Anal. Chem. **1996,** *68,* 3845−3850

Simultaneous Mixture Analysis Using a Dynamic Microbial Sensor Combined with Chemometrics

Michael Slama,[†] Christiane Zaborosch,*[,†] Dietrich Wienke,[‡] and Friedrich Spener[†]

Institut für Chemo- und Biosensorik, Mendelstrasse 7, D-48149 Muenster, Germany, and Stuttgart Technology Center, Sony Deutschland GmbH, Stuttgarter Strasse 106, D-70736 Fellbach (Stuttgart), Germany

A biosensor consisting of immobilized microbial cells and an oxygen electrode was used in a flow-through system as a microbial sensor flow injection analyzer (FIA). For different organic analytes, the metabolism of vital cells provides individual time-resolved responses with distinct time-dependent amplitudes. Chemometrical data analysis revealed that the individual responses are additive and depend linearly on single analyte concentrations. Based on these observations, simultaneous multicomponent analysis of organic mixtures was carried out in the FIA's time domain with analytical errors of less than 10%. For mixture analysis and monitoring in processes like enzymatic conversions, the described microbial sensor FIA ("dynamic microbial sensor") offers an alternative to expensive analytical equipment.

Since the first biosensor was described in 1962 by Clark and Lyons,[1] the biological component of conventional biosensors has been chosen as selective as possible for the determination of a single analyte. Most biosensors attain high selectivities if antibodies or enzymes are used as biological components; in many cases those biosensors enable the specific determination of a desired analyte.

Microbial sensors using immobilized microorganisms as recognition elements usually do not have comparably high selectivities. On the other hand, from a practical point of view, they have some advantages in comparison to biosensors using antibodies or enzymes. Microbial sensors often exhibit an increased stability because they use highly integrated systems under quasi-physiological conditions and they are easy to prepare, as protein extraction and purification steps are not necessary. The multireceptor behavior of intact cells enables microbial sensors to recognize groups of compounds yielding complex parameter signals, but at the cost of less selectivity. The lack of selectivity of microbial sensors is often discussed as a disadvantage, and different approaches were applied to improve their selectivity.[2,3] Several microbial sensors for the determination of various organic compounds, groups of substances, and complex parameters have been described in the literature.[3−5] The common transducer of most microbial sensors is a Clark-type oxygen electrode which detects increased respiratory activity of the microbial cells in the presence of analytes. So far, only sensors for the determination of complex parameters, e.g., biochemical oxygen demand,[6−8] and sensors which use the exclusion of undesired analytes by additional membranes[4,9,10] are of importance for a practical application.

In the present work, the development of a procedure for simultaneous mixture analysis is described. Only one single microbial sensor is used in combination with chemometrics, taking into account the multireceptor behavior of whole cells and exploiting the time-dependent responses ("dynamic microbial sensor").

The classical model for simultaneous multicomponent mixture analysis assumes that the total signal response, $y(k)$, provided by the kth channel (wavelength, retention time, etc.) is a linear combination of individual responses,

$$y(k) = y_1(k) + y_2(k) + ... + y_m(k) \qquad (1)$$

of m analytes present in a mixture. Assuming further linearity for each individual analyte response with respect to its concentration, c_i, where $i = 1, ..., m$, for expression (1),

$$y(k) = a_1(k)c_1 + a_2(k)c_2 + ... + a_m(k)c_m \qquad (2)$$

with $a_i(k)$ as sensitivity coefficient for the ith analyte at the kth measurement channel.

In cases of photometry (UV/visible, near-IR, or IR spectrometry), expression (2) corresponds to the Lambert−Beer law, for example. The first photometric multicomponent models were reported in the 1960s.[11] Later, modern calibration methods in latent variables such as principal component regression, partial least-squares regression,[12,13] and canonical correlation analysis[14]

[†] Institut für Chemo- und Biosensorik.

[‡] Sony Deutschland GmbH.

