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Multivariate data analysis of dynamic amperometric biosensor responses from binary analyte mixtures—application of sensitivity correction algorithms

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Abstract

In this paper, it is demonstrated that a single-receptor biosensor can be used to quantitatively determine each analyte in binary mixtures using multivariate data analysis tools based on the dynamic responses received from flow injection peaks. Mixtures with different concentrations of two phenolic compounds, catechol and 4-chlorophenol, were measured with a graphite electrode modified with tyrosinase enzyme at an applied potential of −50 mV versus Ag/AgCl. A correction algorithm based on measurements of references in-between samples was applied to compensate for biosensor ageing as well as differences caused by deviations between biosensor preparations. After correction, the relative prediction errors with partial least squares regression (PLS-R) for catechol and 4-chlorophenol were 7.4 and 5.5%, respectively, using an analysis sequence measured on one biosensor. Additional validation mixtures of the two phenols were measured with a new biosensor, prepared with the same procedure but with a different batch of tyrosinase enzyme. Using the mixture responses for the first sensor as a calibration set in PLS-R, the relative prediction errors of the validation mixtures, after applying correction procedures, were 7.0% for catechol and 16.0% for 4-chlorophenol. These preliminary results indicate that by applying correction algorithms it could be possible to use less stable biosensors in continuous on-line measurements together with multivariate data analysis without time-consuming calibration procedures.

Keywords: Flow injection; Biosensors; Chemometrics; Phenols; Amperometry; Drift correction

1. Introduction

Biosensors represent a potential screening method in environmental studies, for instance in the analysis of phenolic compounds [1]. As an analytical detector, biosensors have advantageous properties such as high selectivity and sensitivity. The production cost is also relatively low and the analysis time is short compared to conventional analytical methods. The traditional direction in the research of biosensors has been to construct devices that are selective towards a single substrate, i.e., cross-reactivity of these sensors is not desirable. In reality, many biological materials are only partially selective in their nature (e.g., an electrode modified with peroxidase enzyme can be utilized for amperometric detection of phenolic compounds [2,3]). However, signals from non-specific sensors can favourably be used for pattern recognition, by applying chemometric (or multivariate data analytical) tools as PCA [4], PLS-R [5] or ANN [6] to an array of sensors where each sensor contains different selectivity for analytes in a sample matrix [7]. The resulting multivariate pattern can be interpreted for qualitative classification of the samples, usually without exact knowledge of the analyte composition, and/or for quantitative determination of specific

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analytes in the samples. In case of electrochemical detection in liquid media, such systems are commonly referred to as electronic tongues [8–11]. The number of publications that can be related to bioelectronic tongues are still relatively few and among them articles can be found based on, e.g., amperometric enzyme-based systems to quantitatively determine biogenic amines [12] as well as phenols [13], enzyme inhibition for detection of pesticides [14,15], lectin-liposaccharide recognition for identification of microorganisms [16,17], or microbial sensors for determination of ethanol and glucose [18,19].

An alternative to use arrays for quantification of mixtures by multivariate data analysis, as described in [13,19], is to use the information that can be received from a whole dynamic flow injection peak response [20] detected with a single sensor. This has been demonstrated with a single amperometric microbial sensor in a couple of articles [21–23]. The variations in the flow injection signal from different substrates at the microbial electrode depend on the oxygen consumption and are due to the multi-receptor behaviour of whole cells. In this way, it was possible to simultaneously determine each component (<11% in relative error) in ternary mixtures (acetate, L-lactate and succinate) with the microbial sensor using time dependent responses at flow injection signals together with a non-linear multivariate calibration model [23]. Further, flow injection peaks from amperometric electrodes modified with a single enzyme (i.e., a single-receptor) have clearly shown that different phenolic compounds give different characteristic response shapes when using a horseradish peroxidase-based [24] or a tyrosinase-based sensor [25]. In this case, the peak variation can be explained by different diffusion rates and reaction kinetics for different phenols. In this work, the dynamic peak responses from a single amperometric tyrosinase-based sensor are used with multivariate data analysis for quantitative determination of catechol and 4-chlorophenol in mixtures.

Non-linearity of the response, baseline drift and loss of sensitivity with time are common problems with biosensors. After purification, tyrosinase itself is an unstable enzyme and its immobilization on an electrode surface is often accompanied by addition of stabilizing polymers [26]. Opposite to chemical or physical stabilization an alternative in the direction of creating more stable and reproducible tyrosinase sensors as well as other types of biosensors could be exploitation of mathematical signal correction procedures. One example of how chemometric tools can be used to correct for long-term drift has been demonstrated for glucose oxidase modified carbon paste electrodes [27].

