Calibration of a Potentiometric Multi-Sensor Array for the Determination of Na⁺, K⁺ and NH₄⁺ Ions by Using Artificial Neural Networks

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A potentiometric multi-sensor array, comprising of all-solid-state selective and non-selective electrodes, was constituted and calibrated *via* artificial neural networks (ANNs) for the determinations of NH₄⁺, Na⁺ and K⁺ ions in aqueous model solutions. Various artificial neural network configurations were constituted and their root mean square errors of calibration (RMSEC) and root mean square errors of prediction (RMSEP) values were calculated. The model which has the lowest RMSECxRMSEP value was preferred as the best model. Artificial neural network models calculated for Na⁺ and K⁺ have nearly similar prediction ability to univariate calibrations which were performed using the main ion concentrations of the calibration solutions and respective ion-selective electrode responses. Artificial neural network model calculated for NH₄⁺ had the superior prediction ability when compared with its univariate calibration model.

Key Words: Potentiometry, Multi-sensor array, Artificial neural networks, Multivariate calibration, Non-linear calibration.

INTRODUCTION

Ion-selective electrodes (ISEs) have widespread applications in many research and medical laboratories due to their main advantages such as low expense, fast response, easy use and non-destructive properties. In recent years, there has been intensive interest in the field of all solid contact ion-selective electrodes[1-4] . The solid contact has been prepared by using a mixture of graphite and epoxy resin in a suitable ratio. In this type of electrodes, solid contact provides a suitable adherent surface for the sensing membrane[5,6]. Employment of solid contact as a conductive material between the sensing membrane and metallic conductor gives extra properties to the ISEs such as extended life-time, robustness, miniaturized construction and low expense.

Mathematical relationship between the activity of a measured ion in a solution and the potential, which is measured between the reference electrode and the ISE, is expressed by the Nernst equation (eqn. 1).

$$E = E^\theta + \frac{RT}{zF} \ln a_A \qquad (1)$$

where E is the cell potential, E^θ is temperature-dependent standard cell potential, R is ideal gas constant, F is faraday constant, a_A is activity of primary ion and z is ionic charge of the primary ion. On the other hand, insufficient selectivity of ISEs is one of the major problems in analytical applications.

The existence of the interferents in the measurement media affects the potentiometric response of the electrode. In the presence of the foreign ions, potentiometric response of the electrode can be expressed by Nikolskii equation given in eqn. 2 according to the IUPAC recommendations[7].

$$E = E^\theta + \frac{2.303RT}{z_A F} \log\left[a_A + \Sigma(K_{A,B}^{pot} a_B^{z_A/z_B}\right] \qquad (2)$$

where a_A is primary ion activity, a_B is interferent activity, z_A is primary ion charge, z_B is interferent activity and $K_{(A,B)}^{pot}$ is selectivity coefficient. There are various calculation methods to determine selectivity coefficients[8]. One of the problems is that $K_{(A,B)}^{pot}$ was originally introduced with the empirical Nikolskii equation, which is now known to be incorrect when two ions of different charges significantly contribute to the potential[9] because, the size of the selectivity coefficients changes depending on the ion charges and composition of the solution. For the correct determination of the selectivity coefficients, all the ion charges and ion concentrations present in the measurement media must be known exactly. In recent years, utilization of potentiometric multi-sensor arrays combined with various multivariate linear and non-linear models instead of classical univariate models have been offered and widely used for the more accurate potentiometric determination of the target ions. In the literature, partial-least-squares (PLS)[10-13] and principle component regression (PCR)[14-17] models were

employed as linear multivariate processing tools for the determination of various ionic substances by using potentiometric sensor arrays and satisfactory results were obtained. Although PLS and PCR are linear methods, they can be used for the modeling of some specific non-linear data. If the form of the non-linear relationship between the dependent and explanatory data is known, a model can be linearized by taking the suitable transform of the original data or adding higher order terms to the regression equation. However, success of these approaches in practical application is mainly limited because the exact form of the non-linear relationship is not known a priori and the number of calibration samples available is not sufficient to fit a complex model with large number of higher order terms.

