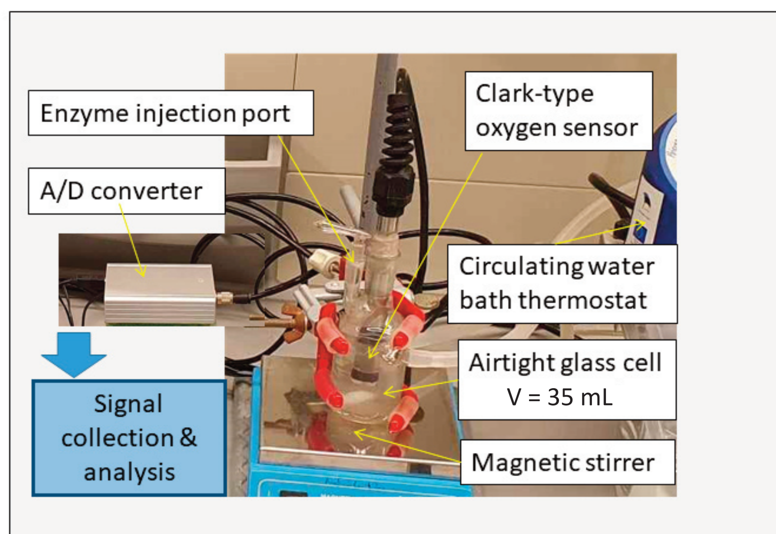

Fig. 1. Scheme of the assay unit.

output current $I(t)/I_0$ or the normalised dissolved oxygen concentration (DOC) $c_{O_2}(t)/c_{O_2}(0)$ ($I(t)$ depends linearly on $c_{O_2}(t)$) is expressed as a three-parameter function of time t:

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

where $I(t)$ is the biosensor output current and $c_{O_2}(t)$ the corresponding DOC at time moment t, I_0 is the output current and $c_{O_2}(0)$ the corresponding DOC at the start of the reaction, and t is time. Parameters A and B characterise the ongoing chemical reaction, and both depend hyperbolically on substrate concentration. Parameter A denotes the maximum signal change in case time $t \to \infty$ (normalised signal change at steady state), and parameter B stands for the kinetic parameter (the initial slope of the enzyme-catalysed process curve). Both A and B are complex parameters, depending on the physicochemical nature of a substrate, e.g. the dissociation constant of the enzyme-substrate complex, and the biosensor setup, as τ_s is the time lag constant of the combined internal processes of oxygen transducer, characterising the inertia of the transducer's (system's) response (Rinken and Tenno 2001).

The most appropriate parameter for the calibration of biosensors is the maximum signal change parameter A. This parameter is calculated from the biosensor transient signal and is insensitive to side reactions, thus minimising the influence of uncontrollable side processes occurring in the system, such as H_2O_2 degradation and oxygen absorption through the liquid–air surface (Rinken 2003). SigmaPlot® 12.3 (Grafiti LLC, USA) and GraphPad Prism® 5.0 (GraphPad Software, USA) software were used for these calculations. All data were normalised to acquire comparable results.

2.2. Correlation analysis

The data of biosensor measurements were collected over a longer period. The results of more than 300 measurements were used to fit experimental data with models (including statistical ones) proposed for biosensor calibration for the multiplex detection of BAs. The multivariate concentration vs biosensor signal correlation analyses were performed with DataFit 9.0 software (Oakdale Engineering, USA).

3. RESULTS AND DISCUSSION

3.1. The activity of diamine oxidase towards different biogenic amines

The activity of PSAO towards the studied BAs was characterised by the normalised maximum signal change parameter A, calculated from experimental data according to Eq. (2). The dependence of parameter A on the concentration of different amines in single substrate solutions is shown in Fig. 2.

As expected, cadaverine, putrescine, and histamine act as normal substrates towards PSAO in single-amine solutions and achieve a saturated state at concentrations considerably exceeding the values of half-limiting constants (Kivirand and Rinken 2009). However, spermidine acts differently: it exhibits inhibiting properties and performs like an inhibiting substrate (Kivirand et al. 2016). The dependence of the spermidine signal had an irregular bell-shaped form with a flat maximum at 6 mM (Kivirand et al. 2016), meaning that in the mixtures of BAs formed during the putrefaction of proteins, the occurrence of spermidine can complicate the determination of other BAs. This phenomenon is often described regarding PSAO-based biosensors (Ben-Gigirey et al. 1998; Hosseini et al. 2013; Özogul et al. 2006; Ruiz-Capillas and Moral 2004).

