

## Nutrient Solution Monitoring in Greenhouse Cultivation Employing a Potentiometric Electronic Tongue

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This work investigates the use of electronic tongues for monitoring nutrient solution compositions in closed soilless systems. This is a horticultural technique in which the nutrient solution is continuously recirculated and an automatic recomposition system maintains the concentration of the different ions in the optimum range for the plants. Electronic tongues used in this study comprised an array of potentiometric sensors and complex data processing by artificial neural networks. A first experiment was able to carry out the simultaneous inline monitoring of ammonium, potassium, sodium, chloride, and nitrate ions during the winter. In the second and third applications, done during summer, some changes were introduced in the sensor array to improve its response toward chloride ions and to incorporate phosphate in the model. This electronic tongue was validated with real greenhouse samples and was also able to detect the variations in the ion concentrations caused by an incorrect configuration of the recomposition system.

**KEYWORDS:** Electronic tongue; potentiometric sensors; artificial neural networks; horticultural monitoring; fertigation; soilless culture

### INTRODUCTION

The constant evolution of our society is causing an increased demand for information about the surrounding environment. In this context, analytical chemistry plays a special role by progressively supplying more and better analytical information (1). Previously, this information was obtained by discrete analysis using available laboratory methods, for example, ionic and high-performance liquid chromatography, spectroscopy, and photometry (2). Recently, analytical procedures capable of obtaining in situ, continuous, and real-time information have appeared to meet some monitoring needs. Because of the complexity of the matrix involved, the measuring principles of these systems must be sufficiently robust and sensitive and have broad selectivity as well.

One area in which the monitoring of the concentrations of analytes in solution is necessary is protected horticulture (3). This work is focused on a particular horticultural technique known as closed soilless culture (4–6). Basically, in this technique, plants grow in substrates such as rockwool, perlite, and peat, which replace natural soil. Nutrients or other necessary elements are supplied through a feed solution. The solution that

is not used by the plants is captured in a drain tank. After filtration and disinfection, it is mixed with clean water and adjusted for pH and electrical conductivity (EC), which determine the addition of nutrient ions. This solution can then be reused. However, a practical problem with these closed techniques appears, which is the alteration in ionic proportions of the recirculated nutrient solution. Existing protocols to control its concentration are based on closed-loop systems employing EC and pH signals. This type of protocol allows for automatically controlling the EC of the nutrient solution by means of diluting the drainage solution with clean water. However, the total conductivity provides no information on the concentration of individual ions, such as nitrate, potassium, phosphate, ammonium, calcium, magnesium, sulfate, sodium, and chloride. In practice, control over these individual ions is achieved by periodic (for example, weekly) laboratory analysis, followed by readjustment of the dosage of fertilizers. Nevertheless, this can only give approximate control because the uptake of ions varies with the time of the day, stage of plant growth, or solution temperature (5). Another disadvantage, which is particularly acute if the clean water source contains appreciable amounts of nonessential ions, such as sodium and chloride, is that these ions may be supplied at a faster rate than they are taken up by the plants and, consequently, they may accumulate in the nutrient solution. Thus, they make an increasing contribution to the overall EC, and the concentration of the fertilizer ions will

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diminish if the conductivity is maintained at a fixed value. Undoubtedly, continuous and real-time measurement of the nutrient solution composition would constitute a clear improvement to the technique.

An alternative to the classical monitoring of such a large number of elements is the application of electronic tongues (7). This novel approach in analysis is based on an array of nonspecific sensors combined with data processing of their complex signals by use of advanced chemometrical tools. Different electronic tongues have been developed to monitor industrial processes (8) and for environmental control (9). In a previous paper (10), we reported an electronic tongue formed by ion-selective electrodes (ISEs) and an artificial neural network (ANN) response model. This was applied to the simultaneous determination of ammonium, potassium, and nitrate, as well as to the undesired saline compounds sodium and chloride, in real greenhouse samples. In addition, it was applied to an inline application in a soilless culture as described above. The results showed that the chloride ion could only be determined semi-quantitatively due to the interference of the nitrate ion. Furthermore, it seemed necessary to improve the method to correct the distortions caused by the response drifts in the sensor array.

In the present work, we study these two aspects by applying two different electronic tongues to the monitoring of the nutrient solution composition used in this type of fertigation system: one for the simultaneous determination of ammonium, potassium, nitrate, sodium, and chloride and the other for the simultaneous determination of these five elements plus phosphate. The five-ion model was tested during the winter and the six-ion model, during the summer. This was intended to study the behavior of the proposed approach in different conditions of the plants' metabolism. We also studied the behavior of the electronic tongue when the closed system is not capable of controlling the concentration of the individual ions in the nutrient solution. In all of the applications developed, special attention was paid to compensating for any effect of temperature changes on the predicted concentrations.

## MATERIALS AND METHODS

**Reagents, Materials, and Apparatus.** The ion-selective polyvinyl chloride (PVC) membranes were prepared from high molecular weight PVC (Fluka, Buchs, Switzerland), using bis(1-butylpentyl)adipate (BPA), dioctyl sebacate (DOS), 2-nitrophenyloctyl ether (NPOE), dibutyl sebacate (DBS), dioctylphenyl phosphate (DOPP), and dibutyl phthalate (all from Fluka) as plasticizers. The recognition elements employed to formulate the potentiometric membranes were the ionophores nonactin (nonactin from *Streptomyces*, Fluka), valinomycin (potassium ionophore I, Fluka), bis[(12-crown-4)methyl]-2-dodecyl-2-methyl malonate (CMDMM, Dojindo, Kumamoto, Japan), tridodecylamine (TDDA, hydrogen ionophore I, Fluka), and tetronasin [provided by the University of Cambridge (11)]. Material for the charged carriers were hemi-calcium bis[4-(1,1,3,3-tetramethylbutyl)phenyl] phosphate(II) (BBTP, Fluka), tridodecylmethylammonium chloride (TDMAC, Fluka), and tetraoctylammonium nitrate (TOAN, Fluka). In addition, two recognition elements with generic response were used: dibenzo-18-crown-6 (Fluka) for cations and tetraoctylammonium bromide (TOAB, Fluka) for anions. Potassium tetrakis(4-chlorophenyl)borate (Fluka) was used when necessary for a correct potentiometric response. All of the components of the membrane were dissolved in tetrahydrofuran (THF, Fluka).

