DEVELOPMENT OF A PHOTOPOLYMERISABLE MEMBRANE FOR CALCIUM ION SENSORS. APPLICATIONS TO SOIL DRAINAGE WATERS

J. Artigas, A. Beltran, C. Jiménez, J. Bartrolí, J. Alonso

Development of a photopolymerisable membrane for calcium ion sensors
Application to soil drainage waters

J. Artigas [a], A. Beltran [a], C. Jiménez [b,*], J. Bartrolí [a], J. Alonso [a]

[a] *Sensors and Biosensors Group, Chemistry Department, Universitat Autònoma de Barcelona, 08193-Bellaterra, Barcelona, Spain*
[b] *Instituto de Microelectrónica de Barcelona (IMB-CNM) CSIC, Campus U.A.B., 08193 Bellaterra, Barcelona, Spain*

Abstract

The fabrication of ion-sensitive electrochemical sensors for calcium is described in the present work. The membrane uses a photocurable polymer based on aliphatic diacrylated polyurethane. The use of photocurable polymers as the support matrix instead of PVC simplifies the preparation and the casting of the developed ion-sensitive membranes. Additionally, these polymers are compatible with the photolithographic fabrication techniques used in the microelectronics industry. This aspect permits the patterning of membranes on wafer level for semiconductor based sensors thus the automatization of the whole sensor fabrication. These polymer matrices show also better adhesion to silanised semiconductor surfaces, such as the gate surfaces of ion selective field effect transistors (ISFETs). Membranes sensitive to calcium ions were optimised according the type of plasticizer and the polymer/plasticizer ratio. Ion selective electrodes (ISEs) with a solid internal reference and ISFETs were used to evaluate the membrane. The resulting sensors were functional for periods of more than 8 months and the resulting sensitivities were quasi-Nernstian (26–27 mV/dec) in a range of 5×10^{-6}–8×10^{-2} M. These sensors were used to measure calcium activity in water samples extracted from agricultural soils. The results were compared with those yielded by standard methods, finding a good correlation between the two analytical procedures. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Calcium sensor; ISE; ISFET; Photopolymerisable membrane; Water soil analysis

1. Introduction

Calcium is an interesting measurand in the field of food science and agriculture. Specifically, calcium is a key component of the cell wall of vegetables and determines the elasticity of cells, providing rigidity to fruits and plants [1]. Calcium also regulates the assimilation of other cations such as K^+, Na^+ and Mg^{2+}. When there is a calcium deficiency roots are smaller, leaves are malformed and fruits become soft and are prone to illnesses such as Biter Pitt [2]. Therefore, electrodes for calcium determination based on poly(vinyl chloride) (PVC) membranes have been developed for biomedical, clinical [3] and environmental [4] samples.

Recent advances in material science and sensor technology have facilitated the development of small and robust sensors that can be mass-produced. This has enabled the appearance of miniaturised analytical instruments [5] using microsensors based on solid-state ion selective electrodes (ISEs) and ion selective field

* Corresponding author. Tel.: +34-935-947700;
fax: +34-935-801496.

effect transistors (ISFETs) [6–8]. The application of ISFETs as electrochemical sensors shows great advantages for some particular applications over the use of ISEs. Their size allows to use small sample volumes; they have low output impedance, show a faster response and are cheaper to fabricate due to the microfabrication techniques employed.

Membranes based on PVC have been traditionally used to modify the selective response of the ISE. However, when this material is used in ISFETs it presents several difficulties [9,10]. First, it shows a poor adhesion to the gate surface so the resulting sensors have a short life and show signal drift. Secondly, these membranes are cast by hand, they require four hours of curing and the use of tetrahydrofuran (THF) as a solvent. These aspects impede the automation of the membrane deposition process.

A viable alternative to PVC is the use of photocurable polymers. These materials are compatible with photolithographic techniques used in the fabrication of semiconductor devices such as ISFETs. This will allow the automatic deposition and patterning of the membrane at the wafer level. Additionally, membrane adhesion is enhanced by silanising the surface where the membrane is cast. Sensors featuring membranes based on photocurable polymers have been described in the literature [11,12]. Among the base oligomers are reported aliphatic polyurethanes [13–17]. These polymers are miscible in the plasticizers more commonly used with PVC and have demonstrated a good performance as matrices for ion-selective membranes.

