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Array of potentiometric sensors for simultaneous analysis of urea and potassium

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Abstract

Urea biosensors based on urease immobilized by crosslinking with BSA and glutharaldehyde coupled to ammonium ion-selective electrodes were included in arrays together with potassium, sodium and ammonium PVC membrane ion-selective electrodes. Multivariate calibration models based on PCR and PLS2 were built and tested for the simultaneous determination of urea and potassium. The results show that it is possible to obtain PCR and PLS2 calibration models for simultaneous determination of these two species, based on a very small set of calibration samples (nine samples). Coupling of biosensors with ion-selective electrodes in arrays of sensors raises a few problems related to the limited stability of response and unidirectional cross-talk of the biosensors, and this matter was also subjected to investigation in this work. Up to three identical urea biosensors were included in the arrays, and the data analysis procedure allowed the assessment of the relative performance of the sensors. The results show that at least two urea biosensors should be included in the array to improve urea determination. The prediction errors of the concentration of urea and potassium in the blood serum samples analyzed with this array and a PLS2 calibration model, based on nine calibration samples, were lower than 10 and 5%, respectively.

Keywords: Urea biosensors; Array of potentiometric sensors; PCR and PLS2 calibration models; Redundant biosensors response; Blood serum analysis

1. Introduction

Urea is the end product of protein metabolism and its determination deserves much interest; for instance, it is monitored in blood as an indicator of renal function. Urease is an important enzyme in biological systems, where it catalyses the conversion of urea to carbon dioxide and ammonia. Coupling of this enzymatic reaction with different transducers has allowed the development of thermal [1–6], amperometric [7–10], condutimetric [11–14], optical [15–18], piezoelectric [19], potentiometric [20–45] and FET [46,47] based biosensors. Potentiometric urea biosensors are among the most attractive due to the simplicity of their construction and general availability of the instrumentation required for their utilization. All-solid-state nonactin-based ammonium ion-selective electrodes are among the most adequate transducers for the development of potentiometric urea biosensors, due to their fast and reproducible response [26] and their long lifetime, up to 12 months [27].

Matrix correction in the determination of urea in blood serum by such potentiometric biosensors is necessary due to the ion composition of the matrix. Usually, the most severe interference is due to potassium because of the lack of selectivity of response of the ammonium-based electrode and of the same order of magnitude of the concentrations of urea and potassium in blood serum. The most effective method for matrix correction has been based on differential measurements [31]. Ideally, for this purpose, these measurements should be made with the same ammonium electrode to ensure that the contribution of the variability in response characteristics of different ammonium electrodes has no influence on the determination of urea. However, this is not generally possible, because it is impossible to remove and apply again the enzymatic layer of the biosensor without destroying it. In this case, a two-electrode approach has been used, and differential mea-

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surements are made relatively to an ammonium ion-selective electrode [31]. However, this approach shows limitations due either to the usual variability of the response of different ion-selective electrodes of the same type, or to changes in the response characteristics of the ammonium electrode to the ions in solution provoked by the enzymatic layer [31].

An alternative generic approach evaluated in the present work uses an array of potentiometric sensors and multivariate inverse calibration models to allow both the correction of the contributions of other ions to the response of the biosensor, and simultaneous determination of urea and its most severe interference, potassium ion, another species with biological interest. Ion-selective electrode arrays and multivariate calibration models [48–63] have already proved to be effective for increasing the selectivity and allowing the simultaneous determination of several analytes in different types of samples, ranging from biological fluids [48–50] to natural water samples [51]. Multivariate data analysis has been processed by several methods including hard modelling algorithms [48], non-parametric multivariate techniques [54,55], multiple linear regression (MLR) [48,56–61], partial least squares (PLS) [51,56,57,62], neural nets [60,63] and genetic algorithms [61].

The main objective of this work was to investigate the use of an array of potentiometric sensors for matrix correction in the analysis of urea in the presence of interfering ions, especially potassium ion, which was assayed in the concentration range 1.30–0.10 mM. This range of concentration, which includes the normal concentration range of potassium in blood serum after ten-fold dilution, was used because it was intended to evaluate the capability of correcting the urea measurements in the presence of larger variation in the potassium concentration. Although the usefulness of arrays of ion-selective electrodes and multivariate calibration models for the analysis of ions in biological fluids has been established, the present case of using potentiometric biosensors based on ion-selective electrodes in an array poses new challenges, because chemical cross-talk between sensors occurs mainly for the ions. In this work, an array of potentiometric sensors, constituted by ion-selective electrodes for potassium, sodium and ammonium and the potentiometric biosensors for urea, based on ammonium ion-selective electrodes with urease immobilized on their sensing membrane by glutaraldehyde crosslinking of urease with BSA were assembled and evaluated. Multivariate calibration models based on principal component regression (PCR) and PLS2 were built and tested for the simultaneous determination of urea and potassium.

The small number of sensors in the array suggests the use of multiple linear regression (MLR) techniques. However, these methods require that all components are known [64] and this is not a simple task for biological fluids. Otto et al. [56,57] showed that for ion-selective electrode arrays, MLR models based on overdetermined systems usually have poorer performance than models based on PCR and PLS. In part, this behaviour results from the mathematics underlying the computations, and consequently models based on MLR usually require variable selection to stabilize calculations. The natural consequence of variable selection (limitation of the number of sensors in the array to the number of analytes) is loss in error detection ability and less precise estimates of the models [64]. Furthermore, Diamond et al. [59] concluded that to improve the analytical performance of the arrays of potentiometric sensors, other factors not considered in the Nikolskii–Eisenmann equation, which are responsible for the concentration dependence of the selectivity coefficients, should be included in the models. In this context, the use of PCR and PLS calibration models for the arrays is preferable because they are a factor-based approach, and take advantage of signal averaging and are more effective in error detection. In the present work, both PCR and PLS calibration models were used. However, usually the price for using PLS is the larger number of samples in the calibration set necessary to develop a good model. Using multivariate standardization techniques circumvents this limitation as demonstrated by Sales et al. [51].