(1) Clark, L. C.; Lyons, C. *Ann. N. Y. Acad. Sci.* **1962**, *102*, 29−45.
(2) Riedel, K. *Bioelectrochem. Bioenerg.* **1991**, *25*, 19−30.
(3) Racek, J. *Cell-Based Biosensors*; Technomic Publishing AG: Basel, 1995.
(4) Karube, I. In *Biosensors—Fundamentals and Applications*; Turner, A. P. F., Karube, I., Wilson, G. S., Eds.; Oxford University Press: Oxford, 1987; pp 13−29.
(5) Riedel, K. In *Food Biosensor Analysis*; Wagner, G., Guilbault, G. G., Eds.; Marcel Dekker: New York, 1994; pp 123−149.
(6) Karube, I.; Matsunaga, T.; Mitsuda, S.; Suzuki, S. *Biotechnol. Bioeng.* **1977**, *19*, 1535−1547.
(7) Riedel, K.; Neumann, B.; Klimes, N.; Fahrenbruch, B.; Scheller, F.; Merten, H.; Klingner, E.; Stein, H.-J. In *Biosensors: Fundamentals, Technologies and Applications*; Scheller, F., Schmid, R. D., Eds.; VCH Verlag: Weinheim, 1992; Vol. 17, pp 51−54.
(8) Riedel, K. *Vom Wasser* **1993**, *81*, 243−256.
(9) Hikuma, M.; Kubo, T.; Yasuda, T.; Karube, I.; Suzuki, S. *Anal. Chim. Acta* **1979**, *109*, 33−38.
(10) Hikuma, M. *Biotechnol. Bioeng.* **1979**, *21*, 1845−1853.
(11) Kortuem, G. *Kolorimetrie, Photometrie und Spektrometrie*; Springer: Berlin-Goettingen-Heidelberg, 1962.
(12) Martens, H.; Næs, T. *Multivariate Calibration*; John Wiley and Sons: New York-Toronto, 1989.
(13) Sjoestroem, M.; Wold, S.; Lindberg, W.; Persson, J.; Martens, H. *Anal. Chim. Acta* **1983**, *150*, 61−70.

S0003-2700(96)00414-3 CCC: $12.00

were reported. A recent overview, for example, can be found in ref 15. Beyond these pure spectroscopic applications, the approach of multivariate calibration also found recent applications in combination with flow injection analyzers (FIAs).[16,17]

However, the present study focuses on organic multicomponent analysis, making use of a microbial sensor in a rather advanced way. It was observed that immobilized microorganisms integrated as an amperometric microbial sensor in a flow-through system show an individual time-dependent response y in oxygen consumption for different pure organic analytes. Further, it was found that this behavior is conserved also when the biosensor is exposed to a mixture of these organic analytes. The cells respond with a total signal $y(k)$ according to the linear combination (2), where k corresponds to a channel on the time axis or to a sensor with a particular type of microorganisms. The present study is focused on the case of a single sensor which records at distinct time channels $k = 1, ..., p$ during a FIA run. These findings were exploited by multivariate calibration using partial least-squares regression.

EXPERIMENTAL SECTION

Reagents. The bisulfite-blocked prepolymer for microorganism immobilization was a research product from the Bundesforschungsanstalt für Landwirtschaft (Braunschweig, Germany). Tris(hydroxymethyl)aminomethane (ultrapure) was obtained from USB (Cleveland, OH). Sodium gluconate (herein referred to as gluconate), sodium acetate trihydrate (herein referred to as acetate), L-serine, and L-threonine were purchased at analytical grade from Sigma (Deisenhofen, Germany). The remaining chemicals were obtained from Merck (Darmstadt, Germany). All sample solutions were prepared by diluting aliquots of stock solutions with 50 mM Tris-HCl buffer (pH 7.2). The same buffer was also used as carrier stream in the flow-through system.

Microorganisms and Culture. For all experiments, *Alcaligenes eutrophus* KT02 (DSM 6519) was used which was isolated from a sewage plant near Göttingen, Germany.[18] This strain carries plasmids encoding for heavy metal resistances against nickel, cobalt, zinc, copper, and cadmium and was already used within a microbial sensor for the estimation of biochemical oxygen demand (BOD) in the presence of heavy metal ions.[19] Cells were grown on a rotary shaker at 30 °C in a Tris mineral medium,[20] supplemented with 0.4% sodium gluconate and 20 mM nickel chloride. The growth of the culture was observed by means of a Klett-Summerson colorimeter (Manostat, New York, NY).

Immobilization. Cells were immobilized within a polyurethane hydrogel.[21] The microorganisms were grown in the described way and harvested at the beginning of the stationary phase. The cell suspension was centrifuged for 20 min at 5000 rpm and 4 °C. Subsequently, the cells were washed in 50 mM potassium phosphate buffer (pH 7.2) and finally resuspended in a minimal amount of the same buffer. To get comparable cell loadings of the microbial sensor, we determined the absorbance (A_{436}) of the cell concentrate and diluted the suspension to $A_{436} = 250$.