A photocurable membrane selective to calcium ions based on an aliphatic diacrylated polyurethane oligomer (Ebecryl 270) was developed. To optimise the composition of the membrane it was evaluated on a solid-state ISE where the internal reference was an epoxy-graphite composite [18,19]. Various plasticizers were tested including dioctyl phenylphosphonate (DOPP), tris(2-ethylhexyl)phosphonate (TOP) and 2-nitrophenyl octyl ether (*o*-NPOE) and several membranes were prepared with different polymer, plasticizer and ionophore contents. Interference, lifetime and pH effect studies were carried out. The optimised membranes were cast on the gate surface of ISFETs and the response characteristics of the resulting sensors were evaluated. Finally, a comparative study of the response from these sensors and standard methods

for water samples extracted from flower cultures was performed.

2. Experimental

2.1. Reagents and solutions

Bis-di(4-1,1,3,3-(tetra-methylbutyl)phenyl)phosphate ionophore from Fluka was used in the preparation of the membrane. DOPP, TOP and *o*-NPOE solvent mediators from Fluka were also used. An aliphatic diacrylated urethane oligomer (Ebecryl 270) and an hexanediol diacrylate (HDDA) crosslinker were used for the membrane matrix, both donated by UCB Chemicals. The photoinitiator 2-2′-dimethoxy phenylacetophenone (Irgacure 651) was obtained from Ciba-Ceigy.

The epoxy resin was prepared by mixing Araldit M and the hardener HR from Ciba-Geigy in a 5:2 weight ratio. This was mixed with graphite powder from Merck in a 1:1 weight ratio to obtain the epoxy–graphite composite used to build the ISEs.

All reagents were analytical grade. The aqueous solutions were prepared with deionized water.

2.2. Membrane preparation and deposition

The prepolymer solution was obtained by mixing Ebecryl 270 and HDDA in an 87:13 ratio, respectively. This solution was mixed with the plasticizer, the ionophore and the photoinitiator. 100 mg of this mixture was dissolved in 150 μl tetrahydrofuran and stirred in an ultrasonic bath until homogenised.

Three electrodes were prepared for each membrane composition. To obtain the calcium ion selective electrodes, 20 μl of the membrane cocktail were cast on the epoxy–graphite surface and then exposed to UV light for one minute (365 nm, 22 mW cm^{-2}). To obtain the calcium ISFETs, 2 μl of membrane cocktail were deposited on the ISFET gate and exposed for 30 s to UV light (Fig. 1). The unpolymerised membrane was removed with ethanol. The resulting layers were 100–120 μm thick for ISEs and 20–50 μm thick for ISFETs. Both types of sensors were conditioned for 1 h in 0.1 M CaCl$_2$ and they were stored between calibrations in a 10^{-3} M solution.

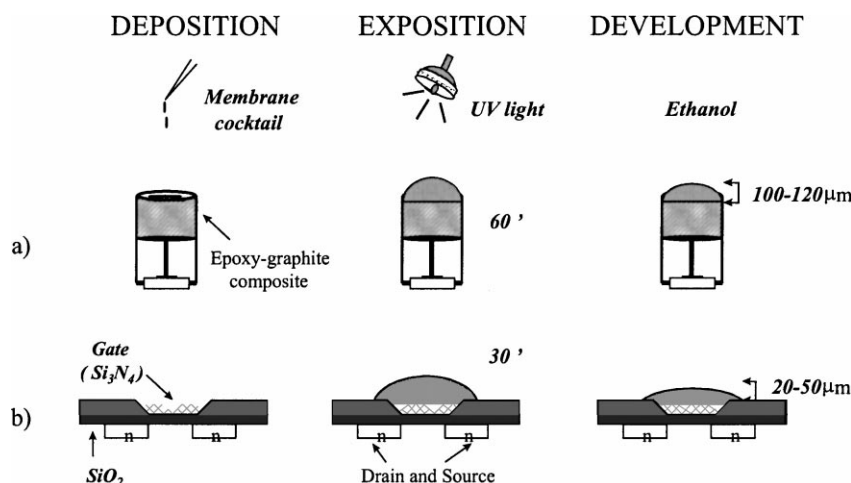DEPOSITION   EXPOSITION   DEVELOPMENT

Fig. 1. Scheme of deposition process of photocurable membranes for (a) ISE and (b) ISFET.