To characterise the specific activity of PSAO towards individual amines, we determined the limiting values of parameter A considering the hyperbolic dependence of parameter A on substrate concentration (Rinken 2003):

$$A = \frac{Mc_S^{bulk}}{K_S + (M+1)c_S^{bulk}}, \quad (3)$$

where coefficient M is a combination of three kinetic constants and the enzyme total amount is as follows:

$$M = \frac{k^{*}_{cat}[E]_{total}}{k^{O_2}_{diff} K_{O_2}}. \quad (4)$$

In Eqs (3)–(4), c_S^{bulk} is the substrate concentration and K_S the dissociation constant for the enzyme-substrate complex, k^{*}_{cat} is the catalytic constant of the reaction, $k^{O_2}_{diff}$ is the diffusion constant of oxygen, K_{O_2} is the dissociation constant for the enzyme-oxygen complex, and $[E]_{total}$ is the overall concentration of enzyme in the solution, kept constant in all measurements.

The limiting values of parameter A for the studied BAs and the relative activity of PSAO towards these BAs were calculated from the experimental data (Table 2).

PSAO has the highest specific activity towards cadaverine, while its activity towards putrescine, histamine, and spermidine is lower. Based on spectrophotometric measurements, the specific activity of PSAO towards histamine has been reported to be considerably higher, at 27% (Medda et al. 1995). However, the data available about the catalytic properties of PSAO are controversial (Pietrangeli et al. 2007; Stránská et al. 2007).

In practice, we have to analyse the mixtures of BAs, which result from the decarboxylation of amino acids originating from different proteins and where the inhibition of PSAO by a competing substrate takes place

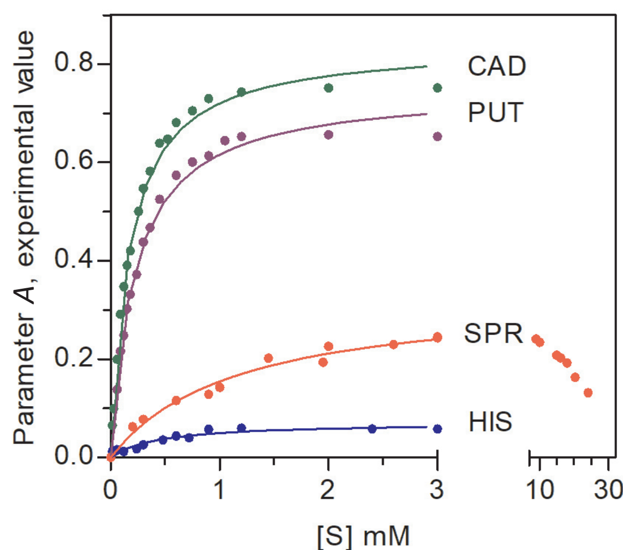

Fig. 2. The maximum signal change parameter A at different cadaverine (CAD), putrescine (PUT), spermidine (SPR), and histamine (HIS) concentrations ([S]). All measurements were carried out in 0.1 M phosphate buffer (pH 7.00) at 25 °C, [PSAO] = 0.108 IU/mL.

Table 2. The limiting values of parameter A for different amines

Amine	Limiting value of A_{max}	Relative activity, %
Cadaverine	0.841 ± 0.078	100
Putrescine	0.752 ± 0.057	89.4
Histamine	0.071 ± 0.007	8.4
Spermidine*	0.332 ± 0.016	39.5

* The limiting value of parameter A for spermidine was determined from the measurements at spermidine concentrations below 6 mM.

(Wójcik et al. 2021). It has been found that the presence of different BAs influences the specific activity of diamine oxidase towards single BAs, e.g. histamine degradation is affected by cadaverine, putrescine, and spermidine (Sánchez-Pérez et al. 2022). We have also demonstrated that in the mixtures of putrescine and cadaverine, the resulting biosensor signal is considerably higher than the signals of cadaverine and putrescine individually, but 1.14 times lower than the summarised parameter *A* values for the single substrates (Rinken et al. 2011). When histamine is also present, no histamine effect on the parameter *A* value is detectable, even at low cadaverine and/or putrescine concentrations (Rinken et al. 2011). In case spermidine is present along with other BAs, it acts as an inhibitor (Kivirand et al. 2016), and its concentration fluctuates due to non-catalytic oxidation or the action of microorganisms (Ben-Gigirey et al. 1998; Hosseini et al. 2013; Özogul et al. 2006; Ruiz-Capillas and Moral 2004; Křížek et al. 2011).

3.2. Multivariate correlation

To minimise the effect of side reactions and the inhibiting effect of spermidine, the parameter *A* values calculated from the pre-steady-state data were considered as the biosensor response in a particular experiment. We used the results of more than 300 BA biosensor measurements in single amine solutions and different mixtures of multiple BAs for the multivariate correlation analyses (Appendix). The BA biosensor output signals in the presence of multiple BAs were fitted to two different models using both physicochemical and statistical approaches.