In this work, a PLS2 calibration model for urea and potassium in the normal blood serum range of concentrations, based on a small set of calibration samples (nine samples) was constructed and used for simultaneous analysis of urea and potassium in blood serum samples. Up to our knowledge, this approach has not been evaluated before for the determination of urea and potassium, but it should allow a more robust determination of both analytes. However, coupling of biosensors with ion-selective electrodes in arrays of sensors raises a few problems related to the limited stability of response and unidirectional cross-talk of the biosensors, and this matter was also subjected to investigation in this work, as well as the usefulness of using redundant biosensors in the array.

2. Experimental

2.1. Reagents and solutions

For preparing the ion-selective electrode sensing membranes, the following reagents from Fluka-Selectophore were used: ammonium ionophore I, dibutylsebacate, 2-nitrophenylethylether, potassium ionophore I, sodium ionophore X, tetrachlorophenylborate and PVC. The composition of the sensing membranes is summarized in Table 1. For dissolving the PVC in the sensor cocktails, tetrahydrofuran (THF) p.a. from Merck was used. For the preparation of the conductive support of the electrodes, graphite powder (<50 μm) ref. 4206, from Merck, and H54-UNF, from Epoxy Technology Inc. (graphite to epoxy weight proportion 1:1), were used.

For the preparation of the biosensors, the following reagents were used: bovine serum albumin (BSA) fraction V from Sigma, glutharaldehyde solution ref. 4239 from Merck, glycine from Merck and urease type III (E.C.3.5.1.5) from Sigma.

All the other reagents used for dissolving BSA and preparing of pH buffer solutions and calibration samples were of

Table 1
Ion-selective electrode membrane composition[a]

Electrode	Sensor		Solvent mediator		PVC
	Component	% (m/m)	Component	% (m/m)	% (m/m)
Ammonium	Nonactin/monactin	4.7	DOS	69.8	24.5
	TCFB	1.0			
Potassium (array1)	Valinomycin	3.6	DOS	67.6	27.4
	TCFB	1.4			
Potassium (array 2)	Valinomycin	3.6	DOA	67.6	27.4
	TCFB	1.4			
Sodium	Ionophre X	1.8	NPOE	69.4	27.8
	TCFB	0.9			

[a]TCFB: potassium tetraquis(4-chlorophenyl)borate; DOS: bis-(2-ethylhexyl)sebacate; DOA: bis-(2-ethylhexyl)adipate; NPOE: 2-nitrophenyloctylether.

analytical grade, p.a. or equivalent. For the preparation of all the solutions, deionised water with resistivity higher than 4 mΩ cm^{-1} was used.

2.2. Construction and evaluation of the ion-selective electrodes, biosensors and array

2.2.1. Construction of the ion-selective electrodes

All-solid-state ion-selective electrodes with sensor cocktails dispersed in a PVC membrane, applied on a conductive epoxy support [65], were used. The PVC membranes, with a diameter of 6 mm, were constituted by evaporation of the THF from the sensor solution (Table 1).

2.2.2. Enzyme immobilization and assembling of the biosensors

The BSA was dissolved in Tris buffer (0.2 M, pH 8.5) to obtain a 15% solution. Five miligrams of the urease was dissolved in 50 μL of the BSA solution, and 5 μL of this solution was placed on the surface of the electrode. Three microliters of glutharaldehyde were mixed with the enzyme solution and the mixture was spread over the surface of the ammonium electrode. The mixture was allowed to react for about 15 min, and then the electrode was washed by immersion in water for about 15 min, followed by immersion in 0.1 M glycine solution for 15 min. When not in use, the electrode was stored in a refrigerator in 0.1 M Tris pH 7.5 buffer solution.

2.2.3. Calibration parameters and response time of the biosensors

For the evaluation of the response characteristics, four biosensors of the same type were studied in parallel. The response characteristics were evaluated by calibration in response to urea, in the concentration range 0.001–10.0 mM. These calibration curves were obtained by titration of 20 mL of a 0.1 M (pH = 7.5) Tris buffer with 0.1 M standard solution of urea prepared in the same buffer. In the experiments, for the determination of the response time, a similar procedure was used but the response potentials after addition were registered along the time. All experiments were made at 25 °C.

2.2.4. Constitution of the array

The array was constituted by three ion-selective electrodes for potassium, ammonium and sodium, and three identical urea biosensors. The electrodes were introduced in a home-made support with the reference electrode in the centre.

2.3. Multivariate calibration

2.3.1. Experimental design

The composition of the samples used for studying the array is summarized in Table 2 . The concentration levels of urea and potassium in samples 1–9 (see Table 2) were based on a full factorial design with two factors and three levels. The experimental values of the concentration of urea and potassium in these solutions include the normal concentration ranges of both analytes in serum after ten-fold dilution. The concentration levels of sodium and ammonium in all the solutions were randomly assigned within narrow concentration ranges that include the normal concentration of these ions in blood serum, after ten-fold dilution.