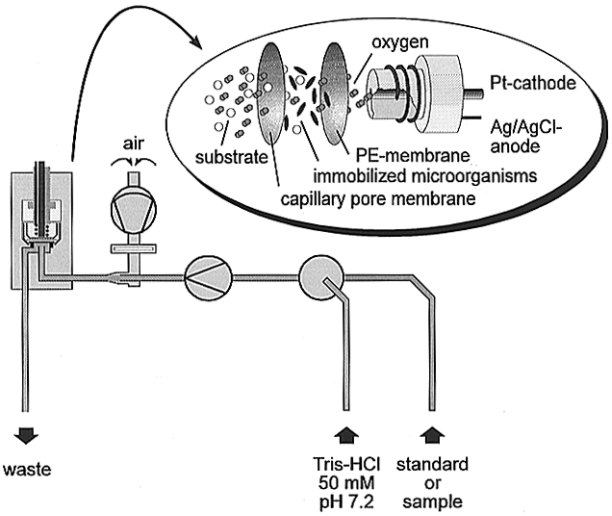

Figure 1. Experimental setup of the flow-through system with the amperometric microbial sensor.

The immobilization procedure was based on previously described methods.[22−24] A 250 mg sample of the prepolymer (33.4%) was mixed with 250 μL of 50 mM potassium phosphate buffer (pH 7.2). This hydrosol was cross-linked and neutralized up to a pH between 6.4 and 6.5 by stepwise addition of 10 μL amounts of poly(ethylene imine) (2.5% (w/v) in water) under constant mixing. The pH was controlled with a Biotrode pH electrode (Hamilton, Darmstadt, Germany). After short centrifugation to remove already polymerized particles, 160 μL of the solution was mixed with 40 μL of cell suspension, resulting in a final cell concentration of $A_{436} = 50$. An aliquot of 5 μL of this mixture was spread onto a 7 mm^2 spot of a gas-permeable polyethylene (PE) membrane (thickness 10 μm; Metra, Radebeul, Germany) and incubated for 30 min at 30 °C in a water vapor-saturated atmosphere, where the polymerization to the hydrogel was completed.

Microbial Sensor. An oxygen electrode (Pt cathode with a diameter of 0.5 mm and Ag/AgCl reference anode; PGW Medingen, Dresden, Germany) was covered first with a gas-permeable PE membrane, onto which the immobilized microorganisms were adhering, and then with a capillary pore membrane (pore diameter 0.6 μm, thickness 10 μm; Oxyphen, Dresden, Germany) to increase mechanical stability. The sensor was installed in a flow-through system (BOD module; PGW Medingen, Dresden, Germany), with an alternating flow of 50 mM Tris-HCl buffer (pH 7.2) and analyte solution (Figure 1). All measurements were taken at 31 °C and a constant flow rate of 100 mL/h. The platin cathode was polarized at −800 mV vs an Ag/AgCl anode. The sample time was, in the case of gluconate/acetate measurements, 30 s and, in the case of L-serine/L-threonine measurements, 25 s.

Chemometrics. Two binary mixtures, gluconate/acetate and L-serine/L-threonine, were considered. Mixtures with distinct

(14) Wienke, D.; Kateman, G. *Fresenius J. Anal. Chem.* **1992**, *343*, 797−808.
(15) Danzer, K. *Fresenius J. Anal. Chem.* **1995**, *351*, 3−10.
(16) Beck, H. P.; Wiegand, C. *Fresenius J. Anal. Chem.* **1995**, *351*, 701−707.
(17) Hernandez, O.; Jimenez, A. I.; Jimenez, F.; Arias, J. J. *Anal. Chim. Acta* **1995**, *310*, 53−61.
(18) Timotius, K.; Schlegel, H. G. *Nachr. Akad. Wiss. Göttingen, III. Math.-Phys. Kl.* **1987**, *3*, 15−23.
(19) Slama, M.; Zaborosch, C.; Spener, F. In *Heavy Metals in the Environment*; Wilken, R.-D., Förster, U., Knöchel, A., Eds.; CEP Consultants Ltd.: Hamburg, 1995; Vol. 2, pp 171−174.
(20) Mergeay, M.; Nies, D.; Schlegel, H. G.; Gerits, J.; Charles, P.; Gijsegem, F. V. *J. Bacteriol.* **1985**, *162*, 328−334.
(21) Vorlop, K.-D.; Muscat, A.; Beyersdorf, J. *Biotechnol. Tech.* **1992**, *6*, 483−488.
(22) Muscat, A.; Beyersdorf, J.; Vorlop, K.-D. *Biosens. Bioelectron.* **1995**, *10*, xi−xiv.
(23) Kotte, H.; Gründig, B.; Vorlop, K.-D.; Strehlitz, B.; Stottmeister, U. *Anal. Chem.* **1995**, *67*, 65−70.
(24) König, A.; Zaborosch, C.; Muscat, A.; Vorlop, K.-D.; Spener, F. *Appl. Microbiol. Biotechnol.* **1996**, *45*, 844−850.