Silver foil (Ag, Aldrich, Milwaukee, WI) of 99.9% purity and 0.5 mm thick was used to prepare a Ag/AgCl based sensor for chloride. Also, cobalt wire (Goodfellow, Huntingdon, U.K.) of 99.99% purity and 0.5 mm diameter was used to prepare a sensor for phosphate.

**Table 1.** Formulation of the Ion Selective Membranes Employed in the Construction of the Potentiometric Sensor Arrays

sensor	PVC (%)	plasticizer (%)	recognition element (%)	ref
NH <sub>4</sub> <sup>+</sup>	33	BPA (66)	nonactin (1)	13
K <sup>+</sup>	30	DOS (66)	valinomycin (3) <sup>a</sup>	14
Na <sup>+</sup>	22	NPOE (70)	CMDMM (6) <sup>a</sup>	15
H <sup>+</sup> <sub>c</sub>	32.8	DOS (65.6)	TDDA (1) <sup>a</sup>	16
Ca <sup>2+</sup> 1 <sup>c</sup>	33	NPOE (66)	tetronasin (1) <sup>a</sup>	17
Ca <sup>2+</sup> 2 <sup>c</sup>	30	DOPP (65)	BBTP (5)	18
generic 1	29	DOS (67)	dibenzo-18-crown-6 (4)	19
NO <sub>3</sub> <sup>-</sup>	30	DBP (67)	TOAN (3)	20
Cl <sup>-</sup> <sup>b</sup>	30	NPOE (65)	TDMAC (5)	21
generic 2	29	DBP (65)	TOAB (4)	22

<sup>a</sup> The formulation includes potassium tetrakis(4-chlorophenyl)borate as additive.

<sup>b</sup> Sensor used only during the first application: winter. <sup>c</sup> Sensor used only during the second application: summer.

The materials used to prepare the solid electrical contact were Araldite M and Hardener HR epoxy resins (both from Vantico, Barcelona, Spain), and graphite powder (50 μm, BDH Laboratory Supplies, Poole, U.K.) for conducting filler.

All other reagents used for the preparation of the training and testing solutions were of high purity, analytical grade, pro analysis or equivalent.

Real water samples and "inline" applications were conducted in a greenhouse located near Barcelona, in Maresme county (41° 25' N and 2° 23' E), on the central coast of Catalonia (Spain). Two types of water samples were studied: nutrient solution and drainage solution.

Potentiometric measurements were performed with a measurement system developed in the laboratory. Each channel has a conditioning stage using an INA116 (Texas Instruments, Dallas, TX) instrumentation amplifier for adapting the impedance of each sensor. Measurements were differential versus the reference electrode (double junction Ag/AgCl electrode, model 90-02-00, Thermo Electron, Waltham, MA) and grounded with an extra connection in contact with the solution through a stainless steel wire. All channels were noise-shielded with their signal guard, and the outputs of each amplifier were filtered with a second-order active low-pass filter with -3 dB, 2 Hz cutoff frequency, using a UAF42 (Texas Instruments) universal filter. These filtered outputs were connected to an MPC506 (Texas Instruments) 16-channel analog multiplexer. Digitalization was performed by an ADS7804 (Texas Instruments) A/D converter. The complete data acquisition system was controlled using an AT90S8515 (Atmel, San Jose, CA) microcontroller, which also supplied the RS-232-C serial communication. This microcontroller was programmed making use of the ImageCraft Development Tools' interface employing language C. The program's main tasks were the multiplexer control that selects each channel, data acquisition with the analog to digital converter, and transmission/reception of words as well as data control. This instrument system has been recently described for its use in different environmental applications (9). For the construction of the chloride sensor, an Autolab PGSTAT (Eco Chemie, Utrecht, The Netherlands) was used for the AgCl electrodeposition.

**Sensor Arrays.** The sensors used were all solid-state ISEs with a solid contact made from a conductive epoxy composite. This is the usual configuration in our laboratories (12). The PVC membranes were formed by solvent casting the sensor cocktail dissolved in THF. The formulation of the different membranes used is outlined in **Table 1**.

During the winter application, the sensor array used comprised eight sensors: one ISE for ammonium, one for potassium, one for sodium, one for nitrate, and two for chloride; in addition, two generic membrane formulations were used (one for alkaline ions employing the electroactive element dibenzo-18-crown-6 and another for anions employing TOAB). The TDMAC-based chloride sensor was duplicated because of the large interference effect shown by chloride ISE due to nitrate ion. This effect is characteristic when PVC membrane carrier-based sensors are used with samples having high levels of nitrate (19).