Table 2
Composition of solutions used for multivariate calibration of the array

Solution no.	Concentration (mM)			
	Potassium	Urea	Sodium	Ammonium
1	**1.20**	**5.00**	**13.00**	**0.005**
2	**1.10**	**0.50**	**14.00**	**0.001**
3	**1.30**	**0.08**	**15.00**	**0.003**
4	**0.50**	**5.20**	**13.00**	**0.002**
5	**0.40**	**0.35**	**15.00**	**0.005**
6	**0.45**	**0.12**	**14.00**	**0.003**
7	**0.12**	**4.80**	**17.00**	**0.003**
8	**0.09**	**0.40**	**13.00**	**0.002**
9	**0.10**	**0.10**	**14.00**	**0.005**
10	1.00	4.50	13.00	0.005
11	0.80	0.55	14.00	0.002
12	1.10	0.30	15.00	0.003
13	1.50	1.50	16.00	0.002
14	0.40	5.00	13.00	0.002
15	0.30	0.40	14.00	0.003
16	0.50	0.45	13.50	0.003
17	0.45	0.10	12.00	0.002
18	0.10	4.80	14.00	0.002
19	0.12	0.45	13.50	0.003
20	0.15	0.32	13.00	0.002
21	0.20	0.12	14.00	0.003

In bold: solutions used for model building (see text).

To establish the upper limit of potassium concentration and the lower limit of urea concentration in these solutions, two preliminary experiments were made with an array constituted by four sensors, specifically a urea biosensor and ion-selective electrodes for ammonium, potassium and sodium. In the first preliminary experiment, PCR and PLS2 calibration models for potassium and urea were built based on a set of 25 solutions with composition designed according to a full factorial design with two factors and five levels with potassium and urea concentration in the ranges 10.20–0.08 and 0.05–4.995 mM. The results obtained showed that the PCR and PLS2 models obtained for potassium were adequate, but the models for urea were unacceptable. Further analysis of the models based on the normal probability plot of the residuals for urea showed that that the upper level of concentration of potassium and the lower level of urea in the design of the calibration set were excessive. In the second experiment, a set of solutions with composition designed according to a full factorial with two factors and four levels was used with potassium and urea of concentration ranges 3.20–0.080 and 0.158–4.995 mM, respectively. The analysis of the PCR and PLS2 models showed that also in this case, the potassium calibration models are adequate and a significant improvement in the urea calibration model was observed. A more detailed analysis of the results showed that samples corresponding to the two lower levels of urea concentration (0.158 mM) and the two higher levels of potassium (3.15 and 0.80 mM, respectively) were not adequately fitted to the PCR and PLS2 calibration models, suggesting that narrowing the range of concentration of potassium in the calibration set to lower levels should improve the performance of the calibration model.

2.3.2. Calibration

The electrodes of the array were introduced in the sample solution in a thermostated vessel and the values of response potential were acquired with the automatic system. Then the electrodes were removed from the solution, and the vessel and the electrodes were washed with deionised water and dried with smooth absorbent paper. Following this procedure, the whole set of samples in Table 2 was assayed within 120 min.

Solutions 1–9 in Table 2 were used for calibration of the array, and the remainder (solutions 10–21) were used for external validation. The two blocks were assayed consecutively and separately, and the samples were randomly assayed within each block. The statistical correlation of the response of the sensors in the calibration set was calculated and these responses were also treated by PCA. The results obtained in these experiments were also used for the construction of PCR and PLS2 calibration models of the array.

2.4. Analysis of blood serum

The array of potentiometric sensors was calibrated and a PLS2 model for potassium and urea was obtained. Then, 1.00 mL of blood serum samples was diluted with 9.00 mL of a pH 7.5 Tris 0.1 M buffer, and the response potentials of the sensors in the array were measured at 25 °C. The values obtained were used to determine the concentration values of potassium and urea in the blood serum samples with the calibration model.

The concentration values of potassium in the ten-fold diluted serum samples were also determined with the potassium ion-selective electrode, a calibration curve previously obtained, in the concentration range 0.1–1.0 mM of potassium, in a matrix solution constituted by 0.014 mM sodium chloride in 0.1 M (pH = 7.5) Tris buffer.

The Laboratory of Clinical Analysis of the Faculty of Pharmacy of Porto, which kindly provided the samples, performed the determination of urea by spectrophotometry, in a Cobas Mira S analyser.

2.5. Equipment

The electrodes were calibrated at 25.0 ± 0.2 °C. An Orion 90–02 double junction reference electrode with 0.1 M Tris buffer (pH 7.5) solution in the external compartment was used.

The calibrations were carried out with an automatic system controlled by a PC. The values of difference of potential (ddp) were acquired with the AD converter of a Lab Master DMA (Scientific Solutions Inc.) card, through a high-impedance circuit.

A Crison Microbu-2030 microburete, controlled via a RS 232C interface of the computer, was used for addition of the standard solutions.

2.6. Calculations

For the calculation of the regression parameters of the calibration curves for the biosensors, a worksheet of the program Excel from Microsoft was used. For all other calculations, the program "The Unscrambler", version 7.6 from Camo-ASA was used. For the construction and testing of the PCR and PLS2 multivariate calibration models of the array, logarithms of potassium and urea concentration were used. Pre-processing of data involved mean centering. For validation of the multivariate calibration models of the array, both external validation and cross validation were used. Root mean squared error of prediction (RMSEP) was calculated by:

$$\mathrm{RMSEP} = \left(\frac{1}{m} \sum_{i=1}^{m} (y_i - \boldsymbol{y}_i)^2 \right)^{1/2} \quad (1)$$

Table 3
Coefficients of statistical correlation between the responses of the potentiometric sensors in the array

	K^+	NH_4^+	Urea1	Urea2	Urea3	Na^+
K^+	1.0000					
NH_4^+	0.6318	1.0000				
Urea1	0.3592	0.7893	1.0000			
Urea2	0.4223	0.5922	0.8656	1.0000		
Urea3	0.3906	0.8474	0.9919	0.8224	1.000	
Na^+	0.1263	0.6611	0.4403	0.2388	0.5026	1.0000

where m is the number of samples in the test set, and y_i and $\boldsymbol{y}_i$ are the true and estimated value for sample i in the test set, respectively.