2.3. Apparatus

The potentiometric response of ISEs was measured with a Crison Instruments 2002 potentiometer. For the evaluation of ISFETs a meter built in our labs, based on the source and drain follower circuit was used. The drain current was $100 \, \mu A$ and the drain voltage was 0.5 V. An Ag/AgCl double junction electrode (ORION 90-00-02) was used as the reference electrode. KNO_3 was the internal reference solution. ISFETs with a pH-sensitive silicon nitride (Si_3N_4) gate were fabricated following the usual NMOS technology [8].

2.4. Evaluation methodology

Sensors were evaluated by adding a known volume of a concentrated stock solution to 25 ml of a background electrolyte solution under constant stirring. Tris 0.01 M at pH 7.55 was used as the background for ISEs and deionized water for ISFETs. To avoid the interfering effect of lipophilic anions, a stock solution of $CaCl_2$ was used for the calibration of the sensor.

Calibration curves were obtained for a range of 10^{-7}–10^{-1} M. The activity was calculated using the Debye–Hückel approximation. To obtain selectivity coefficients, the Mixed Solution method was used. To study the pH effect, tris 0.01 M was used as a background solution and volumes of 2 and 0.2 M of HCl were used to change pH.

Samples from soil were analysed with EDTA at pH 12 using NET as an indicator.

3. Results and discussion

3.1. Plasticizer study

The response characteristics of the ion-selective electrodes are dependent on the composition of the membrane, particularly of the polymer to plasticizer ratio and the type of the plasticizer used. Therefore, first studies were leaded to determine the type of plasticizer. The oligomer was Ebecryl 270 and the ionophore was bis-di(4-1,1,3,3-(tetra-methylbutyl)-phenyl)phosphate.

The composition of first membranes with different plasticizers is detailed in Table 1. The plasticizers were of common usage in calcium membranes based on PVC: dioctyl phenylphosphonate (DOPP), tris(2-ethylhexyl)phosphonate (TOP) and 2-nitrophenyl

Table 1
Composition (wt.%) of calcium membranes with different plasticizers

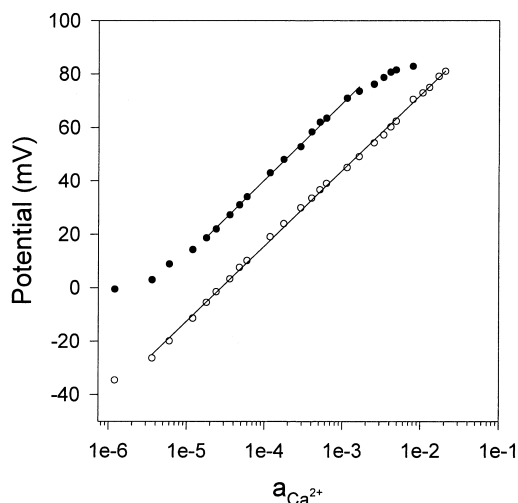|	TOP	*o*-NPOE	DOPP
Prepolymer (Ebecryl 270/HDDA)	36.9	37.0	36.3
Plasticizer	56.5	56.5	57.2
Photoinitiator	2.4	2.1	2.1
Ionophore	4.2	4.4	4.4

Fig. 2. Calibration curves of a calcium electrode with a photocurable membrane with (●) TOP and (○) DOPP as a plasticizer.

octyl ether (*o*-NPOE). The behaviour of these different plasticizers was evaluated using the epoxy graphite ISEs.

It was observed that membranes with *o*-NPOE did not polymerised properly. This was due to the nitro groups present in this plasticizer that act as inhibitors of polymerisation [20]. No significant differences were noticed between the sensitivity of sensors when DOPP or TOP plasticizers were used. In both cases the sensitivity was 25–26 mV/dec. On the other hand, differences in the linear response range were observed. This was 10^{-5}–10^{-3} M for TOP sensors and 5×10^{-6}–8×10^{-2} M for DOPP sensors. Fig. 2 shows two calibration runs carried out with these sensors.