The sensor array used during the summer application was an evolution of the previous one. First, the two chloride sensors based on TDMAC were replaced by a Ag/AgCl electrode to improve the response to chloride. This new chloride sensor was formed by AgCl electrodepo-

**Table 2.** Ranges of Variation of the Concentration of the Analytes in the Solutions Used for the Training Process

species	first application (mol L <sup>-1</sup> )	second application (mol L <sup>-1</sup> )
ammonium	$7.7 \times 10^{-4}$ – $6.2 \times 10^{-3}$	$3.0 \times 10^{-5}$ – $1.5 \times 10^{-2}$
potassium	$4.2 \times 10^{-3}$ – $2.0 \times 10^{-2}$	$3.0 \times 10^{-4}$ – $1.5 \times 10^{-2}$
sodium	$2.0 \times 10^{-3}$ – $1.2 \times 10^{-2}$	$5.3 \times 10^{-4}$ – $1.5 \times 10^{-2}$
chloride	$2.0 \times 10^{-3}$ – $1.2 \times 10^{-2}$	$5.3 \times 10^{-4}$ – $1.5 \times 10^{-2}$
nitrate	$5.3 \times 10^{-3}$ – $2.1 \times 10^{-2}$	$1.5 \times 10^{-3}$ – $1.5 \times 10^{-2}$
phosphate	–	$9.0 \times 10^{-5}$ – $1.5 \times 10^{-2}$

sition on a disk of Ag, 5 mm in diameter. To obtain a homogeneous deposition, a 0.1 mA current was passed through the electrolysis cell containing  $10^{-1}$  mol L<sup>-1</sup> NaCl for 1 h. Second, four new sensors were included to complete the array. A 1 cm length and 0.5 mm diameter cobalt wire based phosphate sensor was constructed (23). A TDDA-based ISE for pH was included because the response of the phosphate sensor is strongly dependent on the pH. Finally, two different electrodes for calcium ion, one of each type, were added to correct the effect of this cation on the rest of the sensors. Thus, the array comprised 11 electrodes altogether.

**Training and Measurement Procedure.** To verify the correct functioning of the prepared sensors and how they were affected by the inline experience, they were calibrated to their corresponding primary ion in 25 mL of doubly distilled water by sequential additions of standard solutions before and after the winter application. The generic response sensors were calibrated with potassium and nitrate, respectively. Also, before and after the summer application, the newly incorporated sensors were calibrated: the Ag/AgCl sensor in response to chloride and the cobalt sensor to phosphate.

Before any application, the response of the system had to be assessed employing an ANN model. Measurements for training were done with solutions with a defined background. To compensate for the matrix effect, the background has to be as similar as possible to the real sample. In this way, because of the complexity of the real conditions, we decided to use a 1/2 (v/v) mixture of nutrient solution from the greenhouse/doubly distilled water instead of generating it completely in the laboratory.

Using this background, different mixtures were prepared by additions of stock solutions of the different considered ions according to a statistical experimental design. For the first application (winter), 27 solutions were defined from a fractional factorial design with three levels of concentration and five factors (the five considered ions,  $3^{5-2}$ ). For the second application (summer), 27 solutions were also defined from a fractional factorial design with three levels of concentration and six factors (the six considered ions,  $3^{6-3}$ ). The ranges of variation of the concentration for the analytes in these solutions, which correspond to expected variations, are summarized in **Table 2**.

To correct for possible drifts, the inputs in the neural network were relative measurements of each sensor with respect to a periodically checked reference solution. In fact, this reference solution was checked every 10 samples during the training procedure and once every day during the inline experiments. The composition of this reference solution was  $10^{-4}$  mol L<sup>-1</sup> for ammonium and phosphate and  $10^{-3}$  mol L<sup>-1</sup> for the rest of the considered ions (potassium, sodium, chloride, and nitrate). These are the minimum concentrations present in the nutrient solution to compensate for any hysteresis effect of the electrodes. We also included as input the solution temperature to compensate for any influence on the response of these potentiometric sensors. Therefore, a laboratory-made temperature probe based on an LM35 integrated circuit (National Semiconductor, Santa Clara, CA) was employed together with the array of electrodes.

For the proper verification of the electronic tongue performance, a new set of solutions was used, the test set, which did not participate in the training process. The test set was formed by 10 synthetic solutions prepared in the same way as the training ones, but with concentrations generated randomly inside the training space.

These 37 prepared solutions, 27 for training and 10 for testing, were measured in three turns: one with all of the solutions at room temperature (around 24 °C); another with half of the solutions at lower

temperature (around 10 °C); and the third with the other half at higher temperature (around 35 °C). This experimental sequence was designed with the goal of including temperature effect in the response model. Thus, the ANN model was built using 54 (27 solutions  $\times$  2) points for training and 20 (10 solutions  $\times$  2) points for testing.

**Software.** The ANNs tested were trained and evaluated using the routines available to the Neural Network Toolbox v. 4.0, which are optional add-ons in the Matlab v. 6.1 (Mathworks, Natick, MA) environment. Sensor readings were acquired in the PC by using custom software written in VisualBasic (Microsoft, Seattle, WA).

**Application with Real Samples.** A set of real samples (nutrient solution and drainage water) was collected from a rose recirculating soilless culture described below and measured by the electronic tongue used in the summer application. To obtain a greater variability of the concentration of the analytes, samples were diluted with different amounts of clean water and presented to the system as well. Given that there were not enough data to make a direct comparison between real and predicted concentrations in the inline experiments, this study would serve to validate the proposed electronic tongue in the inline conditions. Therefore, measurements were done in a pipe installed in the greenhouse to imitate the conditions of the monitoring application. Results were compared with those determined by reference methods of widespread use: ammonium was determined by the Nessler reaction; potassium and sodium were quantified by atomic absorption spectroscopy; and chloride, nitrate, and phosphate, were determined by ion-exchange chromatography.