3. Results and discussion

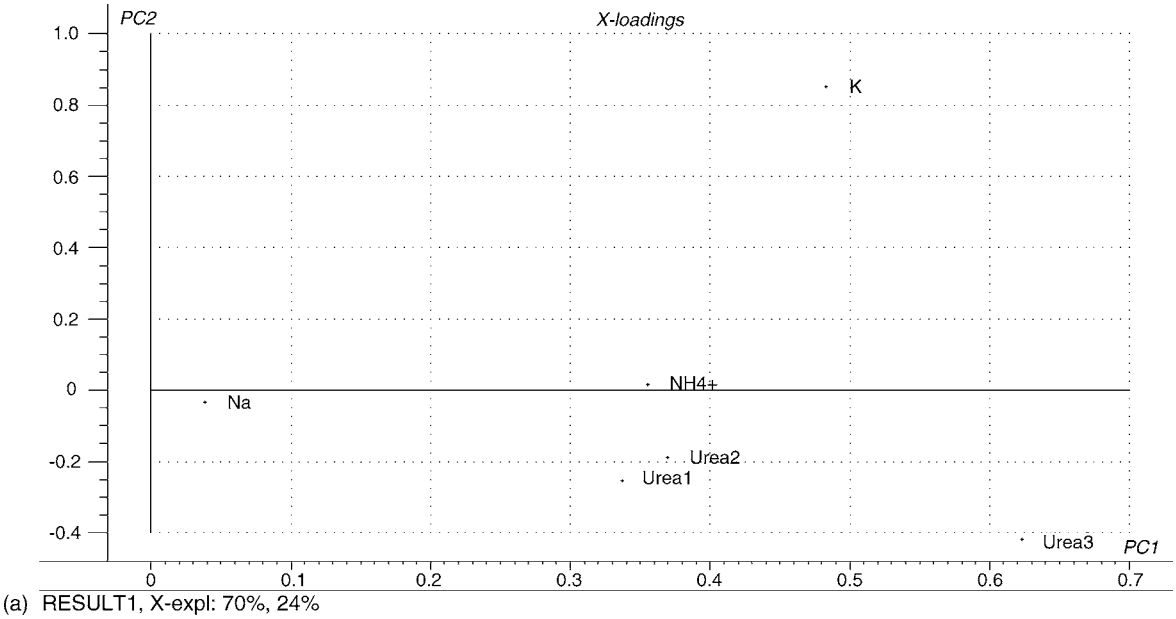
3.1. Response characteristics of the biosensors

Biosensors showed a lower limit of linear response (potential versus logarithm of urea concentration) of 10^{-4} M, and the slope of response to urea varied between 50 and 52 mV decade^{-1}. The coefficient of variation of the response of the biosensors obtained for 0.1 and 6.5 mM urea solutions in five replicate experiments, was 2.4 and 5.6%, respectively. The response time of the biosensors to urea was usually in the range 3–5 min.

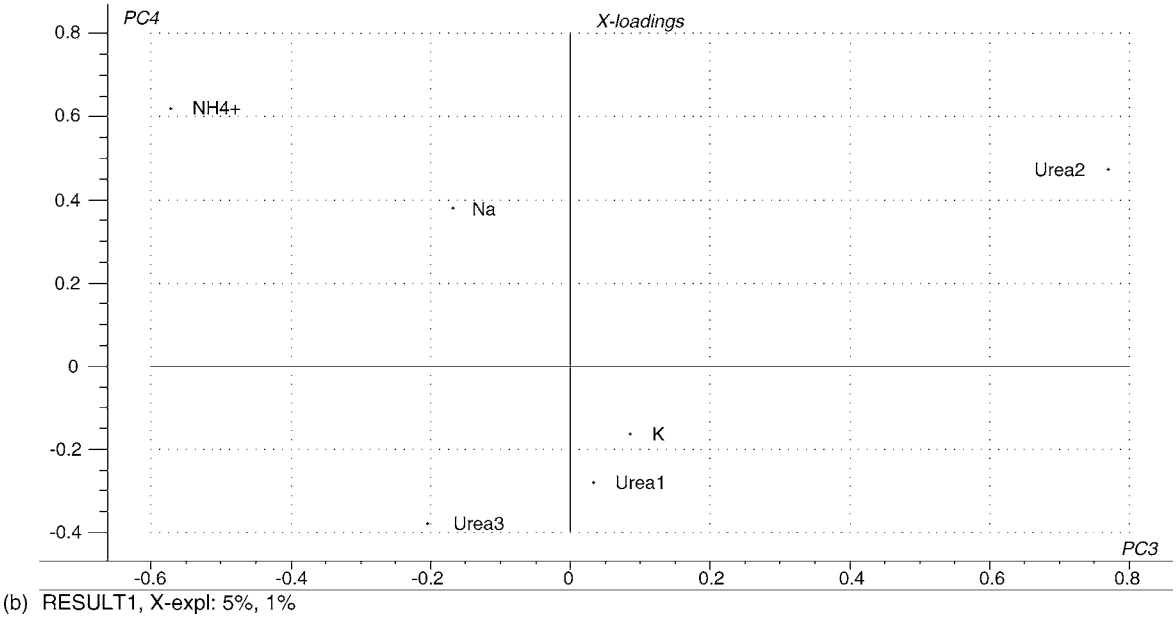
3.2. Multivariate calibration of the array