3.2. Study of the polymer/plasticizer ratio

Next studies were performed with DOPP plasticizer and several membranes were prepared with different polymer/plasticizer ratios. In Table 2, it is shown these membrane compositions and the response characteristics obtained from the corresponding calcium sensors.

A high plasticizer content (DOPP7) produced pliable membranes with a low rigidity that broke easily. On the other hand, a high polymer content produced a negative response from the sensor. This effect arises from the permselectivity of the polymer. Since the polymer has some affinity for anions and cations, a high percentage of the polymer reduces the calcium selectivity of the membrane vis a vis chloride ions, especially at high $CaCl_2$ concentrations. The results obtained with membranes containing 25.7% plasticizer (DOPP1) show this effect clearly. The same effect is observed for the DOPP2 membrane (Fig. 3). These results confirmed that the optimal plasticizer content was 56–66% in weight, as shown by the results for membranes DOPP3, 4, 5 and 6.

When ionophore content was changed between 3 and 4%, no significant differences were observed in the characteristics of the sensor. However, the range of the linear response was slightly better when 4% ionophore was used. Therefore, sensors DOPP3 and DOPP6 were used in latter studies.

The optimisation of the membrane includes an interference study. Considering that the final application of the sensor would be the analysis of soil samples, we studied the effect of pH and other interferents commonly found in this medium. With the exception

Table 2
Membrane composition (wt.%) and response characteristics for calcium sensors using different polymer/plasticizer ratios

	DOPP1	DOPP2	DOPP3	DOPP4	DOPP5	DOPP6	DOPP7
Membrane composition							
Prepolymer	68.7	53.2	36.3	35.7	28.6	27.8	18.2
DOPP	25.7	41.1	57.1	58.5	65.7	65.8	75.8
Irgacure 651	2.0	2.2	2.2	2.4	2.1	2.1	2.4
Ionophore	3.6	3.5	4.4	3.4	3.6	4.3	3.6
Response characteristics							
Sensitivity[a]	−13 ± 8	12 ± 2	26 ± 5	29 ± 3	21 ± 2	27 ± 4	–
(mV/dec)	($n = 6$)	($n = 6$)	($n = 6$)	($n = 18$)	($n = 12$)	($n = 48$)	
Linear	10^{-5}–10^{-2}	10^{-5}–10^{-2}	5×10^{-6}–8×10^{-2}	10^{-5}–5×10^{-2}	5×10^{-5}–5×10^{-3}	5×10^{-6}–8×10^{-2}	–

[a] Sensitivities are given as the mean of *n* determinations and the uncertainty intervals are calculated at 95% confidence level.
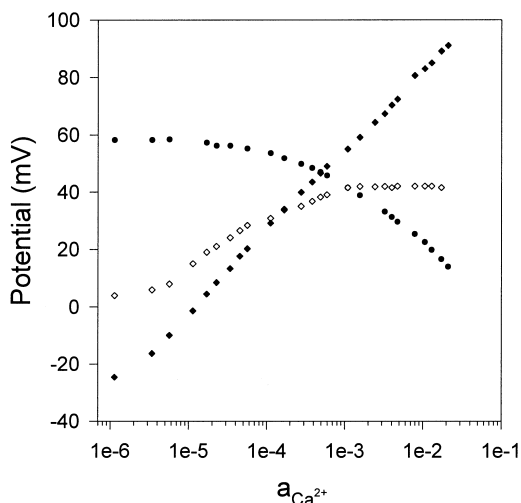
Fig. 3. Calibration curves of calcium sensors showing the influence of the polymer-plasticizer ratio. Plasticizer percentage was (●) 25.7%, (○) 41.1% and (◆) 57.1%.

of swamp terrain and alkaline soils, the expected pH range in soils would be 4–8. Table 3 shows that sensors DOPP3 and DOPP6 cover this pH range.