Table 1. Sample Set for Mixture Analysis of Gluconate and Acetate

sample no.	concn (mg/L)		sample no.	concn (mg/L)	
	gluconate	acetate		gluconate	acetate
1[a,b]	0	0	11	0	5
2	5	0	12[a]	5	5
3[a]	10	0	13	10	5
4	20	0	14[a]	20	5
5[a,b]	40	0	15	40	5
6	0	2	16[a,b]	0	10
7[a]	5	2	17	5	10
8	10	2	18[a]	10	10
9[a]	20	2	19	20	10
10	40	2	20[a,b]	40	10

[a] Samples used for calibration with set size $n = 10$. [b] Samples used for calibration with set size $n = 4$.

Table 2. Sample Set for Mixture Analysis of L-Serine and L-Threonine

sample no.	concn (mg/L)		sample no.	concn (mg/L)	
	L-serine	L-threonine		L-serine	L-threonine
1[a]	0	0	14	10	5
2	2	0	15[a]	15	5
3[a]	5	0	16	0	10
4	10	0	17[a]	2	10
5[a]	15	0	18	5	10
6	0	2	19[a]	10	10
7[a]	2	2	20	15	10
8	5	2	21[a]	0	15
9[a]	10	2	22	2	15
10	15	2	23	5	15
11	0	5	24	10	15
12	2	5	25[a]	15	15
13	5	5			

[a] Samples used for calibration with set size $n = 10$.

concentration ratios were prepared with the concentrations shown in Tables 1 and 2.

From each mixture, the response of the biosensor in the flow-through system was recorded in the time interval between 0 and 240 s, with a time resolution (step width) of 5 s. Measurements of each analysis were repeated three times, and curves were averaged. For each analyzed sample in this case, a concentration vector $\mathbf{c} = [c_1, c_2]$ and a measurement vector $\mathbf{y} = [y_1, y_2, y_3, ..., y_p]$ were stored. A part of the sample mixtures served for multivariate calibration. The remainder were used as independent test samples. All measurements were done in random order. Additionally, the number p of time channels within a fixed time range (time resolution) was varied to look for smaller sets of variables with a goal of improving analyzing speed for future on-line applications. For multivariate calibration, the software package ICB-PLS (Intelligent Calibration of Biosensors-PLS, developed by D. Wienke) was applied, which uses the PLS algorithm.[12,14] Several cross-validation schemes, easy file handling, and graphical tools allow user-friendly calibration and analysis on a personal computer (DOS).

RESULTS AND DISCUSSION

After the sensor was integrated in a flow-through system, it was discovered that the microbial sensor shows an individual and time-dependent response concerning different analytes (Figure 2). The baseline recovery was always reached after 250 s, and thus a sample throughput of about 12 determinations per hour was possible. No changes in the signal characteristics were observed within a period of 24 h. It is obvious that the dynamic patterns of gluconate and acetate differ in shape and size. This observation formed the bases for a multivariate calibration experiment using *multivariate linear calibration* (MLR) or, in its advanced form, *partial least-squares regression* (PLS).