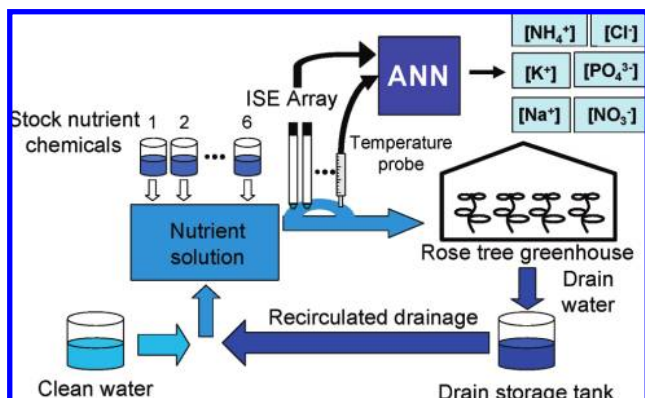
**Inline Application in a Soilless Culture.** The inline measurements were taken in a soilless rose crop (*Rosa indica* L. cv. Lovelly Red), in a 270 m<sup>2</sup> air-conditioned greenhouse at the IRTA site in Cabrils (41° 25' N, 2° 23' E). They were done in two turns: between November and December of 2005 and in July of 2006. The growing medium was expanded medium-grade perlite for half of the greenhouse and coconut fiber for the other half. Irrigation was triggered automatically every time the accumulated radiation reached 200 W m<sup>-2</sup> h, leaving a leaching fraction of around 20% of the applied water. The leachates of both types of substrate were collected together and reused for the production of a new nutrient solution. The recomposition of nutrient solution consisted of the dosification of the volumes of leachate, clean water, and six concentrated solutions (potassium nitrate and ammonium nitrate; potassium sulfate; monopotassium phosphate; magnesium nitrate; microelements and nitric acid). This dosification was controlled by a programmable logic controller (PLC) MCU "Ferti" (Multi Computer Unit; FEMCO, Damazan, France) based on pH (measured by a pH sensor from Broadley James, Irvine, CA), EC, and predefined ratios between the volumes of each concentrated solution. The ratios of injection of the diverse concentrated solution were modified, if necessary, according to laboratory analysis of the leachate performed every 2 weeks (5).

In a similar manner to the monitoring experiment when the system was working correctly, we did a third application during the summer. On some days we changed the ratios of the stock solutions (used by the controller to recompose the nutrient solution) to investigate the response of the electronic tongue when the system is not able to maintain the concentrations at a constant value.

The array of sensors and the temperature probe were installed in the pipe between the tank of recomposed nutrient solution and the irrigation pump. One measurement per sensor was done every minute. The location of the ISE array and the temperature probe inside the system is shown in **Figure 1**.

## RESULTS AND DISCUSSION

**Sensor Performance.** The prepared sensors were evaluated in doubly distilled water to establish if they were suitable for constituting the different arrays. The sensitivities to the primary ion before and after the inline application are summarized in **Table 3**. The results obtained were close to the corresponding theoretical value for monovalent ions (59.1 mV/dec) in the calibrations before the inline monitoring. The sensors with generic response showed the lowest sensitivity due to their



**Figure 1.** Block diagram of the whole system, in-line insertion of the electronic tongue inside the closed soilless strategy. During the winter application, the concentrations of ammonium, potassium, sodium, chloride, and nitrate ions were monitored. During the summer application, we included a sixth ion, phosphate, in the model.

**Table 3.** Sensitivities to the Primary Ion Obtained for Each Sensor before and after Inline Application

sensor	based on	before inline application (mV/dec)	after inline application (mV/dec)
NH <sub>4</sub> <sup>+</sup> <sup>a</sup>	nonactin	56.2	56.4
K <sup>+</sup> <sup>a</sup>	valinomycin	55.4	36.5
Na <sup>+</sup> <sup>a</sup>	CMDMM	59.8	60.2
NO <sub>3</sub> <sup>-a</sup>	TOAN	-53.7	-53.8
Cl <sup>-a</sup>	TDMAC	-53.7	-6.4
generic 1 <sup>a,c</sup>	dibenzo-18-crown-6	50.5	43.2
generic 2 <sup>a,d</sup>	TOAB	-48.2	-53.6
Cl <sup>-b</sup>	Ag/AgCl	-58.9	-67.9
PO <sub>4</sub> <sup>3-b</sup>	cobalt	-53.2	-53.7

<sup>a</sup> Values obtained before and after the winter application. <sup>b</sup> Values obtained before and after the summer application. <sup>c</sup> Response to potassium. <sup>d</sup> Response to nitrate.

nonselective nature. The calibrations after the inline experiment showed that the TDMAC chloride based sensor was most affected, having lost practically all response toward chloride. For this reason, in the summer application these TDMAC sensors were replaced by a Ag/AgCl sensor, the responses of which do not present nitrate interference. Also, the potassium sensor and the sensor based on dibenzo-18-crown-6 lost part of their initial response. This may be caused by the interference of alkaline-earth ions, such as calcium, present in the nutrient solution. In the summer application, we tried to correct this effect by adding two different sensors for calcium.

**Building the ANN Models.** ANNs are multicomponent calibration tools for classification and modeling, which are especially useful for nonlinear systems. Their functioning is inspired by the animal nervous system, and their elementary unit is the perceptron or neuron. The properties that characterize a neural network are the transference function used in the neurons, the network topology, and the learning algorithm used. Among the different ANN structures, the multilayer perceptron is the most used. It can be defined as a feed-forward network with one or more layers of neurons between the input and output neurons. These additional layers contain hidden neurons that are connected to either the inputs or outputs by weighted connections.

Because of the difficulty of predicting an optimum configuration in advance, selecting the topology of an ANN is the first obstacle in the proposed approach. The ANN structure for the best modeling of a sensor array is obtained by a trial and error procedure. This process includes a combination of the

number of neurons of the hidden layer and the transference function used within. These characteristics will define the specific combination leading to the best modeling ability (24) because, according to our previous experience, the remaining characteristics are fixed.