Drift in sensors affects the precision of results from pattern recognition. Several drift correction methods using reference samples have been developed to overcome this problem for electronic noses (i.e., gas sensor arrays), e.g., additive, multiplicative and component correction [28,29]. Some of the mentioned procedures have appeared to be successful for electronic tongues also [30]. These correction methods are based on the assumption that drift in between reference samples and analyte samples are linear. Therefore, it is important that the chosen reference sample and the analyte samples have similar response patterns during the analysis sequence. The simple additive correction method [30] is independent of the signal level and has mainly been used to compensate for baseline drift. However, this procedure is generally not enough as the only correction method for biosensors since there is high risk for reduction in sensitivity of the sensor with time. Multiplicative drift correction [28], used in some commercial electronic noses, is a method that makes it possible to compensate for ageing of the sensor, i.e., decreased sensitivity. The sample correction in [28] is based on multiplication of factors derived from an algorithm calculated from a curve fitting of references measured regularly during the analysis. The procedure allows correction within a single measurement sequence as well as between sequences from day-to-day. For the procedure to work in a proper way a high signal to noise ratio is requested to avoid errors due to an inexact curve fitting. An elegant alternative to the discussed correction methods is to apply component correction [29,30] on the data. This correction is based on multivariate methods (PCA and PLS-R) and the main idea is that the sensor drift has a preferred direction in the multivariate space. The correction is accomplished by subtracting the drift direction component of the reference responses from the data. Sample responses with a low signal to noise ratio is not a problem in component correction, but outliers can remarkably affect the drift direction component and thereby result in a poorer correction. The method has in some cases resulted in better precision in the pattern recognition models compared to the other mentioned correction methods [28,30]. On the other hand it will not work well if the drift direction coincides with significant structural information in the data.

As far as we know, this is the first paper showing that it is possible to simultaneously determine the components in binary mixtures using multivariate calibration of whole dynamic flow injection responses from a single-receptor-based biosensor. The paper also demonstrates how a correction procedure, multiplicative drift correction developed for gas sensors [28], can be used on whole peak biosensor responses to compensate for drift arising from ageing of the sensor within a measurement sequence. The method was also used for correction between two sensors, prepared with exactly the same procedure, but from different batches of enzyme.

2. Experimental

2.1. Chemicals

Mushroom tyrosinase, 3620 U mg^{-1}, was purchased from Sigma (St. Louis, MO, USA). A poly(ester-sulfonic acid) polymer, Eastman 55 AQ, was from Eastman Kodak (Kingsport, Tennessee, USA). Na_2HPO_4, NaH_2PO_4 and KCl for preparing buffer solutions were obtained from Merck (Darmstadt, Germany). Stock solutions (0.1 M) of phe-

nol (Merck), catechol (Sigma), *p*-cresol (Merck) and 4-chlorophenol (Merck) were prepared in methanol. The phenolic working solutions were prepared daily by dilution in 20 mM sodium phosphate buffer containing 0.1 M KCl buffer at pH 7. All aqueous solutions were prepared using water purified with a Milli-Q system (Millipore, Bedford, USA).

2.2. Biosensor preparation

Solid graphite electrodes (SGL Carbon, Werke Ringsdorff, Bonn, Germany, type RW001, 3.05 mm diameter) were cut and polished on wet fine emery paper and washed with Milli-Q water. A stock solution of tyrosinase/Eastman AQ was prepared by mixing tyrosinase powder directly into a 2% Eastman AQ solution prepared in 20 mM phosphate buffer containing 0.1 M KCl at pH 7, giving a tyrosinase concentration of 36,200 U ml^{-1}. Eight microlitre of the tyrosinase-Eastman AQ mixture (290 units of tyrosinase) were then applied on top of the electrodes and let to dry for 20 h at 4 °C before use. The dry sensors were washed with Milli-Q water and stored in 20 mM phosphate buffer at pH 7 at 4 °C.

2.3. Equipment

The tyrosinase modified graphite electrode was fitted into a PTFE holder and placed into a flow through wall jet-amperometric cell [31]. The enzyme electrode was used as the working electrode, an Ag/AgCl (0.1 M KCl) electrode as the reference electrode and a platinum wire as the counter electrode. The electrodes were connected to a three-electrode potentiostat (Zäta Elektronik, Lund, Sweden) and the currents were registered on a strip chart recorder (Kipp and Zonen, Netherlands) and on a computer running Gilson Unipoint software version 3.0 (Gilson, Villiers-le-Bel, France). All measurements were performed at an applied potential of −50 mV versus Ag/AgCl. A peristaltic pump (Gilson minipuls 3) transported the carrier, degassed 20 mM phosphate buffer (pH 7) containing 0.1 M KCl, into the amperometric wall jet-cell at a flow rate of 0.3 ml min^{-1}. The samples were injected using a 50 μl injection loop by a fully automated flow injection system, Gilson ASTED XL Autoinjector. In digitalised form, each recorded peak current has been represented by 260 current values evenly distributed over the entire profile of the current peak signal.

2.4. Experimental design

Binary mixtures of catechol (0, 5, 10, 15, 20 and 25 μM) and 4-chlorophenol (0, 15, 30, 45, 60 and 75 μM) were used in a full factorial design giving 6 × 6 = 36 mixtures totally. Each mixture component was chosen from the linear concentration range of the calibration curve of the tyrosinase/Eastman AQ sensor. The different concentration ranges covering linearity for catechol and 4-chlorophenol is based on the fact that the maximum concentration of each phenol that is needed, before non-linearity in the current signal output occurs, differs from phenol to phenol at the tyrosinase-based sensor. The 36 samples were injected randomly into the flow injection system.