Artificial neural networks (ANNs) are known to be one of the most powerful non-linear data processing techniques. For linear systems, ANNs also perform to the same extent as PCR or PLS[18]. There are many examples of the ANN configurations which have been recently used combined with different potentiometric sensor arrays to relate concentrations of the species to the potentiometric signals[19-23]. Artificial neural networks are employed to relate a set of independent input variables (explanatory variables) with one or more dependent output variables. Fig. 1 illustrates the general structure of an ANN.
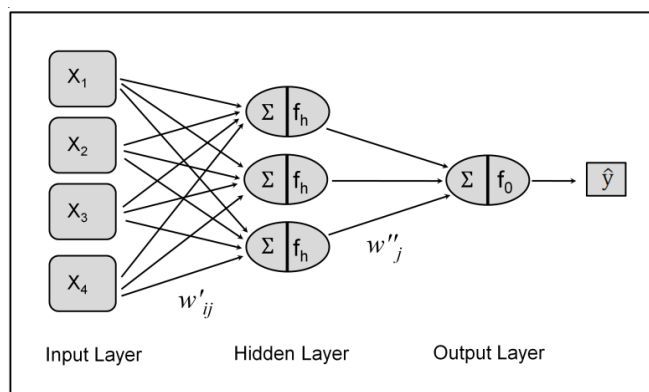

Fig. 1. General structure of an artificial neural network

The nodes are connected together by weights. w'_{ij} is the weights for connecting nodes in the input layer to nodes in the hidden layer and w''_j is the weights for connecting the nodes in the hidden layer to the nodes in the output layer. The nodes in the hidden and output layers each perform two actions. The first is to sum the weighted input signals received from the input layer. The second is to take this sum and project it through a transfer function to produce "activation". The utilized transfer functions can be in different forms such as linear, sigmoid or hyperbolic tangent function[24]. The relationship between the output response $\hat{y}$ (the predicted) and the input variables x_i in an ANN model can be expressed as in the equation given below.

$$\hat{y} = f_o(\beta' + \Sigma_{j=1}^{nh} w''_j \cdot f_h(\Sigma_{i=1}^{nd} w'_{ij} \cdot x_i + \beta)) \qquad (3)$$

The transfer functions for the output and hidden nodes are represented by f_o and f_h, respectively. β' and β are biases and are calculated during the training processes of the network. The weights and the biases are designated random values on the first iteration and thus are adjustable. Each iteration involves

2 steps. At the first step, estimations of the dependent variable are made by using the training set and then compared with the true value. At the second step, the errors between the estimated and the true values are used to regulate the weights. The iterations are repeated until the errors have reached a predefined low level.

NH_4^+-selective electrodes available in the literature generally has low selectivity, especially towards K^+ (selectivity coefficients for K^+ are generally around 10 folds). In present study, we aimed to determine NH_4^+ more correctly together with Na^+ and K^+ in aqueous model solutions by using various ANN models. Therefore, a potentiometric sensor array comprising 5 electrodes was constituted. Three-level full factorial design with three-factor was used for the experimental design of the calibration set. Prediction powers of the constituted ANN models were evaluated comparing the product of their root mean square errors of calibration (RMSEC) and root mean square errors of prediction (RMSEP) values.

EXPERIMENTAL

The following reagents were used for the preparation of the sensing membranes: tetrahydrofuran (THF), high molecular weight poly(vinyl chloride) (PVC), potassium *tetrakis* (*para*-chlorophenyl)borate (KTpClPB), graphite and ionophores (Na^+ ionophore X, K^+ ionophore I, NH_4^+ ionophore I, monensin and dibenzo-18-crown-6) were purchased from Fluka (Bucks, Switzerland). 2-Nitrophenyloctylether (NPOE), dioctyl sebacate (DOS) and dibutyl phthalate (DBP) were used as plasticizers and all were obtained from Fluka.