In the aqueous solution of soil there is a wide range of cations that may interfere with the membrane. The more common cations in soils are Ca^{2+}, Mg^{2+}, K^+,

Na^+ and NH_4^+ and to a lesser extent Fe^{3+}, Al^{3+}, Zn^{2+}, Cu^{2+} and Mn^{2+}. Table 3 shows the results obtained for the interference studies. It can be observed that magnesium is the strongest interferent for both membranes (DOPP3 and DOPP6). Considering that the concentration of magnesium in soils is usually three or four times lower than that of calcium, it can be assumed that magnesium interference is negligible. It is important to point out that after 4 months of continual use, the selectivity coefficients observed were within the same order of those observed at the start of the study. This indicates no leaching of membrane compounds for this period of time.

The lifetime of the sensors was studied by observing the evolution of their sensitivities and detection limits. Fig. 4 shows this evolution for several DOPP6 units. During this time the sensitivities obtained were nearly Nernstian 26 ± 5 mV/dec for sensor DOPP3 and 27 ± 4 mV/dec for sensor DOPP6. The linear range for both sensors was 5×10^{-6}–8×10^{-2} M during this time. According to the limit of detection it is observed a slight change during the first days due to the different studies of interference effects carried out with these sensors. Membranes stayed well adhered for 8–9 months. This long lifetime is comparable with that of PVC membranes for epoxy–graphite ISES used.

Table 3
Response characteristics for calcium sensors with DOPP3 and DOPP6 membranes

	DOPP3	DOPP6
Sensitivity[a] (mV/dec)	26 ± 5 ($n = 57$)	27 ± 4 ($n = 48$)
Linear response (M)	5×10^{-6} to 8×10^{-2}	5×10^{-6} to 8×10^{-2}
Detection limit (M)	$4 \times 10^{-6} \pm 2 \times 10^{-6}$	$4 \times 10^{-6} \pm 3 \times 10^{-6}$
pH range	3.5–10	4–8
Lifetime (months)	8–9	8–9

Selectivity coefficients

Ion	C_j (M)	$-\log K_j^{POT}$	
K^+	0.1	−3.4	−3.4
Na^+	0.1	−3.1	−3.2
NH_4^+	0.1	−3.0	−3.2
Mg^{2+}	10^{-3}	−2.0	−1.4
Fe^{3+}	0.1	−3.0	−3.1
Al^{3+}	0.1	−1.5	−1.4
Zn^{2+}	0.1	−3.0	−3.0
Cu^{2+}	0.1	−1.6	−1.5
Mn^{2+}	0.1	−2.5	−2.6

[a] Sensitivities are given as the mean of n determinations and the uncertainty intervals are calculated at 95% confidence level.
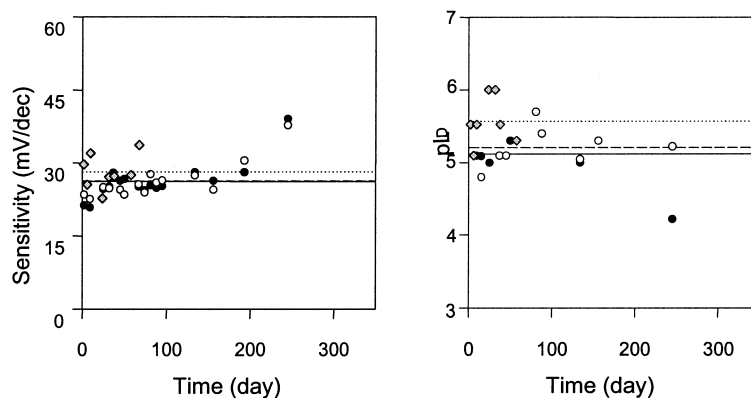
Fig. 4. Evaluation of long-term stability expressed as the variation of sensitivity and the detection limit along time for several units of DOPP6 sensors stored with (◇) (○) 10^{-3} M of CaCl$_2$ and (●) dry stored. Dotted lines indicate the mean of sensitivity.

A study of the storage influence on the sensor response was performed as well. Some ISEs were stored both dry and in a 10^{-3} M de CaCl$_2$ solution. For the 9 months that the study lasted, no significant differences were observed in the sensitivity and the detection limit between the two methods.

The results presented allowed us to establish an optimal composition of the calcium sensitive membrane: prepolymer (Ebecryl 270 and HDDA) in a range of 27–36%; dioctyl phenylphosphonate (DOPP) 56–66%; 2-2'-dimethoxy phenylacetophenone (Irgacure 651) 2%; bis-di(4-1,1,3,3-(tetra-metilbutil)phenil)phosphonate 4%. These membranes correspond to DOPP3 and DOPP6 sensors, whose did not presented significant differences.