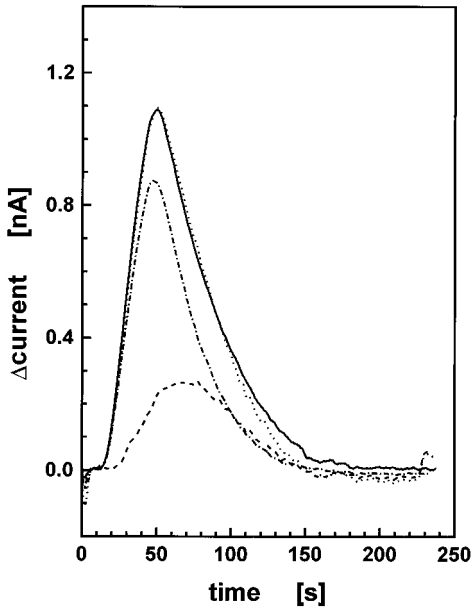

Figure 2. Comparison of the numerical sum (dotted line) of individual time response curves of the dynamic microbial sensor for two analytes gluconate (5 mg/L; dashed line) and acetate (5 mg/L; dash-dotted line) with the measured response of the same sensor to a mixture of both analytes (solid line).

Additivity and Linearity. Before application of multivariate calibration, we investigated whether the prerequisites for this algorithm, such as additivity of signal responses to different analytes and linearity of signal height at distinct time channels, are fulfilled. Additivity of the signal responses was tested first by measuring the signal responses to each pure analyte. Afterward, the signal response was measured to the binary mixture of both analytes in their concentrations as used before (Figure 2). The nearly identical patterns of the numerical sum and the experimental sensor response to the mixture are a first indicator for reasonable additivity at different time channels k according to (1). The linearity of signal height was examined and found to be adequate by determining the signal response to each pure analyte at four representative time channels versus varied concentrations (Table 3). The sensitivity of the sensor for the two analytes gluconate and acetate differs at all selected time channels. While the maximum sensitivity of the signal response to acetate is reached early and has a steep drop, the signal response to gluconate gains the maximum later and does not show a comparable steep drop (compare Figure 2 and Table 3). From these significant differences of the patterns, no further problems with collinearity will be expected.

To test whether additivity and linearity are fulfilled also for mixtures, the complete sample set listed in Table 1 was tested at one representative time channel ($k = 70$ s, Figure 3). The signal heights behave almost linearly for the case of one component in the presence of a constant concentration of the second component. To check the additivity, the slopes have to be considered.

Table 3. Linearity at Four Different Time Channels for a Series of Pure Analyte Solutions

	linear regression[a]			
	slope (pA·L·mg^{-1})	intercept (pA)	standard deviation (pA)	sample size
		$k = 30$ s		
gluconate	12.33 (0.43)	6.39 (8.86)	13.58	5
acetate	76.26 (6.67)	19.81 (37.86)	50.22	4
		$k = 50$ s		
gluconate	36.18 (1.18)	57.56 (24.34)	37.33	5
acetate	152.92 (7.95)	35.37 (45.12)	59.86	4
		$k = 70$ s		
gluconate	42.79 (1.20)	43.22 (25.78)	38.01	5
acetate	101.96 (2.81)	−7.31 (15.94)	21.15	4
		$k = 120$ s		
gluconate	24.28 (0.58)	−25.54 (11.97)	18.36	5
acetate	17.07 (1.32)	−12.70 (7.48)	9.93	4

[a] Standard deviation is given in parentheses.

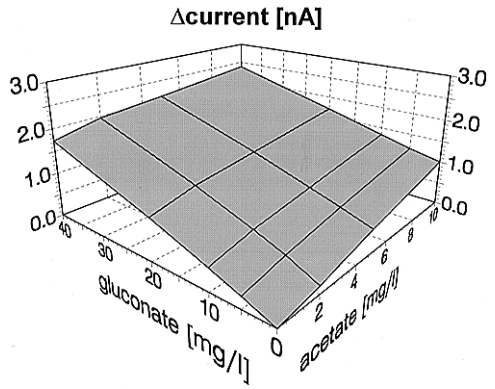

Figure 3. Typical total sensor responses y(k) for 20 mixtures of gluconate and acetate, taken at the time channel $k = 70$ s, showing only slight deviations from additivity and linearity. Corner points and line crossings at the shaded surface, down-projected to the concentrations plane, indicate individual mixtures (cf. Table 1).

Although there might be visible small effects of saturation, the additivity of signal responses is sufficiently fulfilled also in case of mixtures. The PLS algorithm was able to tackle these slight deviations with a few additional latent variables. The appropriate number of latent PLS variables has been determined by PRESS criterion and another two heuristical measures in combination with cross-validation. These three PRESS criteria are part of the ICB-PLS software package. Usually, for the present three-component system (gluconate, acetate, buffer), three dominant latent variables plus four minor components were found by the PRESS criteria, indicating the already-mentioned slight deviation from linearity and additivity.