3.2.1. Physicochemical model

In single-substrate solutions, the maximum signal change parameter *A* of the BA biosensor depends hyperbolically on the substrate concentration (Eq. (3)). For spermidine, this assumption is valid at low concentrations (<6 mmol/L) (Kivirand et al. 2016). Considering that the spermidine concentration in foods is commonly lower than 3 mmol/L (Muñoz-Esparza et al. 2019), the inhibiting effect of spermidine can be omitted for practical analysis in case the calculated parameter *A* is used as a biosensor response. The resulting biosensor response for four competing substrates is defined as a multivariable function of four variables and eight coefficients as follows:

$$A = \sum_{i=1}^{4} \frac{m_i x_i}{K_i + (m_i + 1) x_i}, \tag{5}$$

where x_i are the variables denoting the concentrations of cadaverine, putrescine, histamine, and spermidine, respectively, and K_i and m_i are appropriate coefficients. Here, the effect of each variable is described by two coefficients, which is the minimal number of coefficients to describe a hyperbolic dependence (Rinken 2003). In the case of four substrates, the overall number of coefficients required for this fitting is eight.

When fitting Eq. (5) to the experimentally obtained biosensor data, the standard error σ of the estimated biosensor maximum signal change parameter *A* was 0.127 (measurements where the spermidine concentration exceeded 6 mmol/L were excluded) and the adjusted coefficient of multiple determination R^2 was 0.77. The graphical correlation of the calculated and experimental parameter *A* values is shown in Fig. 3, with the ideal coincidence indicated by a solid line. There are no systematic drifts throughout the whole biosensor working range, where the parameter *A* value ranges from 0 to 1.

The acquired eight coefficient values for this fitting are shown in Table 3. As can be seen from Table 3, some coefficients for histamine and spermidine turned out to be negative, indicating a very strong interaction of BAs. The absolute values of both coefficients for histamine were notably high, which, according to the mathematical form of Eq. (5), eliminates the effect of histamine on the calculated parameter *A* value. For spermidine, the model fitting indicated an apparent overall negative *K* value, even at concentrations below 6 mmol/L, where spermidine was considered to act as a substrate (Kivirand et al. 2016). However, a negative *K* value is meaningless in physicochemical terms.

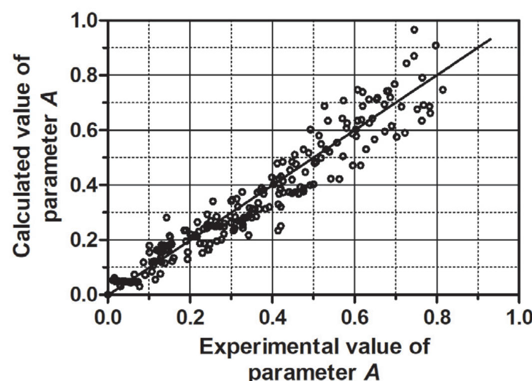

Fig. 3. Calculated parameter *A* values obtained from the physicochemical model and experimental parameter *A* values for BA biosensor.

Table 3. Values of coefficients *K* and *m* for studied BAs

	K	*m*
Cadaverine	3.61 ± 0.81	0.93 ± 0.28
Putrescine	1.42 ± 0.26	0.50 ± 0.15
Histamine	>>10e70	<<–10e70
Spermidine	–0.17 ± 2.65	0.39 ± 0.28

3.2.2. Formal models

In addition, a formal model was applied to characterise the signal change parameter *A*. This model includes numerous addendums or 'descriptors' to characterise the resulting biosensor signal of ongoing reactions generated by different BAs and their interferences. The simultaneous oxidation processes and counteractions of different BAs were described as a sum of four different types of 'descriptors', namely the single substrate concentrations, the cross terms of substrate concentrations, the quadrates, and the exponents of each single BA concentration:

$$A = a + \sum_{i=1}^{4} b_i x_i + \sum_{\substack{i,j=1 \\ i \neq j}}^{4} c_{ij} x_i x_j + \sum_{i=1}^{4} d_i x_i^2 + \sum_{i=1}^{4} f_i e^{x_i}, \tag{6}$$

where a is an absolute term, x_i and x_j are the concentrations of single BAs, and coefficients b_i....f_i denote the impact of each 'descriptor'.

We fitted the experimental data with six different formal models, each comprising different numbers and combinations of 'descriptors' that were symmetrical across all BAs. The simplest model, Model 1, consisted of only five addendums, adding up the single BA concentrations and an absolute term corresponding to conventional linear biosensor calibration in case there is no interaction between different BAs. As expected, the correlation coefficient for this model was relatively low ($R^2 = 0.54$), and the standard error of the estimate was rather high ($\sigma = 0.179$). On the other hand, the most complex model, Model 6, included all proposed addendums, meaning that 19 different coefficients were required for this fitting. Using Model 6 for practical applications implies conducting at least 19 measurements at different BA concentration ratios to determine these coefficients and calibrate the BA biosensor towards four BAs.