Epoxy (Macroplast Su 2227) and hardener (Desmodur RFE), used in the preparation of conductive solid contact, were purchased from Henkel (Istanbul, Turkey) and Bayer AG (Darmstadt, Germany), respectively.

For the preparation of all the aqueous solutions throughout the study, distilled deionized water with resistivity of 18.2 MΩ was used. All cation solutions were prepared from the analytical grade nitrate salts of the relevant cations.

Potentiometric measurements were recorded by using a laboratory-made computer-controlled high-input impedance multi-channel potentiometric system. The system had a home-made software program. Throughout the measurements, double-junction saturated calomel electrode (Gamry) was used as reference electrode. For the calculations of the ANN model parameters, SPSS Statistics 17.0 (SPSS Inc., USA) software was used.

Construction of the ISE array: Sensor array used in the present study was comprised of 5 electrodes based on all-solid state technology. Three of them were selective for Na^+, K^+ and NH_4^+. Two of them were non-specific electrodes. All of the electrodes were prepared as described earlier[6]. A mixture of conductive material consisting of 50 % (w/w) graphite, 35 % (w/w) epoxy and 15 % (w/w) hardener was prepared by mixing in sufficient THF. Then, the mixture was allowed to stand for 10-20 min in air. When the appropriate viscosity was attained, a shielded copper wire was dipped into this mixture a few times to obtain a uniform solid contact with coating thickness of *ca.* 0.2 mm and then allowed to stand overnight in an oven at 40 °C. The membrane cocktails given in Table-1 were coated

TABLE-1
MEMBRAN COMPOSITIONS OF THE POTENTIOMETRIC PVC-MEMBRANE ELECTRODES USED IN THIS STUDY

Electrode	Ionophore (w/w)	Ionic additive (w/w)	Plasticizers (w/w)	PVC (w/w)
Sodium	1.5 % Na^+ Ionophore X	–	60.0 % DOS	38.5 %
Potassium	2.0 % K^+ Ionophore I	0.5 % KTpClPB	64.70 % DOS	32.8 %
Ammonium	4.0 % NH_4^+ Ionophore I	1.0 % KTpClPB	67% NPOE	28.0 %
Generic 1	4.0 % Dibenzo-18-Crown-6	–	67.0 % DOS	29.0 %
Generic 2	3.0 % Monensin	–	70.0 % DBS	27.0 %

on the surface of the conductive material by dipping 4-5 times. The electrodes were left to be dried overnight at laboratory conditions. Finally, the dried PVC membrane electrodes were conditioned by soaking into their 0.1 M main ion solutions for at least 6 hours before use.

All the electrodes were inserted into the holes which were opened circularly onto the inert polymer block. The reference electrode was also inserted into the hole opened in the centre of the polymer block and thus, the ISE array was constructed. The schematic representation of the multi-sensor array and the measurement system used in the present study were given in Fig. 2.
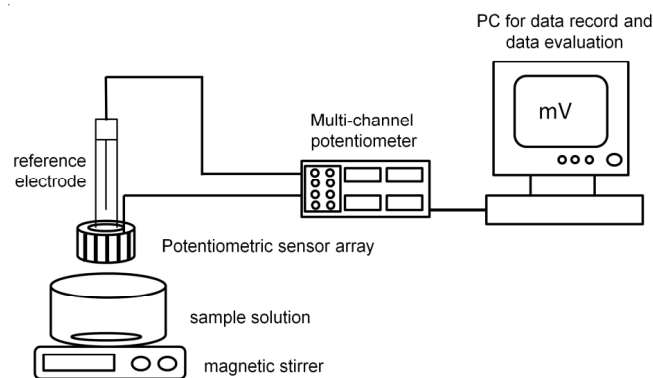