In the same concentration range, 18 new validation sample mixtures, chosen by a reduced factorial design, were measured another day with a new sensor, prepared exactly in the same way as the first one, but from a new batch of tyrosinase enzyme.

In order to correct for drift and decreased sensitivity of the sensor, references of catechol (20 μM) and 4-chlorophenol (60 μM) were injected in the beginning and at the end of the measurement sequences, and after every fourth sample mixture.

2.5. Pre-processing of data

All peaks from each of the two measurement sequences were corrected for shift in baseline and aligned to the time of injection. For compensation of decreased sensor responses within an analysis sequence, a multiplicative drift correction method, similar to what was described in [28], was performed on the binary peaks with help from the references. To simplify the correction procedure, responses for 24 evenly distributed time variables (i.e., time points) from 0.1 to 2.4 min were chosen from the original 260 time variables building up every peak response. For the references of (i) catechol; (ii) 4-chlorophenol; and (iii) additive reference responses of catechol and 4-chlorophenol, the decrease in sensitivity was evaluated by plotting the decay in response for each of the 24 chosen time variables. All 24 time variables were included in the correction since the decrease in response with time for biosensors generally is higher at peak maximum compared to variables chosen before and after peak maximum. Each variable showed nearly a linear dependence in the decay of response with time and thus linear equations were fitted to the data points. The slope and intercept were calculated and used for correction of the same 24 time variables selected from the sample mixtures according to Eq. (1):

$$\text{response}_{\text{corr}} = \text{response}_{\text{org}} \times \frac{a}{a + bt} \quad (1)$$

where $\text{response}_{\text{corr}}$ is the response of the corrected variable, $\text{response}_{\text{org}}$ the response of the original variable, a and b the intercept and slope, respectively, of the linear equation and t the elapsed analysis time.

After the individual correction step within the two analysis sequences, compensation was performed of the responses from the second sensor to fit the sensitivity levels of the first sensor. Each of the 24 chosen time variables from the 18 additional validation mixtures was adjusted for sensitivity differences to the time variables from the 36 calibration mixtures. The correction was performed according to Eq. (2) where a_c and a_v are the intercepts for the linearly fitted calibration and validation reference samples, respectively, $\text{response}_{\text{corrv}}$ the response for the actual validation variable and $\text{response}_{\text{corrc}}$ the calculated validation response corrected against the first

sensor

$$\text{response}_{\text{corrc}} = \text{response}_{\text{corrv}} \times \frac{a_c}{a_v} \quad (2)$$

The pre-processing procedure was performed in Microsoft Excel 2000.

2.6. Multivariate data analysis

Two common multivariate analysis methods have been used to linearly decompose the data, principal component analysis (PCA) [4] and partial least squares regression (PLS-R) [5]. The data to be analysed is collected in an *X*-matrix, which is made up of *n* objects (the number of mixtures) and *p* variables (the 24 currents values reflecting the shape of the flow injection peak responses).

In PCA, the *X*-matrix is approximated to a product of score vectors (*T*) and loading vectors (*P*) containing a simplified distribution pattern of the objects and the variables, respectively. The main idea with PCA is that the most structural information in the *X*-matrix can be found in the directions where the data have the largest variances. The product of *T* and *P* build a new orthogonal coordinate system where the axes are latent variables, i.e., principal components (PCs). The first PC explains the largest variation in the data (*X*), the second PC the second largest variation etc. The highest possible number of PCs that can be used is $n - 1$ (number of objects − 1) or *p* (number of variables), depending on which is the smaller, but usually only some few PCs are needed to visualize hidden structural information and relations in the data.

PLS-R describes with latent variables how two data matrices *X* and *Y* are related to each other by regression. In case of the tyrosinase biosensor, the sensor responses to the mixtures build up the *X*-matrix whereas the *Y*-matrix contains the real concentrations of the analytes in the mixture. There are several methods for validation of a calculated PLS-R model. For the 36 mixtures detected with the first sensor, cross-validation [32] was used in which the mixtures that build up the PLS-R model is reused for the validation step. In full cross-validation, one of the objects is systematically left out from the modelling. The excluded object is used for testing. For the 36 objects (mixtures), each object is left out one by one, 36 sub-models are then calculated where each model is made of 35 objects. The validation was evaluated for each phenol in the mixture by an estimation of the linear correlation coefficient between predicted concentration values versus reference concentration values and by calculation of the root mean square error of cross-validation (RMSECV) (see Eq. (3))

$$\text{RMSECV} = \sqrt{\frac{\sum_{i=1}^{n} (y_{\text{pred}} - y_{\text{ref}})^2}{n}} \quad (3)$$

where y_{pred} is the predicted concentration values, y_{ref} the reference (real) concentration values and *n* the number of samples. A better but more time-consuming validation is to perform a test set. The 18 additional responses detected with the new sensor was used as a test set and the concentrations of each component were predicted using the PLS-R calibration model based on calculations of the previous 36 mixtures recorded with the first sensor. In this case, the precision of the prediction is estimated, similar to RMSECV, by root mean square error of prediction (RMSEP).

For the multivariate data analysis, a computer running Unscrambler software 7.6 (CAMO A/S, Trondheim, Norway) was used.