The σ and R^2 values for different fittings, along with the number of 'descriptors' used, are shown in Table 4. As can be seen from the data in Table 4, the correlation of the fitting with the experimental data was not sufficiently improved with the addition of the cross and/or exponential terms. However, the addition of the quad-

Table 4. Values of correlation coefficients and standard deviations for different fittings

Model		n^*	σ^{**}	R^{2***}
1	$A = a + \sum_{i=1}^{4} b_i x_i$	5	0.179	0.54
2	$A = a + \sum_{i=1}^{4} b_i x_i + \sum_{i,j=1; i \neq j}^{4} c_{ij} x_i x_j$	11	0.170	0.59
3	$A = a + \sum_{i=1}^{4} b_i x_i + \sum_{i=1}^{4} d_i x_i^2$	9	0.135	0.74
4	$A = a + \sum_{i=1}^{4} b_i x_i + \sum_{i=1}^{4} d_i x_i^2 + \sum_{i=1}^{4} f_i e^{x_i}$	13	0.124	0.79
5	$A = a + \sum_{i=1}^{4} b_i x_i + \sum_{i=1}^{4} d_i x_i^2 + \sum_{i,j=1; i \neq j}^{4} c_{ij} x_i x_j$	15	0.122	0.79
6	$A = a + \sum_{i=1}^{4} b_i x_i + \sum_{i=1}^{4} d_i x_i^2 + \sum_{i,j=1; i \neq j}^{4} c_{ij} x_i x_j + \sum_{i=1}^{4} f_i e^{x_i}$	19	0.106	0.85

* number of coefficients, ** standard error of the estimate, *** adjusted coefficient of multiple determination

Table 5. Coefficient values for Model 3. The order of BAs in the table is the following: 1) cadaverine, 2) putrescine, 3) histamine, 4) spermidine

$A = a + \sum_{i=1}^{4} b_i x_i + \sum_{i=1}^{4} d_i x_i^2$									
Coefficient	a	b_1	b_2	b_3	b_4	d_1	d_2	d_3	d_4
Value	0.178	1.079	0.769	0.161	–0.136	–0.442	–0.287	–0.100	1.52
σ^*	0.01	0.06	0.06	0.07	0.02	0.04	0.04	0.03	0.002

* standard error

ratic terms to the model improved the fitting with the experimental data. The best ratio of the correlation coefficient to the number of fitting coefficients was obtained for Model 3. The values of coefficients for this most optimal model are shown in Table 5.

For cadaverine, putrescine, and histamine, the coefficient values were positive for substrate concentration ‘descriptors’ and negative for quadratic terms of substrate concentrations, while for spermidine, it was the opposite, indicating that spermidine does not act as a typical substrate. The overall fittings of the formal statistical models with experimental data are shown in Fig. 4.

The correlation of the formal models with the experimental data indicates that the best fitting was achieved at medium parameter *A* values, while at higher parameter *A* values, the dispersion of the results was relatively high (Fig. 3). At parameter *A* values below 0.2, the deviation of the fittings from experimental data, contrary to the hyperbolic model, was systematic, especially with Model 1 and Model 2, resulting in higher parameter

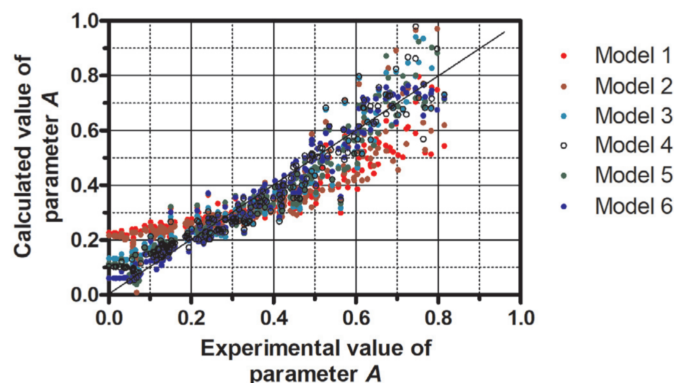

Fig. 4. Overall fittings of formal models.

A values than those obtained experimentally. This indicates that the catalytic activity of PSAO towards different BAs depends on the BA concentration.

The applicability of the proposed chemometric approach was tested for detecting BAs in Estonian white-flesh fish samples, e.g. in Northern pike, European flounder, etc. The biosensor results were in good correlation with the results obtained with LC–MS, especially regarding putrescine concentrations calculated using the physicochemical model (Eq. (5)). The preliminary results show that the proposed models can be used for biosensor signal analysis for the rapid biosensor-based assessment of BAs and fish freshness (unpublished data).

4. CONCLUSIONS

The current research considers the modelling and calibration of biosensors for the multiplex detection of BAs in their mixtures. Fitting experimental data from over three hundred biosensor measurements in different mixtures of four major BAs with two different modelling approaches indicated correlation coefficients between 0.54 and 0.85. With the physicochemical approach, the correlation coefficient of 0.77 was obtained using eight independent BA-related coefficients, while the most optimal formal model resulted in a correlation coefficient of 0.74 using nine coefficients.