Fig. 2. Schematic representation of the utilized measurement system

Preparation of the solutions: Full factorial designs measure response variables using every combination of the factor levels. Therefore, the concentration levels of the calibration samples were adjusted according to three-level full-factorial design with three-factors ($3^3 = 27$). The concentrations of the studied ions in the calibration set were changed in the range of 10^{-2}-10^{-4} M. Thirteen external test solutions were also prepared for the validation of the calibrations. Ion compositions of the test solutions were generated randomly in the ion concentrations range of the calibration set. Five of them had the same ion composition and were prepared in the same way to test whether the solution preparation processes were a significant source of errors. Distributions of the ion contents of the calibration and test set solutions were indicated as a three-dimensional plot in Fig. 3. 10 mM *tris*-HCl at pH = 7.0 were used as background in the preparation of all calibration and test set solutions.

Procedure: The general measurement procedure was as follows: The multi-sensor array was dipped into the solution and the sensor potentials were recorded when the each electrode potential was reached a stable potential. Next, the sensor array was removed from the solution, washed with deionized water and dried with smooth adsorbent paper. Then, the whole samples were assayed.
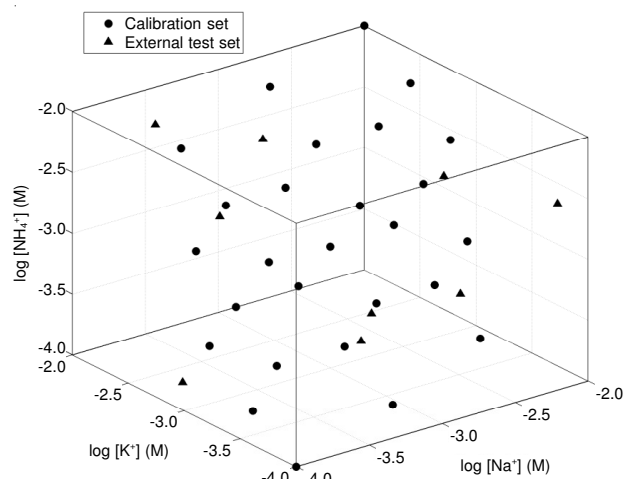

Fig. 3. Demonstration of the ion concentrations of the calibration and test set solutions in three-dimensional space.

RESULTS AND DISCUSSION

According to Vlasov *et al.*[25] applying sensor arrays with low selectivity can results in multicomponent analysis, which can be superior to that performed by using selective sensors. The non-specific sensors of the array have to present high stability and cross-sensitivity to different species. The cross-sensitivity response can be caused by significant differences in sensing mechanism, non-linearity of sensor responses, multiple ion interactions or ionic strength effects[25]. From this point of view, we include two non-specific sensors in the present sensor array, one is based on dibenzo-18-crown-6 and the other is based on monensin. The potentiometric responses of the mentioned generic electrodes to various cations depending on the concentration are illustrated in Figs. 4 and 5, respectively.
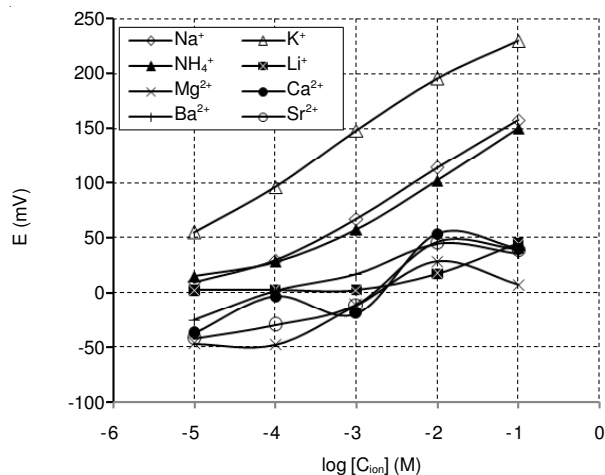