3. Results and discussion

Mushroom tyrosinase is a tetrametric protein containing two active sites; each consists of two copper atoms coordinated with histidines [33]. Tyrosinase is a phenol oxidase that catalyses the oxidation of monophenols and *o*-diphenols into their corresponding *o*-quinones, at the expense of reducing oxygen to water. At a tyrosinase modified electrode for amperometric measurements [26,34], the enzymatic reactions are followed by an electrochemical step at the electrode surface where the enzymatically produced *o*-quinone is reduced to the *o*-diphenol at an applied potential of −50 mV versus Ag/AgCl. The enzymatic oxidation and the electrochemical reduction form a reaction cycle that results in an amplification of the signal response to phenolic compounds. The reaction cycle for both the enzymatic and the electrochemical steps are shown in Fig. 1.

To be able to use multivariate analysis for quantitative determination of different phenols in binary mixtures with the tyrosinase biosensor, it is important that differences occur in the shape of the flow injection peaks. Preliminary studies clearly show that such differences in current-time response-curves could easily be noticed, as shown in Fig. 2. Especially, catechol and 4-chlorophenol show large peak shape differences and were thus chosen for a first attempt to perform a separation by pattern recognition. The stability of the sensor was relatively poor; after 10 h of measurements the sensitivity for catechol was reduced by 50%. Instead of putting efforts into producing a more stable sensor, the approach in this work was to find mathematical correction procedures that compen-

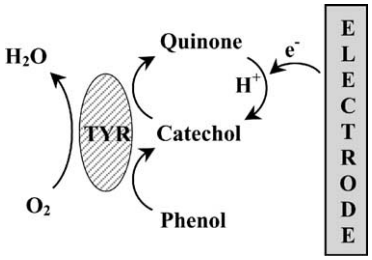

Fig. 1. Schematic representation of the mechanism for phenol at a tyrosinase (TYR)-modified graphite electrode at an applied potential of −50 mV vs. Ag/AgCl.

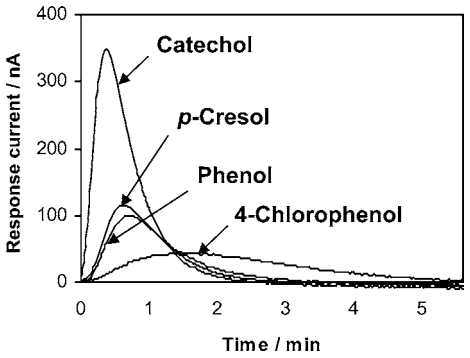

Fig. 2. Flow injection peak shapes for four different phenolic compounds (25 μM concentrations) obtained with a tyrosinase/Eastman AQ modified electrode at −50 mV vs. Ag/AgCl using a flow rate of 0.3 ml min^{-1}.

sate for the decay of sensor sensitivity. The reason for this is that even if a biosensor has shown good and robust properties in a couple of laboratory experiments, this does not guarantee that it will be the same in other situations, e.g., if the enzyme originates from different batches or if changes occur in the environmental conditions (change in pH, temperature, analysis matrix, etc.). Thus, mathematical correction procedures are important factors in the development of commercial biosensor devices.

3.1. Multivariate analysis of responses within an analysis sequence

The 24 time variables, from each of the 36 mixtures of catechol and 4-chlorophenol detected with the first sensor, were used to evaluate the effect of correction due to decreased sensitivity. The responses for the samples and the corresponding PCA score plot before correction for sensitivity are shown in Fig. 3. The down-going response of the additive time variables (current values) obtained for catechol and 4-chlorophenol references, shown in Fig. 3a, clearly reflect the sensor instability. The responses from the 36 mixtures in Fig. 3b were used for multivariate analysis. The PCA score plot in Fig. 3c shows that almost all of the structural information can be explained by two PCs describing 78 and 21% of the variation in data. The points in Fig. 3c represent the concentration in μM of catechol/4-chlorophenol and the arrows show the direction of samples with increased concentration of each phenol. The more or less orthogonal placement of the two arrows point to that the signal responses from the two phenols can be separated by PCA. However, at ideal circumstances the scores would have formed a uniform distribution of points in Fig. 3 due to the chosen evenly distributed phenol concentrations. This is not the case here. As it will be demonstrated below, the non-even distribution of points can partly be explained by the continuously decreasing sensor activity. The PCA score plot of all 260 time variables resulted in the same pattern (not shown) as compared to using only 24 time variables. Thus, no information is lost after reduction of the number of variables.