3.3. Application of the optimised membranes to ISFETs

To study the response characteristics of the calcium ISFETs, membranes of the same composition than those of the ISE study (DOPP3 and DOPP6) were deposited on semiconductor devices. In this manner, sensors DOPP3-ISFET and DOPP6-ISFET were built. The results of the response characteristics are detailed in Table 4. No significant differences were observed for the sensitivity, the linear range, the detection limit and the working pH range between ISEs and ISFETs using the same membranes. However, significant differences were noticed in their lifetimes. The membranes with a harder or less pliant look, with 56%

Table 4
Response characteristics of calcium ISFETs

	DOPP3-ISFET	DOPP6-ISFET	
Sensitivity[a] (mV/dec)	-26 ± 1 ($n = 60$)	-26 ± 1 ($n = 27$)	
Linear range (M)	7×10^{-6} to 8×10^{-2}	9×10^{-6} to 8×10^{-2}	
Detection limit (M)	$5 \times 10^{-6} \pm 2 \times 10^{-6}$	$8 \times 10^{-6} \pm 3 \times 10^{-6}$	
pH range	4–10	4–8.5	
Lifetime (months)	9–10	3–4	
Selectivity coefficients			
Ion	C_j (M)	$-\log K_j^{\mathrm{POT}}$	
K$^+$	0.1	-3.1	-2.8
Na$^+$	0.1	-2.8	-2.6
NH$_4^+$	0.1	-2.6	-2.5
Mg^{2+}	10^{-3}	-1.9	-1.3

[a] Sensitivities are given as the mean of n determinations and the uncertainty intervals are calculated at 95% confidence level.

Table 5
Mean of calcium measurement (M) for three determinations and for different aqueous samples from soil using developed ISEs and standard method of titration with EDTA[a]

Sample	Titration	ISE
$M1$	$14.5 \times 10^{-3} \pm 0.8 \times 10^{-3}$	$18 \times 10^{-3} \pm 5 \times 10^{-3}$ (I)
$M1$	$14.5 \times 10^{-3} \pm 0.8 \times 10^{-3}$	$20 \times 10^{-3} \pm 10 \times 10^{-3}$ (S)
$M2$	$37 \times 10^{-3} \pm 2 \times 10^{-3}$	$44 \times 10^{-3} \pm 5 \times 10^{-3}$ (S)
$M3$	$26 \times 10^{-3} \pm 2 \times 10^{-3}$	$20 \times 10^{-3} \pm 3 \times 10^{-3}$ (I)
$M3$	$26 \times 10^{-3} \pm 2 \times 10^{-3}$	$24 \times 10^{-3} \pm 3 \times 10^{-3}$ (S)

[a] For the calculation of calcium concentration with ISEs, two methodologies were used: interpolation in a calibration curve (I) and standard addition (S); t-test: $t_{calculated}$: 0.66; $t_{critical(P=0.05)}$: 2.78.

of plasticizer, adhered better to the silanised surface of ISFETs and presented a lifetime of 9–10 months while membranes with 66% plasticizer showed a lifetime below 4 months.

An interference study yielded results similar to those from the ISE study. For DOPP6-ISFET these values were slightly lower.

3.4. Analysis of drain waters from flower cultures

Preliminary tests were carried out to establish the behaviour of developed membranes in aqueous samples extracted from soil. Sensors with the membrane DOPP3 were applied to the analysis of drain water from flower cultures. Results were compared with those obtained following a complexiometric evaluation using EDTA at pH 12. Two methodologies were followed for the potentiometric measurement of calcium concentration: a standard addition method and by interpolation in a calibration curve. Table 5 shows the results of this study.

The data was analysed by the student t-test paired. The results obtained for t calculated and the critical value (see Table 5) demonstrated that no significant differences were found between two measurement techniques.