3.2.1. Data examination

The statistical correlation coefficients between the responses of the six sensors in the array are summarized in Table 3. As expected, they show that the highest values for



(a) RESULT1, X-expl: 70%, 24%



(b) RESULT1, X-expl: 5%, 1%

Fig. 1. X-loadings for PC1 and PC2 (a) and PC3 and PC4 (b) obtained in the PCA in of the response of the array to the calibration set (see composition in Table 2).

the correlation coefficients correspond to the responses of the three biosensors, all with values between 0.82 and 0.99, but these values suggest some variability in their response to urea. Indeed, a comparative analysis of data in Table 3 for the three biosensors shows that the response of urea1 and urea3 biosensors show similar values for the correlation between their response and the ion-selective electrodes in the array, whereas the response of urea2 electrode is less correlated with the response of the ammonium and sodium electrodes. These results show that in this array, biosensors urea1 and urea3 show identical cross-talk with the other electrodes in the array, but urea2 biosensor shows different characteristics of response and less cross-talk with the other sensors. This is probably a consequence of the procedure used for enzyme immobilization which produced some biosensors with a more irregular enzymatic layer (urea2).

PCA of these data show that five principal components (PC) describe 99.9% of the total variance. The three first PC's describe 98.6% of the total variance in the data, and PC4 and PC5 only 1.2 and 0.2%, respectively. Graphical representation of the PC loadings in Fig. 1 shows that PC1 has mainly contributions of the potassium electrode and the urea3 biosensor; followed by the other urea biosensors, the ammonium electrode and the sodium electrode, which shows

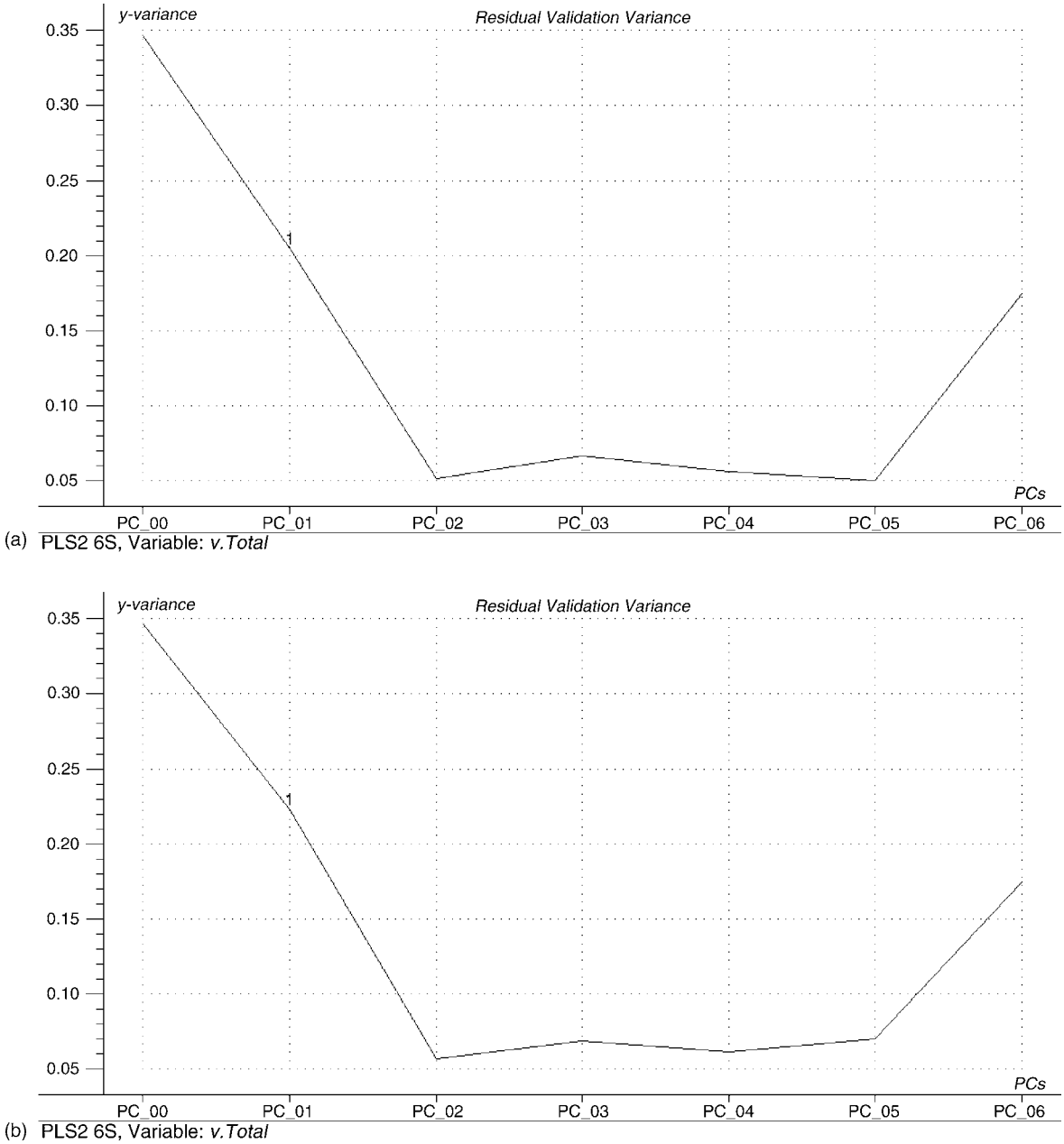


Fig. 2. Residual calibration validation variance vs. the number of factors in the PCR (a) and PLS2 (b) models for the calibration set of nine samples (see composition in Table 2).

a loading close to zero. PC2 is dominated by potassium electrode and urea3 biosensor. The contributions for PC3 and PC4 are mainly from sodium and ammonium electrodes and the biosensors.