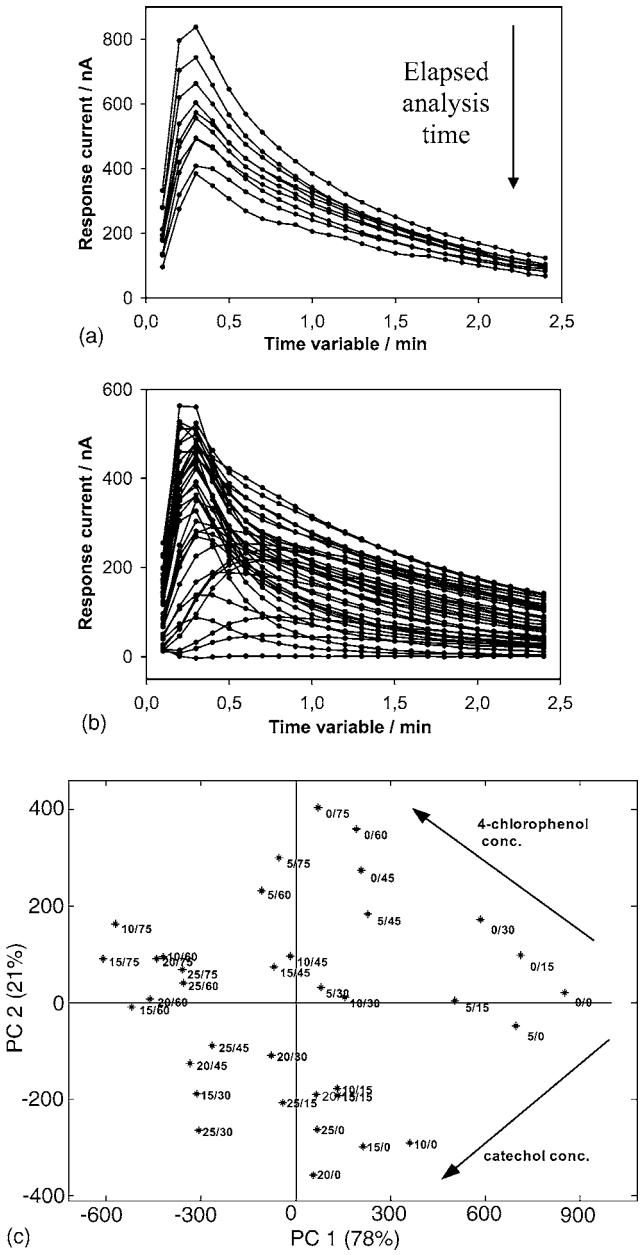

Fig. 3. Current peak-responses (not sensitivity corrected) for 36 different mixtures of catechol/4-chlorophenol, each represented by 24 time variables (points) obtained by flow injection measurements of phenolic solutions. The responses are corrected for shift in baseline and aligned to the time of injection: (a) additive responses for catechol (20 μM) and 4-chlorophenol (60 μM) references; (b) responses for the 36 mixtures; and (c) PCA score plot derived from the 36 mixture responses where PC1 and PC2 describe 78 and 21% of the variation in data, respectively. Points represent concentrations catechol/4-chlorophenol in μM.

Fig. 4 shows the responses and the related PCA score plot after sensitivity correction according to Eq. (1). An obvious improvement of the precision for the additive phenolic references is obtained (Fig. 4a). The slow peak decaying process originally observed (Fig. 3b) for the mixture responses is made faster (Fig. 4b) after the correction procedure. Regarding the PCA score plot (Fig. 4c), a more uni-