The fixed parameters that were common for the two optimized ANN models were a linear transfer function of the output layer (*purelin*) and a single hidden layer of neurons. These selections were based on previous experience with electronic tongues using potentiometric sensors (25). The learning strategy used was Bayesian regularization and employed a learning rate of 0.1 and a momentum of 0.4 selected from preliminary tests for its internal parameters. The modeling capacity of the ANN was examined in terms of the root mean squared error (RMSE).

When compared with others, the strategy selected for the learning process (Bayesian regularization) provided better RMSE value, greater consistency between the predicted and obtained values for the training, and a higher significance for the external test set. In addition, an internal validation subset of samples was not necessary because it avoids overfitting by other means (26). This strategy searches for the simplest model that best fits the experimental function while predicting points that have not participated in the training process. Considering the nonlinear behavior of the sensors, two different nonlinear transfer functions were considered for the hidden layer, a sigma-shaped function called the *tansig* function (27) and a logistic function represented by the *logsig* function.

For the first application, the ANN model had nine input neurons (eight sensors from the array plus the temperature) and five output neurons (the five modeled ions). After the systematic evaluation of configurations, the best training results were obtained with the *logsig* function and seven neurons in the hidden layer. These conditions provided a RMSE value of  $6.46 \times 10^{-3} \text{ mol L}^{-1}$  for the external test set. For the second application, the ANN model had 12 input neurons (11 sensors from the array plus the temperature) and 6 output neurons (the 6 modeled ions). The best training results were obtained with the *logsig* function and seven neurons in the hidden layer, which provided a RMSE of  $8.01 \times 10^{-3} \text{ mol L}^{-1}$  for the test set. Graphs comparing obtained versus expected concentrations for the test set (those solutions that did not intervene in the training process) were produced to check the behavior of the optimized models. **Table 4** shows the parameters of the linear regression of obtained versus expected concentration values for the test set solutions in the two different applications. The accuracy of the models approached ideality, with unity slopes and zero intercepts (all confidence intervals were calculated at the 95% confidence level). Although the confidence interval for chloride includes the unity slope and zero intercept in the first application, the regression coefficient is not significant. This unsatisfactory behavior was corrected in the second application, where chloride sensors based on charged ion carriers were substituted for one sensor based on Ag/AgCl. Furthermore, good correlation parameters were obtained for phosphate using the cobalt-based sensor coupled with the pH sensor. For the rest of the analytes (ammonium, potassium, sodium, and nitrate), the results obtained were comparable and satisfactory in the two applications.

**Application with Real Samples.** Once the applicability of this system was demonstrated, the proposed method was applied to real samples of an existing fertigation experiment. Two groups of samples were studied: nutrient solution and drainage or leachate solution. In addition, these were diluted with clean water by ratios of 1:1, 2:1, and 3:1 and processed in the same way to enlarge the number of available real samples and their

**Table 4.** Linear Regressions of the Comparison Graphs Obtained for the Test Set Solutions in the Two Different Applications Using the Optimized ANN Models

species	first application	second application
NH <sub>4</sub> <sup>+</sup>	$y = (1.11 \pm 0.40)x + (0 \pm 1 \times 10^{-3}), r = 0.806$	$y = (1.10 \pm 0.40)x + (0 \pm 3 \times 10^{-3}), r = 0.835$
K <sup>+</sup>	$y = (1.08 \pm 0.47)x + (3 \times 10^{-3} \pm 4 \times 10^{-3}), r = 0.751$	$y = (0.97 \pm 0.45)x + (1 \times 10^{-3} \pm 3 \times 10^{-3}), r = 0.767$
Na <sup>+</sup>	$y = (0.98 \pm 0.36)x + (0 \pm 2 \times 10^{-3}), r = 0.802$	$y = (1.06 \pm 0.28)x - (0 \pm 3 \times 10^{-3}), r = 0.903$
Cl <sup>-</sup>	$y = (0.53 \pm 0.48)x + (3 \times 10^{-3} \pm 3 \times 10^{-3}), r = 0.480$	$y = (1.00 \pm 0.22)x + (2 \times 10^{-3} \pm 2 \times 10^{-3}), r = 0.926$
NO <sub>3</sub> <sup>-</sup>	$y = (1.11 \pm 0.12)x - (0 \pm 1 \times 10^{-3}), r = 0.980$	$y = (0.93 \pm 0.24)x + (1 \times 10^{-3} \pm 2 \times 10^{-3}), r = 0.904$
PO <sub>4</sub> <sup>3-</sup>	-	$y = (1.19 \pm 0.33)x - (1 \times 10^{-3} \pm 3 \times 10^{-3}), r = 0.905$

**Table 5.** Relative Errors Obtained for the Logarithm of the Concentration, Using the Electronic Tongue, in the Determination of the Six Ions in Real Greenhouse Samples during the Summer Application