Fig. 4. Potentiometric response characteristics of the generic electrode based on dibenzo-18-crown-6
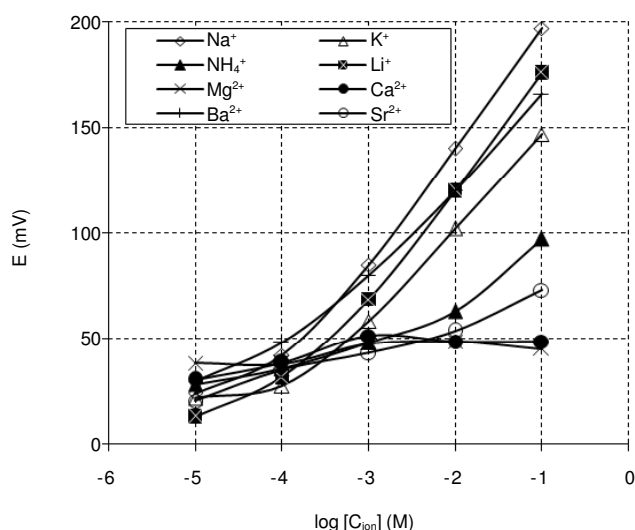
Fig. 5. Potentiometric response characteristics of the generic electrode based on monensin

After the sensor array was constituted, potential values *versus* calibration and test set solutions were gathered from the sensor array and recorded. Various ANN models were constituted by using the ion concentrations and related potential values and model parameters were calculated. In the constitution of all ANN models, standardized electrode responses were used as input values of the network. All the ANN models included single hidden layer. Hyperbolic tangent function was employed as hidden layer activation function. In output layer, identity activation function was used. For the training of the networks, batch training was preferred. Optimization algorithm was scaled conjugate gradient. In the training options, initial λ and initial σ values were chosen as 0.0000005 and 0.00005, respectively. While, in some cases, logarithms of the concentrations were used as output. In the other cases, concentration values were used directly. In some models, species were used alone as output. For the determination of the models which have the best prediction power, RMSEC and RMSEP were calculated. RMSEC values were calculated according to the eqns. 4 and 5 as given below[16]:

$$RMSEC = \sqrt{\frac{1}{m}\Sigma_{i=1}^{m}(\hat{h}_{i,j})^2} \qquad (4)$$

$$\hat{h}_{i,j} = y_{ij} - \hat{y}_{i,j} \qquad (5)$$

where $\hat{h}_{i,j}$ are the residuals of the model, y_{ij} and $\hat{y}_{i,j}$ are the true and predicted values of y for the ith row and the jth column of the calibration set, respectively. $\hat{y}_{i,j}$ is predicted by means of the model which is set by using the m calibration samples.

RMSEP values were also calculated similarly to RMSEC according to the eqns. 6 and 7 as given below[16]:

$$RMSEP = \sqrt{\frac{1}{n}\Sigma_{i=1}^{n}(\hat{g}_{i,j})^2} \qquad (6)$$

$$\hat{g}_{i,j} = y_{ij} - \hat{y}_{i,j} \qquad (7)$$

where n is the number of samples in the test set, $\hat{g}_{i,j}$ are the residuals of the test samples, y_{ij} and $\hat{y}_{i,j}$ are the correct and predicted values of y for the ith row and the jth column of the test set, respectively. $\hat{y}_{i,j}$ is predicted by means of the model which is set by using the m calibration samples.

To evaluate the model's performance, RMSEC and RMSEP values were employed. The model which has the smallest value for the determined species is agreed to be the best model. The created models and related RMSEC, RMSEP and RMSECxRMSEP values are given in Table-2. Some of the lines in the table are left blank because the logarithm values of the predicted concentrations are undefined and cannot be calculated.

The most useful model for the Na+ determination is the model in which log [Na+] values are used as output (RMSECxRMSEP = 0.001110). For the test set solutions; the known Na+ concentrations, predicted Na+ concentrations which are calculated by means of the mentioned model and related relative error percentages are given in Table-3.