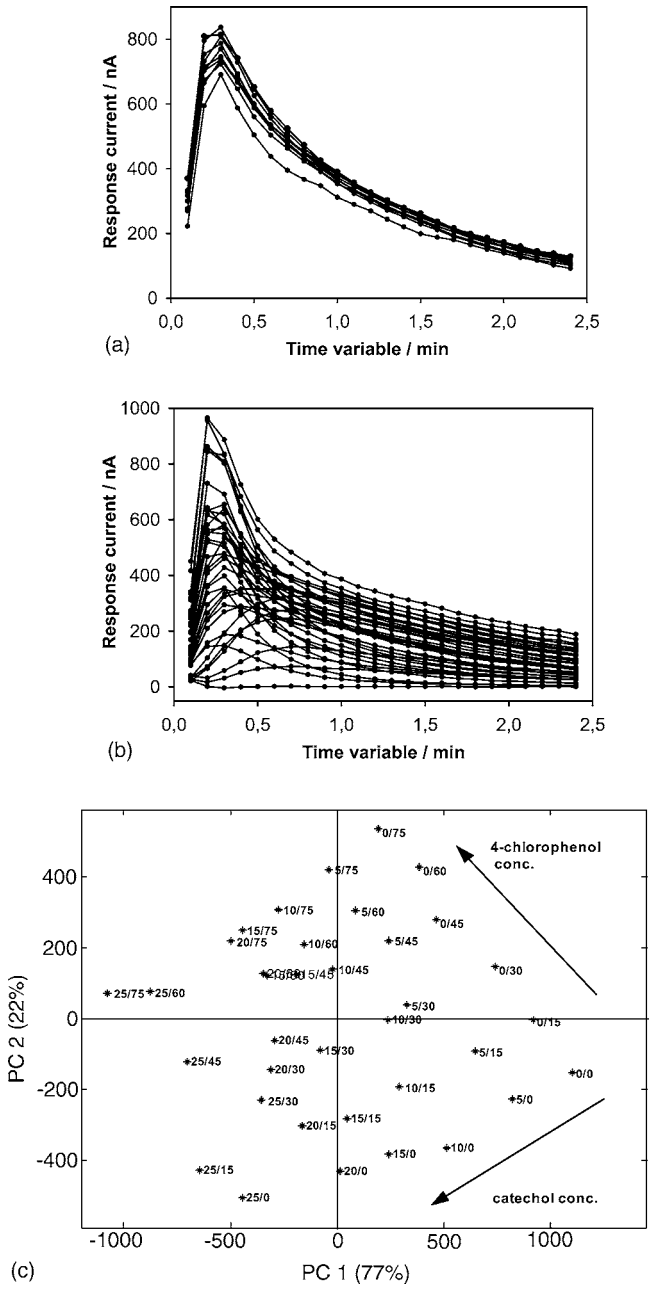

Fig. 4. Sensitivity corrected current peak responses for 36 different mixtures of catechol/4-chlorophenol, each represented by 24 time variables (points) obtained by flow injection measurements of phenolic solutions. The responses are corrected for shift in baseline and aligned to the time of injection: (a) additive responses for catechol (20 μM) and 4-chlorophenol (60 μM) references; (b) responses for the 36 mixtures; and (c) PCA score plot derived from the 36 mixture responses where PC1 and PC2 describe 77 and 22% of the variation in data, respectively. Points represent concentrations catechol/4-chlorophenol in μM.

form systematic pattern of the two phenol concentrations is received.

PLS-R with full cross-validation was used on the 36 mixture responses to evaluate the effect that each reference has on the correction procedure. Besides using the additive references (see Figs. 3a and 4a), corrections were calculated by using individual responses for catechol or 4-chlorophenol. The result is listed in Table 1. It can clearly be seen that any mode of correction improves the prediction of concentrations for both catechol and 4-chlorophenol. The improvement is shown by a lower RMSECV value and thereby a lower relative error, and a higher correlation value (r^2). Better predictions are obtained for 4-chlorophenol than for catechol, before correction as well as after a correction procedure was applied. The best mode of correction seems to be either using only catechol references (relative error is 6.8% for catechol and 5.8% for 4-chlorophenol) or use the correction factors derived from additive current responses of catechol and 4-chlorophenol references (relative error is 7.4% for catechol and 5.5% for 4-chlorophenol). For further analysis the latter method was chosen since it reflects sensitivity changes in both catechol and 4-chlorophenol responses. One can speculate that the results could have been even better if reference measurements were performed more often, i.e., between every sample, and/or the reference correction due to decay in sensitivity by time was made by a non-linear algorithm. However, no clear non-linearity dependence could be observed when studying the corrected responses for the chosen ranges of phenol concentrations, not even at high mixture concentrations of both phenols. Thus, for simplicity reasons the linear algorithm for correction (see Eq. (1)) of the sensitivity loss was chosen.

3.2. Validation of responses detected with one biosensor using a multivariate calibration model from another biosensor

Variation between biosensors must also be taken into account if systematic analysis with pattern recognition methods should give reliable results. Hence, 18 catechol/4-chlorophenol validation mixtures, measured on a new tyrosinase/Eastman AQ sensor, were predicted with the PLS-R calibration model calculated for the 36 mixture responses at the first electrode. A new batch of tyrosinase enzyme was used for this second biosensor. Each analysis sequence was individually aligned to the time of injection and corrected for shift in baseline. Additive reference peaks of catechol and 4-chlorophenol were thereafter used to compensate for the decreasing sensitivity according to Eq. (1). Eq. (2) was used to correct the 18 validation mixture responses against responses measured with the first sensor. Fig. 5a shows the 24 time variables for the 18 validation samples before any sensitivity correction has been made. The responses differ very much from the uncorrected responses that were obtained with the 36 mixtures measured on the first sensor, see Fig. 3b. Responses for the mixtures detected at the second sensor (Fig. 5a) are generally higher in sensitivity and have a slower peak decaying process. Repeated analysis with other newly prepared biosensors resulted in similar response patterns using this new batch of tyrosinase enzyme. However, after all correction steps, the validation sample responses in Fig. 5b looks similar to the 36

Table 1
PLS-R prediction results made with full cross-validation on 24 time variables corrected for decreased sensitivity with different modes to determine catechol and 4-chlorophenol in 36 binary mixtures

Mode of correction	Catechol prediction			4-Chlorophenol prediction		
	RMSECV (μM)	Relative error (%)[a]	Correlation (r^2)	RMSECV (μM)	Relative error (%)[a]	Correlation (r^2)
No correction	2.77	11	0.90	6.79	9.1	0.94
Catechol	1.70	6.8	0.96	4.37	5.8	0.97
4-Chlorophenol	2.45	9.8	0.92	4.14	5.5	0.98
Catechol/4-chlorophenol	1.85	7.4	0.96	4.13	5.5	0.98

[a] Relative error is denoted as RMSECV divided by the maximum concentration; 25 μM for catechol and 75 μM for 4-chlorophenol.

responses at the first sensor received after sensitivity correction in Fig. 4b.