Multivariate Calibration. The following studies focus on multivariate calibration of a data set taken from the responses of a sample set consisting of a two-component mixture of gluconate and acetate (Table 1). An excellent prediction of the composition of unknown binary mixtures of gluconate and acetate was achieved (Figure 4a,b). This good result was obtained using $n = 10$ calibration samples out of a set of 20 (Table 1). All calibrations shown in Figure 4 were performed with response data taken at 25 time channels equally distributed over a time range between 30 and 150 s after injection had started. Even with fewer calibration samples ($n = 4$), very predictive models were obtained (Figure 4c,d). In this case, the prognoses for gluconate (Figure 4c) look quite good, as deviations from the true value are small and equally distributed. The forecast for acetate (Figure 4d) still reveals some weakness. Deviations from true values are sometimes more than 20%, and most concentrations are predicted too high. The mean of deviation from true value is 0.58 ± 0.30 mg/L. A t-test for paired observations indicates a significant difference between true and predicted concentrations (model 1, Table 4). Earlier-observed small nonlinearities and nonadditivities (Figure 3) seem to influence the quality of prediction. The applied linear PLS algorithm allows only partial compensation for these effects. Beside those nonlinearities and a possible sensor drift, the representativity of the calibration data set affects the model quality. A calibration set size of 10 is representative enough to afford a good prediction, as mentioned earlier (Figure 4a,b). In this case ($n = 10$), the root-mean-square error of prediction (RMSEP) is 1.67 mg/L concerning analysis of gluconate and 0.51 mg/L concerning analysis of acetate. The mean of deviation from true value is −0.66 ± 1.16 mg/L in the case of gluconate and 0.16 ± 0.37 mg/L in the case of acetate. A t-test for paired observations provides the result that no significant difference between true and predicted concentrations exists (model 3, Table 4).

Figure 5 shows a three-dimensional plot with the calibration model based on the complete sample set. With this kind of presentation, it is possible to relate the absolute error in estimation (concentration recovered) to a particular mixture. The gluconate error increases with increasing concentrations of gluconate and acetate (heteroscedasticity). In the case of the error of acetate determination, no heteroscedasticity has been observed. However, the relative errors of estimation are, in general, larger for acetate (data not shown).

A more detailed error analysis can be found in Table 4. This table was provided to optimize the calibration parameters and summarizes calibrations with different number n of calibration set size, number p of time channels, and range of selected time channels. Optimization of calibration in this case means finding the best compromise between a quick calibration, short time of analysis, and a low, equally distributed error in prediction of a desired analyte. Besides splitting into a calibration and test data set for each calibration model (models 1−22), additionally, the full data set (calibration and test data set) has been recalculated. Subsequently, in the case of the test data set, the RMSEP was determined, and, in the case of the calibration data set, the root-mean-square error of estimation (RMSEE) was determined. By means of a t-test for paired observations, we examined how far predicted concentrations deviate systematically from the true concentrations. If the relative t-value (t_{calc}/t_{tab}) exceeds a value of 1, a systematic error in prediction is detected.

The main effect is provided by the number of calibration samples used (Table 4, models 1−4). The error generally decreases with an increasing number n of calibration samples, but at the expense of needed time for calibrating. Raising the number p of time channels within a fixed time range ("time resolution") appears also to be an effective method to reduce deviations (Table 4, models 5−13). Such a higher resolution compensates for noise in the recorded signal responses. This improvement does not consume additional analysis time, which is important for an on-line application of the dynamic microbial sensor. The additional increase in computational effort can be ignored. Another way to improve calibration might be the suitable choice of range on the time axis. Its influence was tested for