Similar to the Na+ models, the best model for the K+ determination is the model in which log [K+] values are used as output (RMSECxRMSEP = 0.002159). The known K+ concentrations, predicted K+ concentrations and related relative error percentages were given in Table-3.

The best model for the NH4+ determination is the model in which log [NH4+] values are used as output (RMSECxRMSEP = 0.003776). For the test set solutions; the known NH4+ concentrations, predicted NH4+ concentrations and related relative error percentages were given in Table-3.

The interested ions were also predicted by using classical univariate regression to show the prediction efficiency of the ANN models. For Na+, K+ and NH4+ ions; the known concentrations, the predicted concentrations and related relative error percentages were given in Table-4. The RMSEP values for

TABLE-2
PREDICTION ABILITY OF THE VARIOUS ARTIFICIAL NEURAL NETWORKS CONFIGURATIONS

Predicted species	Output	RMSEC	RMSEP	RMSECxRMSEP
Na+	[Na+], [K+], [NH4+]	–	–	–
	[Na+]	0.1318	0.1376	0.018136
	log[Na+], log[K+], log[NH4+]	0.0422	0.0448	0.001890
	log[Na+]	0.0434	0.0256	0.001110
K+	[Na+], [K+], [NH4+]	–	–	–
	[K+]	–	–	–
	log[Na+], log[K+], log[NH4+]	0.0444	0.0537	0.002385
	log[K+]	0.0630	0.0342	0.002159
NH4+	[Na+], [K+], [NH4+]	–	–	–
	[NH4+]	0.2451	0.1553	0.038072
	log[Na+], log[K+], log[NH4+]	0.0710	0.0610	0.004333
	log[NH4+]	0.0828	0.0456	0.003776

TABLE-3
PREDICTED ION CONCENTRATIONS AND RELATED PER CENT RELATIVE
ERRORS CALCULATED BY USING MULTIVARIATE CALIBRATIONS

Solution	Ion concentrations (mM) and per cent relative errors								
	$[Na^+]_{real}$	$[Na^+]_{pred}$	E (%)	$[K^+]_{real}$	$[K^+]_{pred}$	E (%)	$[NH_4^+]_{real}$	$[NH_4^+]_{pred}$	E (%)
1-a	0.25	0.23	-8.3	6.00	5.85	-2.4	7.00	6.00	-14.3
1-b	0.25	0.24	-4.5	6.00	6.02	0.3	7.00	6.73	-3.9
1-c	0.25	0.26	4.4	6.00	5.93	-1.2	7.00	5.74	-18.0
1-d	0.25	0.24	-4.7	6.00	5.77	-3.8	7.00	6.52	-6.9
1-e	0.25	0.26	3.6	6.00	5.81	-3.2	7.00	6.66	-4.9
2	0.11	0.12	9.6	1.20	1.50	25.0	0.15	0.15	-0.7
3	0.43	0.42	-1.9	0.18	0.16	-6.4	0.50	0.46	-8.6
4	1.00	1.08	8.3	4.00	4.29	7.2	4.00	4.46	11.5
5	1.70	1.76	3.8	0.85	0.88	3.0	0.25	0.25	1.9
6	0.60	0.66	10.7	5.00	5.27	5.4	1.00	1.17	16.6
7	8.50	8.90	4.7	0.15	0.16	9.0	2.50	2.65	6.2
8	3.50	3.58	2.4	0.35	0.32	-8.1	0.43	0.37	-14.1
9	6.00	5.84	-2.6	1.00	0.98	-2.1	2.00	1.95	-2.4