3.2.2. Calibration models

The graphical representations of the residual variance versus the number of factors for the calibration set in PCR and PLS2 models in Fig. 2 show that for these models, the most adequate number of factors is 4 and 5, respectively. However, most of the variance in the data is explained with the first three factors in the PCR model (95.0% of the total variance) and the first two factors in the PLS2 model (94.8% of the total variance).

These models were also tested using the external validation set, constituted by samples 10–21 in Table 2. The results summarized in Table 4 show that the lowest RMSEP values for the prediction of the logarithm of concentration of the analytes in the external validation set depend on the analyte considered. Their minima occur for 3 and 4 PC's, respectively, for urea and potassium in both the PCR and PLS2 models. Analysis of Table 4 also shows that the RMSEP for PCR and for PLS2 are of the same magnitude, and that the RMSEP for potassium prediction is lower than for urea in all of the calibration models.

For the construction of the calibration models, the effect of using upto three biosensors in the calculation was also studied. The analysis of data in Table 4 shows that the higher RMSEP in PCR and PLS2 models, for both potassium and urea, are obtained when the response of only a single biosensor is used for calculation. The RMSEP values for potassium prediction obtained in this case, for PLS2 models, are all in the range 0.049–0.067. The values for the RMSEP of urea are larger than those for potassium, specifically 0.258 and 0.284, when the response of urea3 and urea1 is used, and 0.642 if urea2 is used in the calculation. This large difference in the RMSEP obtained with biosensor urea2 is probably related to the lower cross-talk of its response with the ion-selective electrodes in the array (see Section 3.2.1). The results in Table 4 also show that a significant improvement in the RMSEP for potassium and urea is obtained by using two biosensors simultaneously in the calculation. The lowest value of RMSEP for urea is obtained using urea1 and urea3 biosensors (RMSEP of 0.153) and values of RMSEP of 0.234 and 0.181are obtained when urea2 and one of the other biosensors are included in the calculation. Thus, using urea1 and urea3 biosensors in the calculation lowered the RMSEP of urea by approximately 33% comparatively to the results obtained using only one of these biosensors. The RMSEP for potassium in the PLS2 models based on two biosensors are all in the range 0.032–0.048. Finally, data in Table 4 also show that using three biosensors in the calculation of the PLS2 model minimizes the RMSEP for potassium, but a small increase of the RMSEP of urea is obtained comparatively to the model with urea1 and urea3 biosensors, probably due to the poor performance of urea2. However, the product of RMSEP for both analytes is minimum, and for this reason, the response of the three biosensors was included in the PLS2 and PCR calibration models.

Table 4
Results (RMSEP[a]) for the logarithm of concentration of urea and potassium obtained in the external validation of PCR and PLS2 models

Biosensors	RMSEP–PCR			RMSEP–PLS2		
	PC	Log $\|K^+\|$	Log |urea|	PC	Log $\|K^+\|$	Log |urea|
Urea1				5	0.137	0.683
Urea2	4	0.033	0.315	4	0.032	0.412
Urea3	3	0.165	0.180	3	0.198	0.170
	2	0.400	0.225	2	0.359	0.199
	1	0.306	0.499	1	0.331	0.415
Urea1						
Urea3	3	0.048	0.154	3	0.048	0.153
Urea1						
Urea2	4	0.033	0.208	4	0.038	0.234
Urea2						
Urea3	4	0.032	0.196	4	0.032	0.181
Urea1	3	0.050	0.245	3	0.054	0.284
Urea2	3	0.045	0.530	3	0.067	0.642
Urea3	3	0.046	0.235	3	0.049	0.258

[a] RMSEP, root mean square of prediction (see text).

Table 5
Errors of prediction of the concentrations (mM) of urea and potassium in the set of external validation samples with PCR and PLS2 (three factors for urea and four factors for potassium) calibration models of the array with three urea biosensors