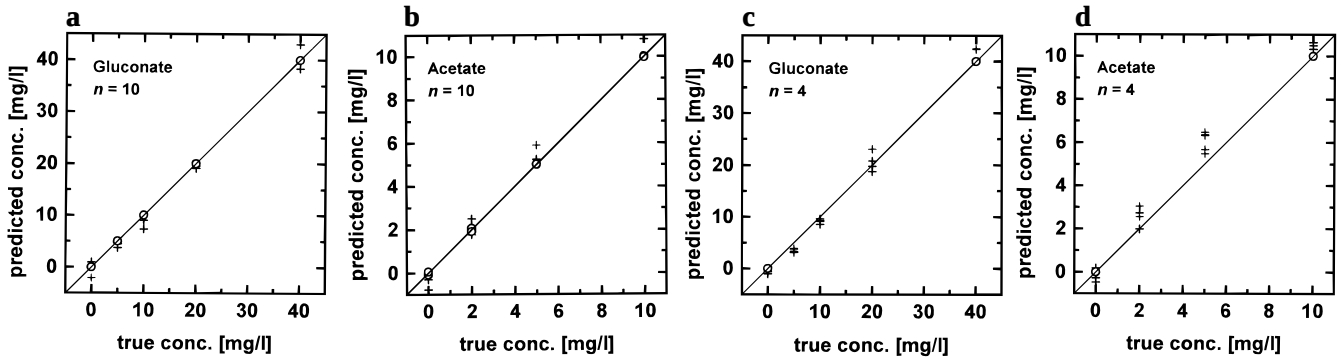

Figure 4. Correlation plot of recovered (○) concentrations for calibration data set and predicted (+) concentrations for remaining test data set (Table 1) versus true concentration obtained by multicomponent PLS calibration for simultaneous analysis of mixtures of gluconate and acetate and for different numbers n of calibration set size (a, b, $n = 10$; c, d, $n = 4$), analyzed by the dynamic microbial sensor. Note that each point represents a distinct mixture of gluconate and acetate.

Table 4. Dependency of Analytical Error on Different Parameters

calibration model no.	number n/n_{pred} of calibration and test samples[b]	number p of time channels	range of selected time channels (s)	root-mean-square error of prediction (RMSEP) gluconate (mg/L)	RMSEP acetate (mg/L)	relative t-test for paired observations (t_{calc}/t_{tab})[c] gluconate	t-test acetate
1	4/16	25	30−150	1.52	0.77	0.29	1.94
2	8/12	25	30−150	1.97	0.48	0.07	0.29
3	10/10	25	30−150	1.67	0.51	0.57	0.42
4	20/0	25	30−150	0.62[a]	0.19[a]	0.00	0.00
5	20/0	5	30−150	0.88[a]	0.32[a]	0.00	0.00
6	20/0	13	30−150	0.74[a]	0.21[a]	0.00	0.00
7	20/0	25	30−150	0.62[a]	0.19[a]	0.00	0.00
8	10/10	5	30−150	0.94	0.53	0.28	0.19
9	10/10	13	30−150	3.01	0.71	0.08	0.22
10	10/10	25	30−150	1.67	0.51	0.57	0.42
11	4/16	5	30−150	1.69	1.15	0.16	3.20
12	4/16	13	30−150	1.55	0.80	0.32	1.92
13	4/16	25	30−150	1.52	0.77	0.29	1.94
14	20/0	15	30−100	0.59[a]	0.21[a]	0.00	0.00
15	20/0	15	55−125	0.66[a]	0.23[a]	0.00	0.00
16	20/0	15	80−150	0.76[a]	0.39[a]	0.00	0.00
17	10/10	15	30−100	1.57	0.53	0.23	0.30
18	10/10	15	55−125	2.31	1.07	0.06	0.12
19	10/10	15	80−150	2.22	0.87	0.44	0.42
20	4/16	15	30−100	1.94	0.88	0.82	1.98
21	4/16	15	55−125	1.62	0.54	0.12	1.07
22	4/16	15	80−150	1.74	1.00	0.24	1.25

[a] Root-mean-square error of estimation (RMSEE). [b] Total set of samples divided into n calibration and n_{pred} test samples. [c] $t_{calc}/t_{tab} = |\bar{d}|/(s_d/n_{pred}^{1/2})t_{(n_{pred}-1,a=5\%)}$, with $\bar{d} = \Sigma(y_{pred,true} - y_{pred,calc})/n_{pred}$.

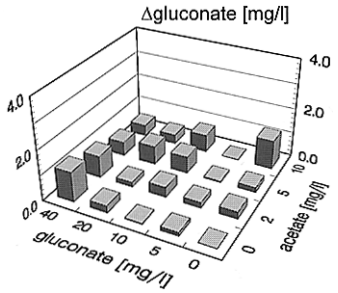

Figure 5. Absolute estimation error of gluconate (concentration recovered) as a function of mixture composition (calibration set size $n = 20$, complete sample set).

different numbers of calibration samples with three overlapping ranges (Table 4, models 14−22). Because of a decreasing signal-to-noise ratio, the range of selected time channels should not be chosen too far from the time of injection (cf. Figure 2).