Considering the deviation of experimental data from the ideal fitting, the physicochemical approach displayed no systematic drifts throughout the whole biosensor working range, contrary to the formal approach, which exhibited a systematic deviation of the fitting at lower biosensor signal values, resulting in higher parameter *A* values than those obtained experimentally. Therefore, the physicochemical model, which considers ongoing processes, the catalytic activity of PSAO towards different amines and the inhibiting effect of spermidine on PSAO, can be deemed more suitable for calibrating biosensors for the multiplex detection of BAs in their mixtures.

Furthermore, it became evident that the catalytic activity of PSAO towards different BAs in mixtures depends on the concentration of a particular BA, which requires using significantly more complex models than the ones employed in the current study to describe the biosensor signals generated by BAs in their mixtures. The proposed approach can also be applied to other low-selectivity biosensors the responses of which responses include the signals of several bio-recognition processes.

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APPENDIX

The experimental values of parameter A in BA mixtures

	Cadaverine	Putrescine	Histamine	Spermidine	A (exp. value)
1.	0.06	0.06	0	0	0.2516
2.	0.09	0.09	0	0	0.3684
3.	0.15	0.15	0	0	0.6073
4.	0.30	0.30	0	0	0.7823
5.	0.90	0.90	0	0	0.9527
6.	2.00	2.00	0	0	0.9827
7.	0.15	0	0.30	0	0.2405
8.	0.12	0	0.30	0	0.2103
9.	0.30	0	0.30	0	0.4512
10.	0.60	0	0.30	0	0.7717
11.	0.15	0	1.20	0	0.2395
12.	0.12	0	1.20	0	0.2021
13.	0.30	0	1.20	0	0.4819
14.	0.60	0	1.20	0	0.7831
15.	0	0	0	0	0
16.	0.015	0	0	0	0.0657
17.	0	0	0.015	0	0.0124
18.	0.03	0	0	0	0.0997
19.	0	0	0.03	0	0.0258
20.	0.06	0	0	0	0.1996
21.	0	0.06	0	0	0.1385
22.	0	0	0.06	0	0.0457
23.	0.09	0	0	0	0.2917
24.	0	0.09	0	0	0.2161
25.	0	0	0.09	0	0.0654
26.	0.12	0	0	0	0.3480
27.	0	0.12	0	0	0.2489
28.	0	0	0.12	0	0.0700
29.	0.15	0	0	0	0.3911
30.	0	0.15	0	0	0.3028
31.	0	0	0.15	0	0.0750
32.	0.18	0	0	0	0.4207
33.	0	0.18	0	0	0.3317
34.	0	0	0.18	0	0.0750
35.	0	0.24	0	0	0.3726
36.	0.255	0	0	0	0.5008
37.	0.30	0	0	0	0.5473
38.	0	0.30	0	0	0.4383
39.	0.36	0	0	0	0.5827
40.	0	0.36	0	0	0.4678
41.	0.45	0	0	0	0.6397
42.	0.60	0	0	0	0.6810
43.	0.525	0	0	0	0.6475
44.	0.90	0	0	0	0.7301

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The experimental values of parameter *A* in BA mixtures. *Continued*

	Cadaverine	Putrescine	Histamine	Spermidine	*A* (exp. value)
45.	1.20	0	0	0	0.7433
46.	1.05	0	0	0	0.7618
47.	0.75	0	0	0	0.7050
48.	2.00	0	0	0	0.7620
49.	0	0.45	0	0	0.5252
50.	0	0.60	0	0	0.5742
51.	0	0.90	0	0	0.6136
52.	0	1.20	0	0	0.6526
53.	0	1.05	0	0	0.6444
54.	0	0.75	0	0	0.6010
55.	0	2	0	0	0.6569
56.	0	0	0.525	0	0.0750
57.	0	0	0.90	0	0.0750
58.	0.015	0	0	0	0.0657
59.	0.03	0	0	0	0.0997
60.	0.06	0	0	0	0.1996
61.	0.09	0	0	0	0.2917
62.	0.12	0	0	0	0.3480
63.	0.15	0	0	0	0.3911
64.	0.18	0	0	0	0.4207
65.	0.255	0	0	0	0.5008
66.	0.30	0	0	0	0.5473
67.	0.36	0	0	0	0.5827
68.	0.45	0	0	0	0.6397
69.	0.60	0	0	0	0.6810
70.	0.525	0	0	0	0.6475
71.	0.90	0	0	0	0.7301
72.	1.20	0	0	0	0.7433
73.	1.05	0	0	0	0.7618
74.	0.75	0	0	0	0.7050
75.	2	0	0	0	0.7620
76.	0	0.06	0	0	0.1385
77.	0	0.09	0	0	0.2161
78.	0	0.12	0	0	0.2489
79.	0	0.15	0	0	0.3028
80.	0	0.18	0	0	0.3317
81.	0	0.24	0	0	0.3726
82.	0	0.30	0	0	0.4383
83.	0	0.36	0	0	0.4678
84.	0	0.45	0	0	0.5252
85.	0	0.60	0	0	0.5742
86.	0	0.90	0	0	0.6136
87.	0	1.20	0	0	0.6526
88.	0	1.05	0	0	0.6444
89.	0	0.75	0	0	0.6010
90.	0	2.00	0	0	0.6569