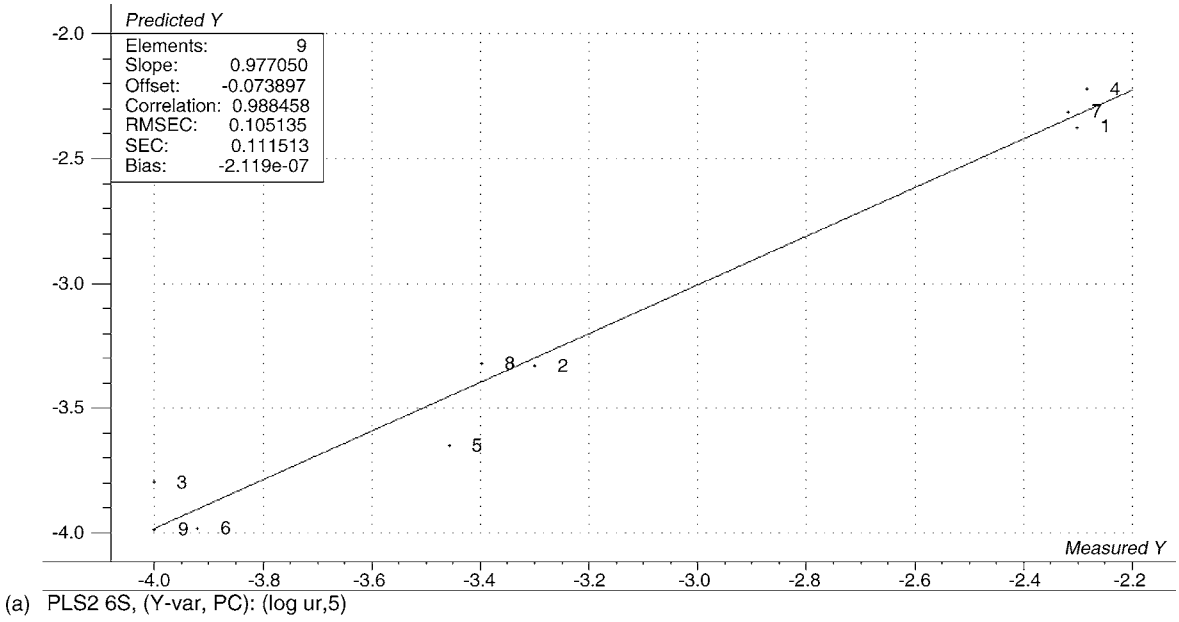
Sample[a]	|Urea|/mM				
	Actual	PCR	Error (%)	PLS2	Error (%)
10	4.50	4.77	6.0	4.73	5.0
11	0.55	0.48	−12.7	0.46	−16.2
12	0.30	0.45	49.4	0.42	40.4
13	0.15	0.42	179.4	0.39	157.2
14	5.00	4.84	−3.3	4.63	−6.3
15	0.40	0.34	−16.2	0.32	−19.2
16	0.45	0.44	−3.1	0.42	−6.4
17	0.10	0.11	6.0	0.10	3.3
18	4.80	7.86	63.8	7.86	63.8
19	0.45	0.36	−19.4	0.35	−21.4
20	0.32	0.24	−24.8	0.24	−25.8
21	0.12	0.20	70.7	0.13	6.0
	$\|K^+\|$/mM				
10	1.00	1.17	17.3	1.17	16.5
11	0.80	0.73	−8.5	0.75	−6.6
12	1.10	1.07	−2.7	1.12	1.6
13	1.50	1.56	4.3	1.60	6.8
14	0.40	0.41	3.4	0.42	4.6
15	0.30	0.30	−1.3	0.30	0.5
16	0.50	0.50	−0.1	0.51	2.2
17	0.45	0.49	9.2	0.50	10.5
18	0.10	0.09	−7.7	0.09	−7.0
19	0.12	0.11	−10.6	0.11	−10.4
20	0.15	0.16	5.3	0.16	3.8
21	0.20	0.20	−2.2	0.20	0.2

[a] See composition of the samples in Table 2.

The graphical representation of the predicted versus the expected values of logarithm of concentration of urea and potassium for the PLS2 model for urea and potassium with three biosensors, in Fig. 3, shows that both give adequate models for the calibration samples. The actual concentration values in the samples and the errors of prediction for the PCR and PLS2 models with the three biosensors are summarized in Table 5, and show that in general, the prediction errors are lower for PLS2 than for PCR. Analysis of the results in this table also show that the prediction errors for samples 14, 15 and 17, which correspond to the normal concentration levels of urea and potassium in blood serum, are lower than 7 and 11%, respectively, for urea and potassium. Furthermore, analysis of data also shows that for samples 10–13, which have the highest levels of potassium (1 mM), the prediction error for urea increases as the concentration of urea diminishes. However, in the presence of 1 mM potassium, the prediction errors in the samples are lower than 17%, provided that the concentration of urea is 0.55 mM or higher.

3.3. Analysis of blood serum samples

The graphical representation of the predicted versus the actual values of urea and potassium obtained in the analysis