sample <sup>a</sup>	rel error, NH <sub>4</sub> <sup>+</sup> (%)	rel error, K <sup>+</sup> (%)	rel error, Na <sup>+</sup> (%)	rel error, Cl <sup>-</sup> (%)	rel error, NO <sub>3</sub> <sup>-</sup> (%)	rel error, PO <sub>4</sub> <sup>-</sup> (%)
NS 1	-10.5	10.9	-2.9	-4.0	13.4	59.1
DS 1	-26.6	-2.0	3.8	-4.1	9.9	35.0
NS 2	-15.9	11.4	1.6	12.3	12.1	30.8
NS:CW 1:1	-14.6	11.1	-1.6	-3.5	13.5	20.4
NS:CW 2:1	-13.4	8.5	-8.3	6.9	14.2	25.5
NS 3	26.0	19.0	13.0	12.3	18.1	40.3
NS:CW 3:1	21.4	17.4	12.7	12.0	21.5	29.1
NS:CW 1:1	14.7	17.6	12.5	11.7	25.8	20.9
NS:CW 2:1	13.2	14.7	12.7	11.9	22.1	25.3
NS:CW 3:1	12.5	12.6	12.7	12.0	20.4	28.2
NS:CW 2:1	11.1	12.4	12.7	11.9	22.1	26.5
NS 4	17.6	7.1	13.0	12.3	15.9	49.7
DS 2	-9.1	-1.7	24.4	28.5	11.5	28.4
NS 5	-16.2	-3.0	-3.2	-6.1	14.0	41.7
NS 6	-8.5	23.9	16.0	14.4	20.8	31.5
NS 7	31.5	28.3	16.7	15.4	11.6	41.8
NS:CW 1:1	22.0	30.9	13.2	11.9	22.9	22.6
NS:CW 2:1	29.3	32.1	14.4	13.2	20.9	25.8
NS:CW 3:1	55.3	33.5	15.0	13.7	19.3	25.9
NS 8	49.5	17.6	16.7	15.4	7.3	34.9
DS 3	23.2	12.0	27.2	-3.2	16.4	26.4
DS 4	13.1	7.0	41.3	0.6	13.1	25.9
NS 9	46.8	17.2	-14.5	15.4	-2.9	45.1
NS:CW 3:1	49.1	5.3	-16.0	-7.7	6.6	35.9
NS:CW 2:1	36.4	-13.5	-18.0	-15.2	10.2	29.7
NS:CW 1:1	31.8	-35.4	-19.7	-17.0	11.0	23.6
mean absolute rel error (%)	23.8	15.6	14.0	11.3	15.3	31.9

<sup>a</sup> NS, nutrient solution; DS, drainage solution; CW, clean water.

variability. These samples were treated separately, and the relative errors of the logarithm of the concentration for each sample were calculated as usual. The results are summarized in **Table 5**. The mean absolute relative error for each considered ion is calculated as the mean of the absolute values of errors presented for that ion. This table shows that the relative errors are in specific cases close to 50%, although much lower on average. For example, we obtained a mean relative error of 11.3% for chloride and very close to 15% for potassium, sodium, and nitrate. For ammonium, the average was 23.8%. Worse results were obtained in the determination of phosphate with a mean error of 31.9%, which was probably due to an uncompensated matrix effect (23). The lower concentrations of phosphate normally used in the nutrient solution also made a good performance difficult for this ion. In any case, this fact proves that the cobalt wire electrode, although conceptually simple, is not a good sensor for the direct measurement of phosphate.

**Table 6** summarizes the comparison of the results obtained using the electronic tongue with those obtained using the Nernstian calibration curve of each specific sensor. This table

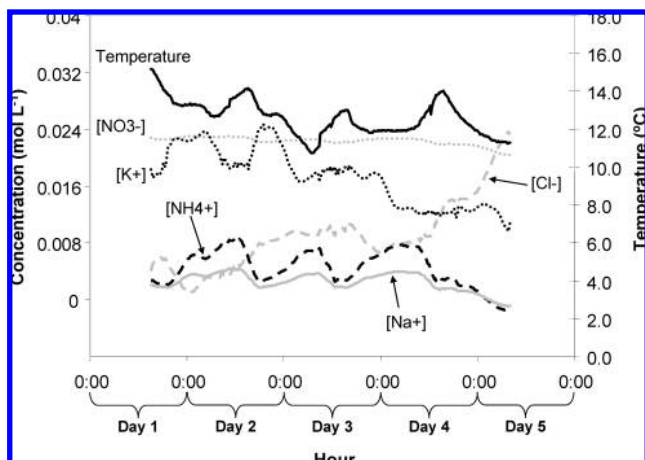
**Table 6.** Relative Errors Obtained for the Logarithm of the Concentration by Direct Interpolation in the Nernstian Model for the Determination of the Six Ions in Real Greenhouse Samples during the Summer Application

sample <sup>a</sup>	rel error, NH <sub>4</sub> <sup>+</sup> (%)	rel error, K <sup>+</sup> (%)	rel error, Na <sup>+</sup> (%)	rel error, Cl <sup>-</sup> (%)	rel error, NO <sub>3</sub> <sup>-</sup> (%)	rel error, PO <sub>4</sub> <sup>-</sup> (%)
NS 1	11.6	29.1	13.9	21.1	22.4	-46.8
DS 1	31.6	36.8	16.2	34.3	30.4	-43.2
NS 2	12.5	28.3	11.2	29.1	34.1	-46.2
NS:CW 1:1	20.0	38.1	12.2	29.4	29.8	-52.5
NS:CW 2:1	15.5	40.2	13.4	27.5	29.3	-51.4
NS 3	15.9	35.0	70.7	26.9	-26.5	-55.0
NS:CW 3:1	19.5	44.2	70.3	26.9	-21.3	-56.5
NS:CW 1:1	24.2	41.5	70.2	31.9	-11.6	-57.9
NS:CW 2:1	20.1	42.1	70.8	32.0	-9.4	-57.0
NS:CW 3:1	18.2	41.2	71.3	31.4	-8.0	-56.5
NS:CW 2:1	19.6	41.2	71.7	31.2	-6.0	-84.3
NS 4	15.5	43.8	72.1	21.9	-14.0	-55.1
DS 2	37.2	34.6	95.2	39.8	-3.0	-54.0
NS 5	34.4	79.1	74.4	17.7	44.0	-50.3
NS 6	40.0	91.4	22.1	18.2	73.5	17.5
NS 7	33.2	73.8	79.7	-54.1	13.1	17.1
NS:CW 1:1	34.2	54.9	81.3	-42.9	6.9	10.6
NS:CW 2:1	37.0	62.2	108.1	-34.3	18.8	13.0
NS:CW 3:1	40.0	65.5	65.7	-22.1	34.0	14.1
NS 8	33.5	74.2	58.8	-17.2	48.6	-39.5
DS 3	42.3	85.2	61.7	-13.8	41.4	-44.3
DS 4	43.5	92.3	61.6	-12.6	50.1	-43.1
NS 9	38.0	74.0	61.5	-21.4	37.3	-33.7
NS:CW 3:1	37.3	65.4	64.7	-22.4	28.1	-39.8
NS:CW 2:1	38.4	62.2	62.7	-21.7	28.9	-38.0
NS:CW 1:1	40.6	54.9	60.0	-20.7	27.2	-34.1
mean absolute rel error (%)	29.0	55.0	58.5	27.0	26.8	42.8

<sup>a</sup> NS, nutrient solution; DS, drainage solution; CW, clean water.

shows that the relative errors have increased for the six ions due to the interferences and the effect of temperature, especially for potassium and sodium.