Finally, an alternative two-component system was studied with the dynamic microbial sensor. Twenty-five mixtures of L-serine and L-threonine (Table 2) were chosen for analysis, which are expected to be rather difficult to analyze because of their similar chemical structures. Particularly at low concentrations, the dynamic FIA curves of both pure analytes show only marginal differences. The multivariate calibration was performed with 10 calibration samples at a time range between 20 and 165 s, resolved into 30 time channels. Even in the case of chemically very similar analytes, the calibration model returns good predictions of the presented 15 "unknown" samples (Figure 6). The RMSEP is 0.85 mg/L for the analysis of L-serine and 1.31 mg/L for the analysis of L-threonine. The predicted values appear to be equally distributed, with a mean of deviation from true value of 0.24 ± 0.45 mg/L in the case of L-serine and of −0.26 ± 0.73 mg/L in the case of L-threonine. For both analytes, no significant difference between true and predicted concentrations can be observed.

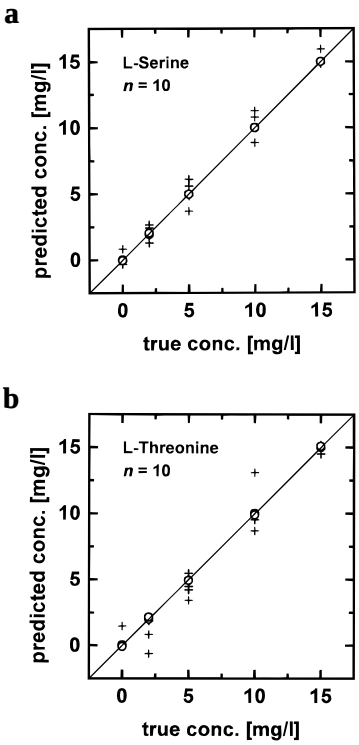

Figure 6. Correlation plot of recovered (○) concentrations for calibration data set and predicted (+) concentrations for remaining test data set (Table 2) versus true concentration obtained by multicomponent PLS calibration for simultaneous analysis of mixtures of L-serine (a) and L-threonine (b), analyzed by the dynamic microbial sensor based on $n = 10$ calibration samples. Note that each point represents a distinct mixture of L-serine and L-threonine.

CONCLUSIONS

The present study leaves the rather classical approach of "univariate" thinking in the design of biosensors. The time axis in a flow injection system is exploited, which makes the sensor "dynamic" and spans a full "multivariate space". This multivariate approach takes into account the cross-sensitivities of a microbial sensor to several analytes in mixture.

It was shown that the described dynamic microbial sensor enables the analysis of a two-component mixture. The average error relative to maximum measured concentration is, in the cases of analyzing gluconate, 4.2%; acetate, 5.1%; L-serine, 5.6%; and L-threonine, 8.7%. This seems to be very promising when it is considered that only one sensor has been used and that the applied classical PLS algorithm enables only small deviations from linearity and additivity to be modeled. PLS already provided an acceptable calibration model, but further improvements seem possible. In the future, alternative methods will be tested, such as nonlinear regression or artificial neural networks which compensate for nonlinearity and nonadditivity of individual time responses. Suitable data preprocessing techniques will also be considered, especially with respect to the time axis (scaling, normalization, optimal selection of time channels, and cross-validation or bootstrapping to estimate the RMSEP more precisely). Another exciting problem is that the distinct time channels are not independent variables in a statistical sense, which would require additional chemometrical research. Further research will focus on an increased number of analytes simultaneously present in the mixture. It is thought that dynamic microbial sensors which contain different microorganisms could be applied and interpreted in parallel ("dynamic microbial sensor array"). In this way, an additional dimension will be added, and the maximum number of simultaneously detectable analytes as well as the number of overall detectable analytes should be increased.

ACKNOWLEDGMENT

This work was supported by grants from the Deutsche Bundesstiftung Umwelt. The authors are grateful to A. König (ICB Münster, Germany) for helpful discussions and K.-D. Vorlop (FAL Braunschweig, Germany) for kindly providing the poly(carbamoyl sulfonate) prepolymer. This paper is part of the doctoral thesis of M. Slama.

Received for review April 25, 1996. Accepted August 7, 1996.[⊗]

AC9604144

[⊗] Abstract published in *Advance ACS Abstracts,* September 15, 1996.