4. Conclusions

The proposed photocurable polymer, based on an aliphatic diacrylated polyurethane, has many advantageous features for the fabrication of calcium sensitive devices based either on ISEs or ISFETs. The main advantage of this novel polymer comparing with PVC includes both the preparation and the casting of the membrane that are easy and quick, requiring only 1 min of curing. The use of the photopolymers is more advantageous when ISFETs are used since this material is compatible with photolithographic techniques and this provides the automatization of all sensor fabrication process. Additionally, it is important to underline that this polymer presents biocompatibility when tested in rats [12] which widens the range of potential applications.

Sensitivity, selectivity and response time of ISEs and ISFETs with this polymeric membrane were similar to those reported when PVC membranes are used [21]. For ISFETs, the adhesion of the membrane to the silanised substrate was much stronger than that of PVC membranes. This is evident in the lifetime of 9–10 months shown by photocurable membranes contrasting to the 2–3 months with PVC membranes [22]. No significant differences were appreciated between the response characteristics of ISEs and ISFETs.

The study of pH effects and interferents showed that the resulting devices could be used in soil analysis and other agricultural samples. The results obtained when analysing aqueous samples from soil show a satisfactory performance of the sensors when compared with the standard methods. This corroborates that the sensors with photocurable membranes are appropriate for this applications and suggest their use to soil analysis. At this moment, the measurement of soil nutrients with ISFETs is under study in our laboratories and preliminary results show great promise.

References

[1] W.J. Bramlage, S.A. Weis, M. Drake, J. Am. Soc. Hort. Sci. 110 (4) (1985) 493.

[2] W.J. Bramlage, M. Drake, J.H. Baker, J. Am. Soc. Hort. Sci. 99 (4) (1974) 376.

[3] S.A.H. Khalil, G.J. Moody, J.D.R. Thomas, Analyst 110 (185) 353.

[4] J. Sander, F. Zuther, K. Cammann, Sens. Actuators B 15/16 (1993) 188.

[5] K. Petersen, Sens. Actuators A56 (1996) 143.

[6] T. Matsuo, K.D. Wise, IEEE Trans. Biomed. Eng. BME-21 (1974) 485.

[7] P. Bergveld, IEEE Trans. Biomed. Eng. BME-19 (1972) 342.

[8] S. Alegret, J. Bartrolí, C. Jiménez, M. Del Valle, C. Domínguez, E. Cabruja, A. Merlos, Electroanalysis 3 (1991) 355.

[9] S.D. Moss, C.C. Johnson, J. Janata, IEEE. Trans. Biomed. Eng. BME-25 (1978) 49.

[10] G.J. Moody, J.D.R. Thomas, J.M. Slater, Analyst 113 (1988) 1703.

[11] P.D. Van der Wal, A. Van der Berg, N.F. De Rooij, Sens. Actuators B18/19 (1994) 200.

[12] J. Muñoz, C. Jimenez, A. Bratov, J. Bartrolí, S. Alegret, C. Domínguez, Biosens. Bioelectronics 12 (7) (1997) 577–585.

[13] C. Puig-Lleixà, C. Jiménez, E. Fabregas, J. Bartrolí, Sens. Actuators B49 (1998) 211–217.

[14] C. Puig-Lleixà, S. Ramírez-Garcia, C. Jiménez, J. Bartrolí, Anal. Chim. Acta 386 (1999) 13–19.

[15] R.W. Cattrall, P.J. Iles, Anal. Chim. Acta 169 (1985) 403.

[16] A. Bratov, N. Abramova, J. Muñoz, C. Domínguez, S. Alegret, J. Bartrolí, Anal. Chem. 67 (1995) 3589.

[17] A. Bratov, N. Abramova, J. Muñoz, C. Domínguez, J. Electrochem. Soc. 144 (2) (1997) 617.

[18] J.L.F.C. Lima, A.A.S.C. Machado, Analyst 111 (1986) 799.

[19] J.M.C.S. Magalhães, F. Céspedes, S. Alegret, A.A.S.C. Machado, Anal. Chim. Acta 355 (1997) 241.

[20] C. Dumschat, R. Frömer, H. Rautschek, H. Müller, H.J. Timpe, Anal. Chim. Acta (1991) 243.

[21] J. Alonso, J. Bartrolí, S. Jun, J.L.F.C. Lima, M.C.B.S.M. Montenegro, Analyst 118 (1993) 1527.

[22] C. Jiménez, I. Marqués, J. Bartrolí, Anal. Chem. 68 (1996) 3801.