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The experimental values of parameter A in BA mixtures. *Continued*

	Cadaverine	Putrescine	Histamine	Spermidine	A (exp. value)
91.	0	0	0.015	0	0.0124
92.	0	0	0.03	0	0.0258
93.	0	0	0.06	0	0.0457
94.	0	0	0.09	0	0.0654
95.	0	0	0.12	0	0.0700
96.	0	0	0.15	0	0.0750
97.	0	0	0.18	0	0.0750
98.	0	0	0.525	0	0.0750
99.	0	0	0.90	0	0.0750
100.	0.06	0.06	0	0	0.2516
101.	0.09	0.09	0	0	0.3684
102.	0.15	0.15	0	0	0.6073
103.	0.30	0.30	0	0	0.7823
104.	0.90	0.90	0	0	0.9527
105.	0.06	0.06	0.06	0	0.2144
106.	0.09	0.09	0.09	0	0.3383
107.	0.12	0.12	0.12	0	0.4362
108.	0.15	0.15	0.15	0	0.5083
109.	0.18	0.18	0.18	0	0.6872
110.	0.06	0	0.06	0	0.1638
111.	0.09	0	0.09	0	0.2423
112.	0.12	0	0.12	0	0.3228
113.	0.15	0	0.15	0	0.4185
114.	0.18	0	0.18	0	0.5058
115.	0.30	0	0.30	0	0.8562
116.	0	0.06	0.06	0	0.1148
117.	0	0.09	0.09	0	0.1687
118.	0	0.15	0.15	0	0.3358
119.	0.0075	0.15	0	0	0.3557
120.	0.03	0.15	0	0	0.3863
121.	0.045	0.15	0	0	0.4432
122.	0.06	0.30	0	0	0.5390
123.	0.06	0.12	0	0	0.4236
124.	0.06	0.195	0	0	0.6427
125.	0.06	0.15	0	0	0.4389
126.	0.075	0.15	0	0	0.5124
127.	0.09	0.30	0	0	0.6241
128.	0.09	0.06	0	0	0.3323
129.	0.09	0.12	0	0	0.4923
130.	0.09	0.39	0	0	0.9566
131.	0.09	0.195	0	0	0.6361
132.	0.09	0.09	0	0	0.3684
133.	0.09	0.15	0	0	0.4893
134.	0.12	0.06	0	0	0.4178
135.	0.12	0.09	0	0	0.4406
136.	0.15	0.30	0	0	0.6259

Continued on the next page

The experimental values of parameter *A* in BA mixtures. *Continued*

	Cadaverine	Putrescine	Histamine	Spermidine	*A* (exp. value)
137.	0.15	0.06	0	0	0.4753
138.	0.15	0.15	0	0	0.6073
139.	0.15	0.015	0	0	0.3959
140.	0.15	0.03	0	0	0.4012
141.	0.15	0.06	0	0	0.4564
142.	0.15	0.09	0	0	0.5085
143.	0.15	0.12	0	0	0.5339
144.	0.15	0.15	0	0	0.6073
145.	0.15	0.30	0	0	0.8365
146.	0.15	0.045	0	0	0.4354
147.	0.15	0.0075	0	0	0.3815
148.	0.195	0.06	0	0	0.5917
149.	0.195	0.09	0	0	0.6218
150.	0.255	0.15	0	0	0.7958
151.	0.30	0.30	0	0	0.7823
152.	0.30	0.06	0	0	0.6984
153.	0.30	0.15	0	0	0.7952
154.	0.30	0.90	0	0	0.9728
155.	0.30	0.09	0	0	0.7827
156.	0.30	0.06	0	0	0.7352
157.	0.30	0.06	0	0	0.7917
158.	0.30	0.15	0	0	0.9054
159.	0.39	0.06	0	0	0.8929
160.	0.39	0.09	0	0	0.9773
161.	0.90	0.30	0	0	0.9647
162.	0.90	0.06	0	0	0.9203
163.	0.90	0.15	0	0	0.9242
164.	0.15	0.15	0	0	0.4428
165.	0.15	0.09	0	0	0.3739
166.	0.15	0.30	0	0	0.5138
167.	0.15	0.21	0	0	0.4548
168.	0.15	0.60	0	0	0.6195
169.	0.09	0.15	0	0	0.3644
170.	0.30	0.15	0	0	0.5952
171.	0.21	0.15	0	0	0.4888
172.	0.60	0.15	0	0	0.6558
173.	0.30	0.90	0	0	0.7455
174.	0.30	0.09	0	0	0.5392
175.	0.30	0.30	0	0	0.6535
176.	0.30	0.21	0	0	0.6438
177.	0.30	0.60	0	0	0.7445
178.	0.09	0.30	0	0	0.5184
179.	0.30	0.30	0	0	0.6353