TABLE-4
PREDICTED ION CONCENTRATIONS AND RELATED PER CENT RELATIVE
ERRORS CALCULATED BY USING UNIVARIATE CALIBRATIONS

Solution	Ion concentrations (mM) and per cent relative errors								
	$[Na^+]_{real}$	$[Na^+]_{pred}$	E (%)	$[K^+]_{real}$	$[K^+]_{pred}$	E (%)	$[NH_4^+]_{real}$	$[NH_4^+]_{pred}$	E (%)
1-a	0.25	0.25	-1.9	6.00	5.59	-6.8	7.00	6.40	-8.6
1-b	0.25	0.26	2.3	6.00	5.59	-6.8	7.00	6.75	-3.6
1-c	0.25	0.28	11.3	6.00	5.84	-2.7	7.00	6.07	-13.3
1-d	0.25	0.26	2.3	6.00	4.92	-18.0	7.00	6.07	-13.3
1-e	0.25	0.28	11.3	6.00	5.14	-14.4	7.00	6.40	-8.6
2	0.11	0.12	8.1	1.20	1.06	-11.5	0.15	0.16	9.1
3	0.43	0.46	8.4	0.18	0.15	-14.5	0.50	0.28	-44.3
4	1.00	1.02	2.4	4.00	3.50	-12.5	4.00	3.57	-10.8
5	1.70	1.69	-0.3	0.85	0.66	-21.8	0.25	0.19	-23.2
6	0.60	0.67	12.1	5.00	4.15	-17.0	1.00	1.17	16.9
7	8.50	8.37	-1.6	0.15	0.16	4.1	2.50	1.79	-28.5
8	3.50	3.46	-1.1	0.35	0.30	-15.5	0.43	0.24	-44.1
9	6.00	5.73	-4.5	1.00	0.82	-17.8	2.00	1.45	-27.7

the Na^+, K^+ and NH_4^+ ions were calculated as 0.0277, 0.0654 and 0.1248, respectively. Artificial neural network models constructed for Na^+, K^+ and NH_4^+ have better prediction ability when compared to the univariate calibrations, especially for NH_4^+. NH_4^+-selective electrode is one of the ISEs with the lowest selectivity among the other electrodes used in the array. Therefore interferents present in the measurement media change the linear response of the electrode towards NH_4^+ ions and the univariate calibration is not an effective way for correct predictions. However, ANN is a more useful calibration tool in the case of multicomponent analysis.

Gallardo et al.[23] utilized a multisensory array to determine Na^+, K^+ and NH_4^+ ions simultaneously by using ANN for the data analysis. Average relative error percentages for Na^+ and K^+ ions were calculated as 83 and 32 %, respectively. There was no information about the average percent relative error of NH_4^+ ion determination, but better prediction was obtained according to the Na^+ and K^+ since the RMSEP value is smaller than Na^+ and K^+. In another study, Mimendia et al.[26] used a sensor array for simultaneous determination of four ions (K^+, NH_4^+, NO_3^- and Cl^-). ANN was used in order to model the sensors' response. Average percent relative errors for K^+ and NH_4^+ ions were calculated as 17.4 and 38.9 %, respectively. In our present study, average percent relative errors for Na^+, K^+ and NH_4^+ were calculated as 5.3, 5.9 and 8.5 %, respectively. These results show that the offered ANN models can be used for the more correct prediction of the mentioned ions.

Conclusion

In the present study, a potentiometric multi-sensor array for the determination of Na^+, K^+ and NH_4^+ ions were constructed and calibrated by using classical univariate and nonlinear multivariate calibration method (ANN). The prediction abilities of the models were compared to each other. It was found that ANN calibration models had better prediction ability in the sensor array applications when compared with the classical univariate calibration models. The average per cent relative errors for Na^+, K^+ and NH_4^+ were calculated as 5.2, 13.0 and 19.3 %, respectively by using ANN models.