Predicted versus reference concentrations were plotted for catechol (Fig. 6a) and 4-chlorophenol (Fig. 6b). For catechol, the model works fine with RMSEP of 1.75 μM (relative error, 7.0%) using three PLS components. These results agree well with the ones received within the first sensor (relative error, 7.4%), see Table 1. Regarding 4-chlorophenol concentrations, a relative error of 16.0% was obtained with two PLS components. From Fig. 6b, it can clearly be seen that overestimations of predicted 4-chlorophenol concentrations in the validation mixtures are obtained. One reason for this can be that not full curves (just 24 points) have been used for correction of continuously decreasing sensitivity of the sensors. No deviation from proportionality between predicted versus reference concentration was seen if the calibration and test set model (corrected according to Eq. (1)) were analysed separately with PLS-R and cross-validation. Thus, the observed overestimations for predicted 4-chlorophenol concentrations seem to depend on the correction step that was made between the two biosensors according to Eq. (2). The main question here is, of course, to understand how much the sensors can differ, that after procedures of cor-

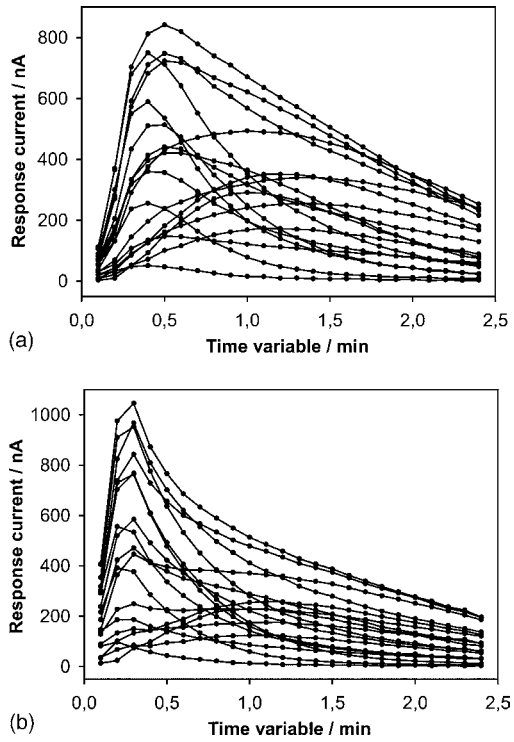

Fig. 5. Responses for 24 time variables obtained from flow injection responses in the second analysis sequence containing 18 validation mixtures of catechol/4-chlorophenol detected on a new sensor modified with a different batch of tyrosinase enzyme. The responses are corrected for shift in baseline and aligned to the time of injection: (a) non-sensitivity corrected peaks and (b) sensitivity corrected peaks adjusted against the first analysis sequence based on the 36 mixture responses using additive references.

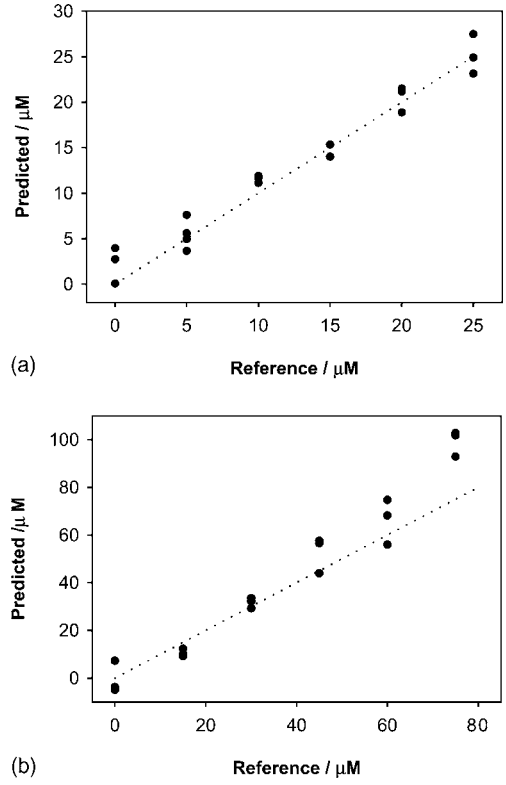

Fig. 6. Predicted vs. reference concentration of (a) catechol and (b) 4-chlorophenol for 18 validation mixtures of these two phenols. Predictions are based on PLS-R models with (a) 3 PLS components and (b) 2 PLS components, using the 36 mixture samples measured on the first sensor for calibration, and 24 time variables from the flow injection responses (the peaks were aligned to the time of injection and corrected for baseline, sensitivity and adjusted against the first analysis sequence using additive references).

rections their results could provide acceptable predictions. To answer this more sensors have to be tested. The results presented here demonstrate the feasibility for sensitivity correction to improve results from pattern recognition using information derived from whole peak responses from unstable biosensors. The correction may work fairly well when small changes in the condition appear. As demonstrated in this case, a new batch of tyrosinase enzyme was used for the second sensor.

4. Conclusion

In this work, it is shown that the information obtained by multivariate data analysis of whole flow injection responses measured with an enzymatic single-receptor biosensor allows quantitative determination of analyte components in binary mixtures. Instability of the biosensor can be adjusted by the use of mathematical correction algorithms obtained from analysis of reference responses. This correction can also easily be automatized for long-time measurements and has a potential to work in case of exchange of biosensors without performing new statistical full-calibration procedures. The multivariate approach needs to be further investigated in more systems and with more complex sample matrices. Handling non-linearity in the response (e.g., using concentration ranges outside the linear range of the calibration curve or creating algorithms that can handle non-linearity differences between biosensors) is one of the future challenges. Incorporation of non-linear "interaction" terms [23] into the model can be a possible way to improve the results. However, this approach requests similar behaviour in the response pattern from sensor to sensor since interaction terms from new calibrations may be necessary to be calculated if changes occur in the conditions. Exploiting a multivariate analysis of the whole dynamic responses from biosensors in arrays will open up more interesting perspectives, such as possible multiway modelling. This work, however, should be well grounded by sufficient knowledge obtained from multivariate data analysis of responses from single biosensors.