Continued on the next page

The experimental values of parameter A in BA mixtures. *Continued*

	Cadaverine	Putrescine	Histamine	Spermidine	A (exp. value)
180.	0.21	0.30	0	0	0.5704
181.	0.60	0.30	0	0	0.7264
182.	0.90	0.30	0	0	0.7974
183.	0.06	0.15	0	0	0.3162
184.	0.06	0.09	0	0	0.2418
185.	0.06	0.30	0	0	0.4804
186.	0.06	0.21	0	0	0.3710
187.	0.09	0.06	0	0	0.2510
188.	0.30	0.06	0	0	0.5067
189.	0.21	0.06	0	0	0.4256
190.	0.60	0.06	0	0	0.6904
191.	0.06	0.06	0	0	0.2016
192.	0.03	0.15	0	0	0.2830
193.	0.03	0.09	0	0	0.1908
194.	0.03	0.21	0	0	0.3450
195.	0.03	0.30	0	0	0.4760
196.	0.03	0.06	0	0	0.1259
197.	0.15	0.03	0	0	0.3574
198.	0.09	0.03	0	0	0.2215
199.	0.21	0.03	0	0	0.4406
200.	0.60	0.03	0	0	0.7026
201.	0.06	0.03	0	0	0.1015
202.	0.03	0.03	0	0	0.0867
203.	0.15	0	0	0.30	0.2333
204.	0.15	0	0	0.60	0.2034
205.	0.15	0	0	0.90	0.1624
206.	0.15	0	0	1.95	0.1610
207.	0.15	0	0	6.00	0.1094
208.	0.09	0	0	0.30	0.1328
209.	0.15	0	0	3.00	0.0663
210.	0.09	0	0	0.60	0.0983
211.	0.09	0	0	0.90	0.0802
212.	0.09	0	0	3.00	0.0740
213.	0.09	0	0	6.00	0.0633
214.	0.30	0	0	0.30	0.3010
215.	0.30	0	0	0.60	0.2276
216.	0.30	0	0	0.90	0.1647
217.	0.30	0	0	1.95	0.0966
218.	0.30	0	0	6.00	0.0642

Continued on the next page

The experimental values of parameter *A* in BA mixtures. *Continued*

	Cadaverine	Putrescine	Histamine	Spermidine	*A* (exp. value)
219.	0.30	0	0	3.00	0.0772
220.	0.60	0	0	0.30	0.4595
221.	0.60	0	0	0.60	0.2979
222.	0.60	0	0	0.90	0.2175
223.	0.60	0	0	6.00	0.0759
224.	0.60	0	0	3.00	0.1069
225.	0.03	0	0	3.00	0.0120
226.	0.03	0	0	1.95	0.0302
227.	0.03	0	0	0.60	0.0360
228.	0	0.15	0	0.30	0.1282
229.	0	0.15	0	0.60	0.0767
230.	0	0.15	0	0.90	0.0644
231.	0	0.15	0	1.95	0.0362
232.	0	0.15	0	6.00	0.0408
233.	0	0.15	0	3.00	0.0213
234.	0	0.09	0	0.30	0.0760
235.	0	0.09	0	0.60	0.0345
236.	0	0.09	0	0.90	0.0291
237.	0	0.09	0	3.00	0.0162
238.	0	0.09	0	6.00	0.0233
239.	0	0.30	0	0.30	0.0983
240.	0	0.30	0	0.60	0.0656
241.	0	0.30	0	0.90	0.0478
242.	0	0.30	0	1.95	0.0244
243.	0	0.30	0	6.00	0.0131
244.	0	0.30	0	3.00	0.0212
245.	0	0.60	0	0.30	0.2053
246.	0	0.60	0	0.60	0.1183
247.	0	0.60	0	1.95	0.0498
248.	0	0.60	0	6.00	0.0381
249.	0	0.03	0	3.00	0.0089
250.	0	0.03	0	1.95	0.0169
251.	0	0.03	0	0.60	0.0174
252.	0.15	0	0	0	0.3248
253.	0.30	0	0	0	0.4927
254.	0.09	0	0	0	0.1786
255.	0.60	0	0	0	0.6281
256.	0.90	0	0	0	0.6734
257.	0.21	0	0	0	0.3880

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The experimental values of parameter *A* in BA mixtures. *Continued*