REFERENCES

1. J. Gallardo, S. Alegret, M.A.D. Roman, R. Munoz, P.R. Hernandez, L. Leija and M. del Valle, *Anal. Lett.*, **36**, 2893 (2003).
2. M.J. Gismera, S. Arias, M.T. Sevilla and J.R. Procopio, *Electroanalysis*, **21**, 979 (2009).
3. M. Gutierrez, S. Alegret, R. Caceres, J. Casadesus, O. Marfa and M. del Valle, *J. Agric. Food Chem.*, **56**, 1810 (2008).
4. M.K. Amini, M. Ghaedi, A. Rafi, M.H. Habibi and M.M. Zohory, *Sensors*, **3**, 509 (2003).
5. I. Isildak, M. Yolcu, O. Isildak, N. Demirel, G. Topal and H. Hosgoren, *Microchim. Acta*, **144**, 177 (2004).
6. F. Coldur, M. Andac, I. Isildak and T. Saka, *J. Electroanal. Chem.*, **626**, 30 (2009).
7. R.P. Buck and E. Lindner, *Pure Appl. Chem.*, **66**, 2527 (1994).
8. Y. Umezawa, P. Bühlmann, K. Umezawa, K. Tohda and S. Amemiya, *Pure Appl. Chem.*, **72**, 1851 (2000).
9. E. Bakker, E. Pretsch and P. Buhlmann, *Anal. Chem.*, **72**, 1127 (2000).
10. M. Gutierrez, S. Alegret and M. del Valle, *Biosens. Bioelectr.*, **22**, 2171 (2007).
11. P. Ciosek, I. Grabowska, Z. Brzozka and W. Wroblewski, *Microchim. Acta*, **163**, 139 (2008).
12. J.M.C.S. Magalhaes and A.A.S.C. Machado, *Analyst*, **127**, 1069 (2002).
13. A. Rudnitskaya, A. Legin, B. Sleznev, D. Kirsanov and Y. Vlasov, *Microchim. Acta*, **163**, 71 (2008).
14. D.P.A. Correia, J.M.C.S. Magalhaes and A.A.S.C. Machado, *Talanta*, **66**, 773 (2005).
15. N.G. Villar, J. Saurina and S.H. Cassou, *Fresenius J. Anal. Chem.*, **371**, 1001 (2001).
16. M. Baret, P. Fabry, D.L. Massart, C. Menardo and M. Fraysse, *Analusis*, **26**, 267 (1998).
17. M. Baret, D.L. Massart, P. Fabry, C. Menardo and F. Conesa, *Talanta*, **50**, 541 (1999).
18. F. Despagne and D.L. Massart, *Analyst*, **123**, 157 (1998).
19. Y.G. Mourzina, J. Schubert, W. Zander, A. Legin, Y.G. Vlasov, H. Lüth and M.J. Schöning, *Electrochim. Acta*, **47**, 251 (2001).
20. M. Gutierrez, J.M. Gutierrez, S. Alegret, L. Leija, P.R. Hernandez, L. Favari, R. Munoz and M. del Valle, *Int. J. Environ. Anal. Chem.*, **88**, 103 (2008).
21. A.V. Legin, B.L. Seleznev, A.M. Rudnitskaya, Y.G. Vlasov and S.V. Tverdokhlebov, *Czech. J. Phys.*, **49**, 679 (1999).
22. M. Cortina, A. Duran, S. Alegret and M. del Valle, *Anal. Bioanal. Chem.*, **385**, 1186 (2006).
23. J. Gallardo, S. Alegret, R. Munoz, L. Leija, P.R. Hernandez and M. del Valle, *Electroanalysis*, **17**, 348 (2005).
24. A.J. Izenman, In eds.: G. Casella, S. Fienberg and I. Olkin, Modern Multivariate Statistical Techniques, New York: Springer Science + Business Media (2008).
25. Y. Vlasov, A. Legin, A. Rudnitskaya, C. Di Natale and A. D'Amico, *Pure Appl. Chem.*, **77**, 1965 (2005).
26. A. Mimendia, J.M. Gutierrez, L.J. Opalski, P. Ciosek, W. Wroblewski and M. del Valle, *Talanta*, **82**, 931 (2010).