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References

[1] C. Nistor, J. Emnéus, Waste Manage. 19 (1999) 147.
[2] T. Ruzgas, E. Csöregi, J. Emnéus, L. Gorton, G. Marko-Varga, Anal. Chim. Acta 330 (1996) 123.
[3] B. Haghighi, L. Gorton, T. Ruzgas, L.J. Jönsson, Anal. Chim. Acta 487 (2003) 3.
[4] S. Wold, K. Esbensen, P. Geladi, Chemom. Intell. Lab. Syst. 2 (1987) 37.
[5] H. Martens, T. Naes, Multivariate Calibration, Wiley, Chichester, UK, 1989.
[6] J. Zupan, J. Gasteiger, Neural Networks for Chemists: An Introduction, VCH, Weinheim, 1993.
[7] K.J. Albert, N.S. Lewis, C.L. Schauer, G.A. Sotzing, S.E. Stitzel, T.P. Vaid, D.R. Walt, Chem. Rev. 100 (2000) 2595.
[8] M. Otto, J.D.R. Thomas, Anal. Chem. 57 (1985) 2647.
[9] K. Toko, Biosens. Bioelectron. 13 (1998) 701.
[10] C. Krantz-Rulcker, M. Stenberg, F. Winquist, I. Lundström, Anal. Chim. Acta 426 (2001) 217.
[11] Y. Vlasov, A. Legin, A. Rudnitskaya, Anal. Bioanal. Chem. 373 (2002) 136.
[12] J. Lange, C. Wittmann, Anal. Bioanal. Chem. 372 (2002) 276.
[13] R.S. Freire, M.M.C. Ferreira, N. Durán, L.T. Kubota, Anal. Chim. Acta 485 (2003) 263.
[14] T.T. Bachmann, R.D. Schmid, Anal. Chim. Acta 401 (1999) 95.
[15] T.T. Bachmann, B. Leca, F. Vilatte, J.-L. Marty, D. Fournier, R.D. Schmid, Biosens. Bioelectron. 15 (2000) 193.
[16] P. Ertl, S.R. Mikkelsen, Anal. Chem. 73 (2001) 4241.
[17] P. Ertl, M. Wagner, E. Corton, S.R. Mikkelsen, Biosens. Bioelectron. 18 (2003) 907.
[18] A.N. Reshetilov, A.V. Lobanov, N.O. Morozova, S.H. Gordon, R.V. Greene, T.D. Leathers, Biosens. Bioelectron. 13 (1998) 787.
[19] A.V. Lobanov, I.A. Borisov, S.H. Gordon, R.V. Greene, T.D. Leathers, A.N. Reshetilov, Biosens. Bioelectron. 16 (2001) 1001.
[20] J. Saurina, S. Hernández-Cassou, Anal. Chim. Acta 438 (2001) 335.
[21] M. Slama, C. Zaborosch, D. Wienke, F. Spener, Anal. Chem. 68 (1996) 3845.
[22] M. Slama, C. Zaborosch, D. Wienke, F. Spener, Sens. Actuators B 44 (1997) 286.
[23] V. Plegge, M. Slama, B. Süselbeck, D. Wienke, F. Spener, M. Knoll, C. Zaborosch, Anal. Chem. 72 (2000) 2937.
[24] E. Dock, A. Lindgren, T. Ruzgas, L. Gorton, Analyst 126 (2001) 1929.
[25] S. Sapelnikova, E. Dock, R. Solna, P. Skladal, T. Ruzgas, J. Emnéus, Anal. Bioanal. Chem. 376 (2003) 1098.
[26] C. Nistor, J. Emneus, L. Gorton, A. Ciucu, Anal. Chim. Acta 387 (1999) 309.
[27] J. Kulys, H.E. Hansen, Biosens. Bioelectron. 9 (1994) 491.
[28] J.E. Haugen, O. Tomic, K. Kvaal, Anal. Chim. Acta 407 (2000) 23.
[29] T. Artursson, T. Eklöv, I. Lundström, P. Mårtensson, M. Sjöström, M. Holmberg, J. Chemom. 14 (2000) 711.
[30] S. Holmin, C. Krantz-Rulcker, I. Lundström, F. Winquist, Meas. Sci. Technol. 12 (2001) 1348.
[31] R. Appelqvist, G. Marko-Varga, L. Gorton, A. Torstensson, G. Johansson, Anal. Chim. Acta 169 (1985) 237.
[32] S. Wold, Technometrics 20 (1978) 397.
[33] S.-Y. Seo, V.K. Sharma, N. Sharma, J. Agric. Food Chem. 51 (2003) 2837.
[34] G. Marko-Varga, J. Emnéus, L. Gorton, T. Ruzgas, Trends Anal. Chem. 14 (1995) 319.