**First Inline Application in the Greenhouse: Winter.** With the previously optimized ANN, the primary data were turned into analytical information. The concentrations of ammonium, potassium, sodium, chloride, and nitrate in the nutrient solution were continuously monitored for more than 20 days during November and December 2005. Three and a half of these days were analyzed in more detail. **Figure 2** shows the concentrations of the considered cations and anions that were predicted by the electronic tongue during these days. In addition, the figure represents the recorded nutrient solution temperature with the three day-night cycles. The recirculation system was able to maintain the concentration of ammonium, sodium, and nitrate in particular, within a narrow range. Because no daily cyclic variation of concentrations was observed for these three ions, we interpret that the electronic tongue corrected the temperature effect. Although there are times in which the concentrations are out of the training space (**Table 2**), the optimized ANN model has the capacity of extrapolating new concentrations. The ammonium concentration was between 0.002 and 0.008 mol L<sup>-1</sup>, the sodium concentration between 0.002 and 0.004 mol L<sup>-1</sup>,

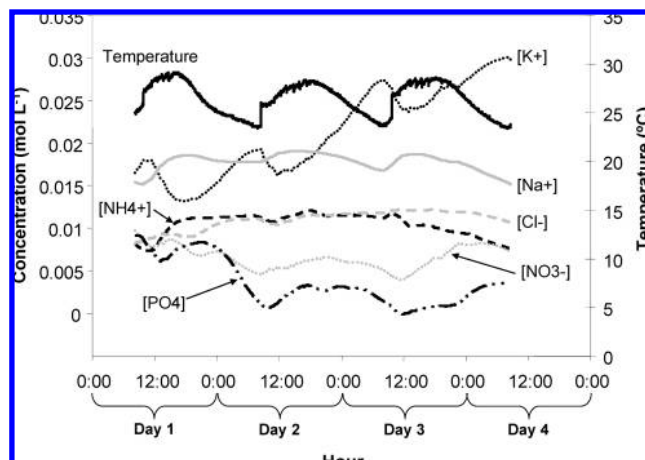


**Figure 2.** Representation of the concentration values predicted by the electronic tongue during the first application, in wintertime, for the considered ions, ammonium, potassium, sodium, chloride, and nitrate, in the nutrient solution during 3.5 days of continuous monitoring.

and the nitrate concentration around  $0.02 \text{ mol L}^{-1}$ . However, the chloride concentration showed a continuous increase up to  $0.02 \text{ mol L}^{-1}$  that can be caused by the degradation of the electrodes for chloride due to the presence of high concentrations of nitrate. On the other hand, the potassium concentration suffered a continuous decrease to  $0.01 \text{ mol L}^{-1}$  at the end of the third day. It is difficult for us to physiologically interpret this decrease in potassium concentration given that the values predicted by the electronic tongue do not directly represent the uptake of the plants, but the imbalance between the ion concentrations added to the nutrient solution and the plant absorption rates of each of them. However, the study could have coincided with the formation of the floral buds, which is the period of maximum absorption of nutrients by the rose tree, especially of potassium (28). In addition to this, a function of the potassium uptake is to protect the plant against low winter temperatures (29).

#### Second Inline Application in the Greenhouse: Summer.

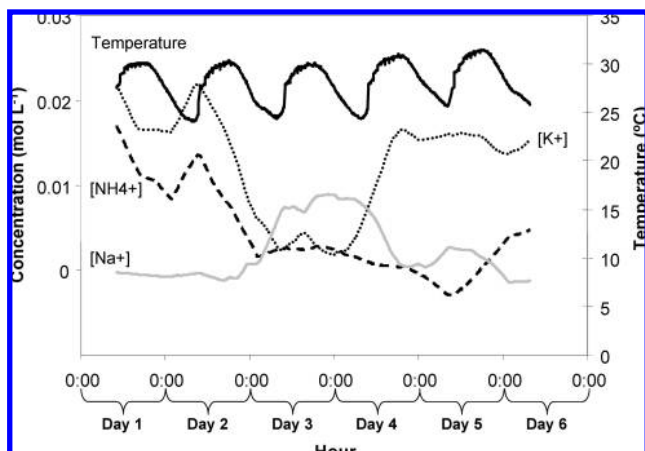
As before, the concentrations of ammonium, potassium, sodium, chloride, nitrate, and phosphate were continuously monitored for 13 days during July 2006. Three of these days were studied in more detail. **Figure 3** shows the concentrations of the cations and anions predicted by the electronic tongue during these days, as well as the temperature of the nutrient solution. In this case, the recirculation system was able to correct the variations of the concentration of ammonium, sodium, chloride, nitrate, and phosphate ions while correcting for the temperature effect as well. In this summer application, the ammonium concentration was around  $0.01 \text{ mol L}^{-1}$ , the sodium concentration between  $0.015$  and  $0.02 \text{ mol L}^{-1}$ , the chloride concentration around  $0.01 \text{ mol L}^{-1}$ , the nitrate concentration between  $0.005$  and  $0.01 \text{ mol L}^{-1}$ , and the phosphate concentration between  $0.001$  and  $0.01 \text{ mol L}^{-1}$ . In this case, the potassium concentration was not in a narrow range and suffered a continuous increase to  $0.03 \text{ mol L}^{-1}$ . This observation is the opposite of the wintertime behavior. During summertime, the autotrophic phase and the constitution of new organic molecules predominate. In summer, the formation of all the photosynthetic system (heterotrophic phase) is brief and happens at the beginning of summer to make better use of the daylight. This is also when potassium consumption is preferred. It is likely that the observed behavior corresponds to a moment when the heterotrophic phase had finished and the dosification system had not been readjusted.