	Cadaverine	Putrescine	Histamine	Spermidine	*A* (exp. value)
258.	1.20	0	0	0	0.7638
259.	0.06	0	0	0	0.1600
260.	0.03	0	0	0	0.0800
261.	0	0.15	0	0	0.2345
262.	0	0.60	0	0	0.6148
263.	0	1.50	0	0	0.7524
264.	0	0.45	0	0	0.5000
265.	0	0.30	0	0	0.3837
266.	0	0.90	0	0	0.6486
267.	0	0.09	0	0	0.1570
268.	0	0.21	0	0	0.3140
269.	0	0.06	0	0	0.1080
270.	0	0.03	0	0	0.0670
271.	0	0	0	0.90	0.1283
272.	0	0	0	3.00	0.2430
273.	0	0	0	6.00	0.2145
274.	0	0	0	9.00	0.2412
275.	0	0	0	1.95	0.1940
276.	0	0	0	0.30	0.0776
277.	0	0	0	0.60	0.1160
278.	0.05	0.05	0.12	1.00	0.2647
279.	0.05	0.10	0.24	1.50	0.3485
280.	0.05	0.20	0.48	2.00	0.3783
281.	0.05	0.30	1.20	3.00	0.3856
282.	0.05	0.60	2.40	6.00	0.4349
283.	0.10	0.05	0.12	1.00	0.4486
284.	0.10	0.10	0.24	1.50	0.4752
285.	0.10	0.20	0.48	2.00	0.4521
286.	0.10	0.30	1.20	3.00	0.4639
287.	0.10	0.60	2.40	6.00	0.3328
288.	0.30	0.05	0.12	1.00	0.6215
289.	0.30	0.10	0.24	1.50	0.6305
290.	0.30	0.20	0.48	2.00	0.6141
291.	0.30	0.30	1.20	3.00	0.4782
292.	0.30	0.60	2.40	6.00	0.3754
293.	0.60	0.05	0.12	1.00	0.8775
294.	0.60	0.10	0.24	1.50	0.7231
295.	0.60	0.20	0.48	2.00	0.6813

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The experimental values of parameter *A* in BA mixtures. *Continued*

	Cadaverine	Putrescine	Histamine	Spermidine	*A* (exp. value)
296.	0.60	0.30	1.20	3.00	0.5901
297.	0.60	0.60	2.40	6.00	0.3820
298.	0.90	0.05	0.12	1.00	0.7935
299.	0.90	0.10	0.24	1.50	0.7402
300.	0.90	0.20	0.48	2.00	0.7298
301.	0.90	0.30	1.20	3.00	0.5598
302.	0.80	0.60	2.40	6.00	0.4282

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Biogeensete amiinide üheaegne määramine biosensoriga: biosensori signaali analüüs ja selle kalibreerimine

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Biosensorite praktiline kasutamine reaalsetes proovides, kus leidub palju mõõdetavat signaali mõjutavaid komponente, on tihti keerukas komponentide vastastikuse mõju tõttu. Siiski võimaldab signaali modelleerimine ja kemomeetriliste meetodite kasutamine biosensorite kalibreerimist mitme analüüdi üheaegseks määramiseks biosensorite väljundsignaali alusel. Üks olulisemaid probleemseid proove, kus on vaja igapäevaselt määrata korraga mitme komponendi sisaldust, on biogeensete amiinide segud, mis tekivad valkude lagunemisel ja kus biogeensete amiinide kontsentratsioon on hea indikaator valku sisaldavate toodete (nt kalatooted, liha, juust, vein) kvaliteedi hindamisel.

Käesolevas töös uuritigi võimalusi diamiini oksüdaasil (EC 1.4.3.22) põhineva biosensori väljundsignaali modelleerimiseks ning selle alusel sensori kalibreerimiseks nelja olulisema biogeense amiini (kadaveriin, putrestsiin, histamiin ja spermidiin) üheaegseks määramiseks nende segudes. Signaali modelleerimiseks pakuti välja kaks erinevat lähenemist: esimene põhines toimuvate füüsikalis-keemiliste protsesside arvestamisel ja teine määratavate biogeensete amiinide kontsentratsioonide erineval formaalloogilisel kombineerimisel. Väljapakutud mudelite korreleerimisel rohkem kui kolmesajal mõõtmisel saadud eksperimentaalsete andmetega leitud korrelatsioonikoefitsientide väärtus oli vahemikus 0,54 kuni 0,85. Selgus, et füüsikalis-keemilise mudeli korral, mis võttis arvesse substraatide difusiooni, kasutatud ensüümi aktiivsust erinevate substraatide suhtes ja spermidiini kõrgemate kontsentratsioonide inhibeerivat mõju ensüümile, olid eksperimentaalsete tulemuste hälbed erinevalt väljapakutud formaalsest mudelist mittesüstemaatilised. Saadud tulemuste alusel võib järeldada, et biosensorite kalibreerimiseks on eelistatud füüsikalis-keemiline mudel. Väljapakutud lähenemine on perspektiivne ka teiste biosensorite kalibreerimiseks, mida kasutatakse mitme analüüdi määramiseks nende segudes.