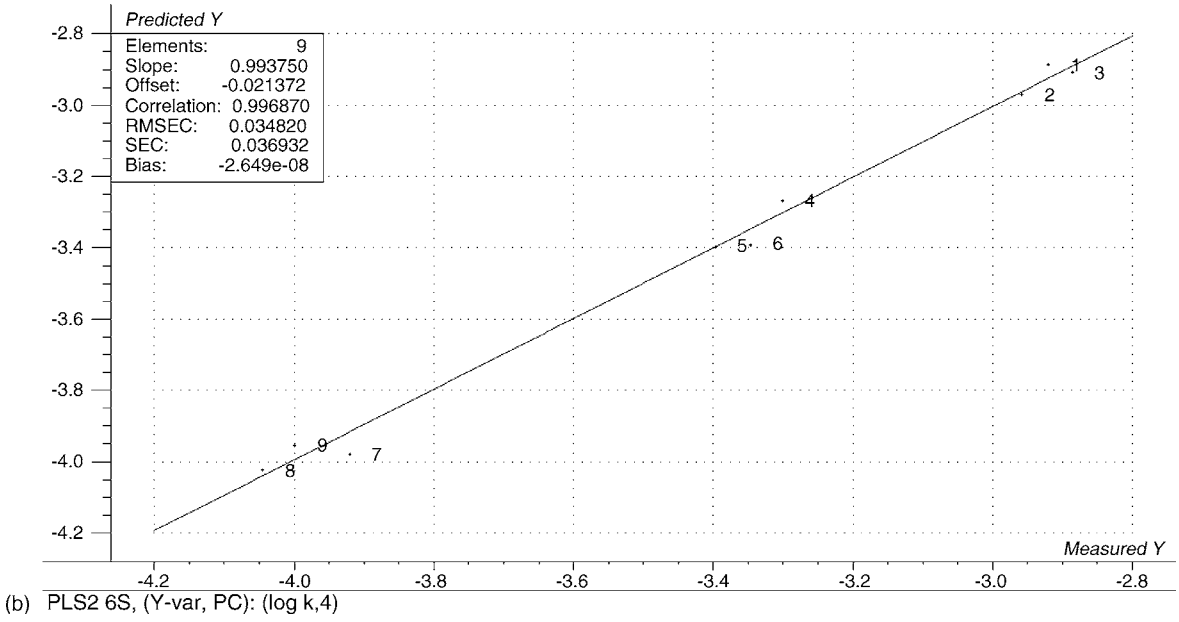


Fig. 3. Predicted values vs. expected values for the calibration set (samples 1–9 in Table 2) by the PLS2 calibration model for the array obtained for urea (a) and potassium (b).

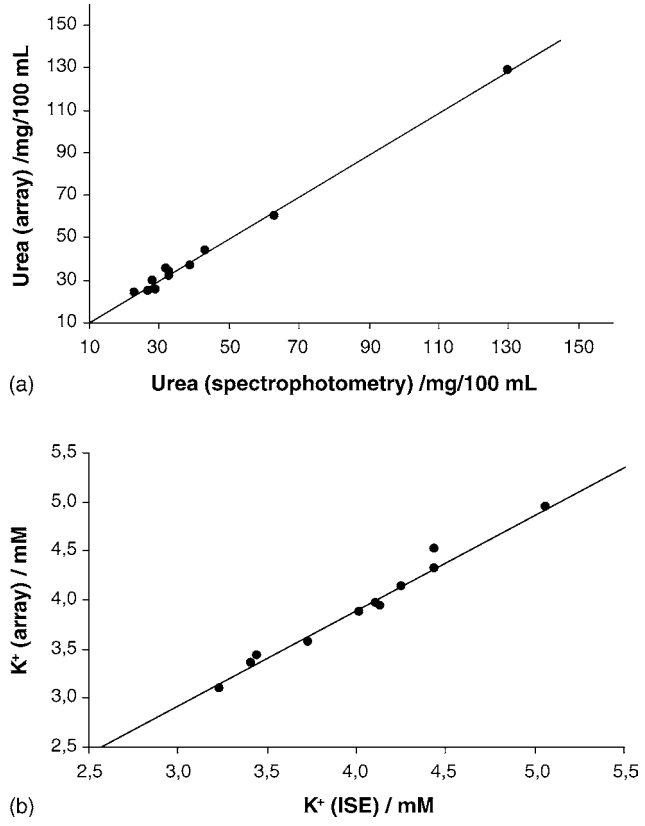


Fig. 4. Graphical representation of the concentration values of urea (a) and potassium (b) in blood serum obtained with the PLS2 calibration model of the array with three biosensors and two factors vs. the values obtained in the Clinical Analysis Laboratory.

of 12 blood serum samples with the PLS2 calibration model of the array is presented in Fig. 4. The prediction errors of the concentration of urea and potassium in the blood serum samples analyzed with this array and a PLS2 calibration model based on nine calibration samples were lower than 10 and 5%, respectively. It should be stressed that this comparative statistical analysis of the urea resulting from the two methods was limited because the values provided by the clinical analysis laboratory had only two significant figures. The value of the correlation coefficient (r) for the least squares linear regression of the urea concentration values determined with the array versus the values provided by the clinical analysis laboratory in this set of samples was 0.998, with a slope of 0.987 with confidence limits (95% level) of 0.94 and 1.04, and an intercept of 0.25 with confidence limits (95% level) of −2.22 and 2.72. The same analysis for the results obtained for potassium in the same samples showed that the value of r was 0.995, the slope was 0.964 with confidence limits of 0.89 and 1.03, and the intercept was 0.04 with confidence limits (95% level) of −0.26 and 0.34. These results suggest that the method of matrix correction based on the PLS2 calibration model of the array produces adequate values for urea and potassium in blood serum samples. This contrasts with the results obtained in previous studies [66] on the determination of creatinine in urine samples with an array of potentiometric sensors and PLS1 calibration models where the results obtained with the array were approximately 10% lower than those based on the Jaffé reaction.

4. Conclusions

Urea biosensors based on urease immobilized by crosslinking with BSA and glutharaldehyde coupled to ammonium ion-selective electrodes were included in arrays together with potassium, sodium and ammonium ion-selective electrodes. The results obtained show that it is desirable to use at least two biosensors in the array to control the quality of response the biosensors and it is possible to obtain a PLS2 calibration model for simultaneous detection of urea and potassium based on a very small set of calibration samples, specifically nine samples. The prediction errors in the concentration of urea and potassium in the blood serum samples analyzed are lower than 10 and 5%, respectively. Moreover, the results show that the use of redundancy of urea biosensors in the array allows for some compensation of poorer response of some of them by PLS2 calculations.

In conclusion, the present work suggests that the coupling of multisensing with redundancy of biosensors and chemometric treatment of multidimensional data allows improvement in the analytical measurement. This result is not unexpected—after all, these features (multiplicity and redundancy of sensors and powerful data treatment) are used by the human body to perform whatever measurements it needs to keep life under control. Therefore, it seems worth trying to implement and improve this style of measurement in analytical chemistry.

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