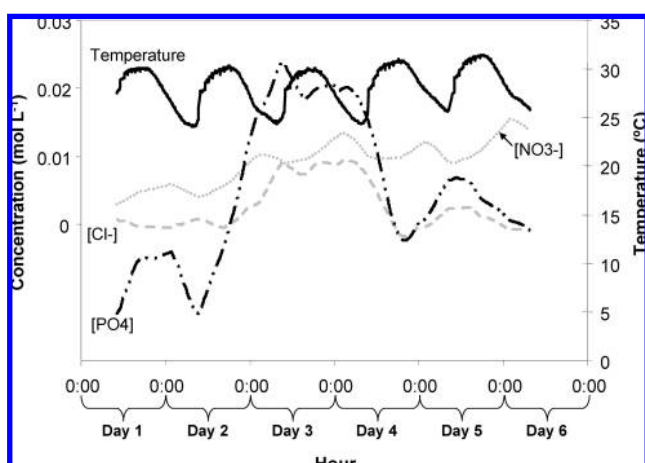
**Figure 3.** Representation of the concentration values predicted by the electronic tongue during the second application, in summertime, for the considered ions, ammonium, potassium, sodium, chloride, nitrate, and phosphate, in the nutrient solution during 3 days of continuous monitoring.

Other differences noted in comparison with the winter application were an increase in the concentrations of the nonessential sodium and chloride ions. This could be caused by the fact that evapotranspiration is higher during the summertime (5). Consequently, the accumulation in the nutrient solution of these ions provided by the drainage solution is also higher. Similarly, a decrease in nitrate concentration is observed. This may be explained by the higher amount of nitrogen that the plants use when diurnal photosynthesis increases (30). Another cause of this decrease can be the fact of maintaining the EC at a fixed value when sodium and chloride are present at high concentration. As we explained in the introductory section, if occurring, it may cause a lesser dosage of fertilizer ions. Similarly to nitrogen, there is a close relationship between plant phosphorus uptake and carbon metabolism. Besides, the concentration of phosphate in the formulation of the nutrient solution is always minority. This fact can explain why phosphate had the lowest concentration of the six considered ions.

**Third Inline Application in the Greenhouse: Incorrect Recomposition of the Nutrient Solution.** An incorrect configuration for the system that controls the recombination of the nutrient solution was established for 5 days to check the ability of the analytical system to detect the anomalies. **Figures 4** and **5** show the concentrations of the cations and anions predicted by the electronic tongue during these 5 days, with the corresponding temperature. The output of the electronic tongue clearly shows a wave of inappropriate composition of the nutrient solution between the second and fourth days of continuous monitoring. More precisely, the anomaly consisted basically of an important decrease of potassium concurrent with an increase in the concentration of the undesired solutes, sodium and chloride. In fact, the autocorrelation matrix constructed with the values of concentration predicted by the electronic tongue for this third application showed that the most significant correlation was between the sodium and chloride ions, with a coefficient value of 0.961. This pattern could be explained as an excessive proportion of exhausted recirculated solution in the reformulated nutrient solution. It is remarkable that this type of anomaly would be "invisible" to the inline measurements used by the dosification equipment, typically based on pH and EC. Furthermore, depending on how frequently the



**Figure 4.** Representation of the concentration values predicted by the electronic tongue during the third application, with incorrect recombination of the nutrient solution, for the considered cations, ammonium, potassium, and sodium, during 5 days of continuous monitoring.



**Figure 5.** Representation of the concentration values predicted by the electronic tongue during the third application, with incorrect recombination of the nutrient solution, for the considered anions, chloride, nitrate, and phosphate, during 5 days of continuous monitoring.

laboratory analysis is performed, its detection could be delayed for up to several weeks.

In conclusion, two electronic tongues were tested in different seasons: winter and summer. They permitted differences to be observed in the calculated concentrations of the ions considered, and we tried to explain these variations using physiological arguments. We also confirmed that the sensor based on Ag/AgCl is a good alternative to the carrier-based sensors for chloride in such a complex matrix. Unfortunately, the cobalt-based sensor for phosphate did not present good results, and we could determine it only semiquantitatively. Finally, in the third inline application, the used electronic tongue was able to detect in real time the anomaly introduced deliberately in the automatic recombination system, demonstrating clearly its utility in control of closed soilless systems.

#### ABBREVIATIONS USED

EC, electrical conductivity; ISE, ion-selective electrode; ANN, artificial neural network; PVC, polyvinyl chloride; BPA, bis(1-butylpentyl)adipate; DOS, dioctyl sebacate; NPOE, 2-nitrophenyloctyl ether; DBS, dibutyl sebacate; DOPP, dioctylphenyl phosphate; CMDMM, bis[(12-crown-4)methyl]-2-dodecyl-2-

methylmalonate; TDDA, tridodecylamine; BBTP, bis(bis(4-1,1,3,3-tetramethylbutyl)phenyl)phosphatocalcium(II); TDMAC, tridodecylmethylammonium chloride; TOAN, tetraoctylammonium nitrate; TOAB, tetraoctylammonium bromide; THF, tetrahydrofuran; RMSE, root mean squared error.

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