APPLICATION OF ION SENSITIVE FIELD EFFECT TRANSISTORS BASED SENSORS TO SOIL ANALYSIS

J. Artigas, A. Beltran, C. Jiménez, A. Baldi, R. Mas, C. Domínguez, J. Alonso

Application of ion sensitive field effect transistor based sensors to soil analysis

J. Artigas [a], A. Beltran [a], C. Jiménez [b,*], A. Baldi [b], R. Mas [b],
C. Domínguez [a,b], J. Alonso [a]

[a] *Grup de Sensors i Biosensors, Departament de Química, Universitat Autònoma de Barcelona,
08193 Bellaterra, Spain*
[b] *Instituto de Microelectronica de Barcelona (IMB-CNM)-CSIC, Campus UAB,
08193 Bellaterra, Spain*

Abstract

Standard methods to measure nutrient levels in soil are complex and time consuming due to the extraction and pre-treatment processes involved. Besides, the instrumentation used for these measurements is also expensive. Therefore, the use of chemical sensors warrants investigation since they can be placed directly in the soil and results can be provided in real or quasi-real time at a moderate cost. The control of nutrients with sensors will permit an optimisation of irrigation and fertilisation management systems and thus will be useful for reducing the environmental impact caused by the runoff of nutrients into surface and ground waters. In this work, the use of chemical sensors based on ion sensitive field effect transistors (ISFETs) for soil analysis is proposed. These devices are fabricated with microelectronic technology – providing some important advantages such as robustness, small size, low output impedance and mass production. Fabrication of pH, Ca^{2+}, K^+ and NO_3^- ISFETs with photocurable polymeric membranes and their evaluation in aqueous solutions is reported. Studies of their response in horticulture soils and comparison with standard methods have been performed. The results confirm the feasibility of ISFET based sensors for in-soil monitoring and the promising future applications they have. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: In-soil monitoring; Nutrient levels determination; Ion sensitive field effect transistor based sensors

* Corresponding author. Tel.: + 34-93-5947700; fax: + 34-93-5801496.
E-mail address: cecilia.jimenez@cnm.es (C. Jiménez).

1. Introduction

Chemical fertilisers are introduced to soils as essential nutrient sources for intensive agricultural production. The uncontrolled addition of these substances leads to runoff of nutrients excess into surface and ground waters causing an undesirable environmental impact (i.e. the effects of nitrate lixiviation are well known), maybe plant damage and a uselessness increasing of production costs as well.

Therefore, monitoring of nutrient levels in soils will provide useful information for the reduction of environmental impact allowing simultaneously the optimisation of the overall production process of a given crop. Additionally, if the information is obtained in real time, it would be possible to design intelligent irrigation and fertilisation management systems.

Systems to measure ions directly in the soil are not commercially available at the present time. All the standard methods are based on extraction processes, pre-treatment of the sample and analysis using atomic absorption spectrometry, molecular emission spectrometry, chromatographic techniques, etc. (Hesse, 1972). These are complex methods, time consuming and require expensive facilities and instruments. Furthermore, the results are not produced in real time so that the use of alarm-driven monitoring is not possible. The use of sensors for soil analysis is interesting since it opens the possibility of using them with on-line systems to produce real (or quasi-real) time results at a moderate cost.

At present, the existing literature on the application of potentiometric sensors for soil analysis is focused on ion selective electrodes (ISEs) used in flow injection analysis (FIA) systems (Ruzicka et al., 1977; Hongbo et al., 1985; Ferreira et al., 1996). These systems measure ions of agricultural interest such as Cl^-, NO_3^-, Ca^{2+}, K^+, Na^+, etc, automatically and continuously but require previous sample extraction and pre-treatment. Other reports published recently use robotic systems for sample extraction and pH measurement (Torres et al., 1993; Brenes et al., 1995). These are complex and also need pre-treatment of sample that makes the analysis time consuming. In both cases, the analysis has to be carried out in the laboratory.

Few papers have been published regarding the application of ISFETs in soil analysis. An interesting paper, reports the use of FIA techniques applied to ornamental plants (Van den Vlekkert et al., 1992). That system uses a closed loop that monitors drainage waters in a hydroponic set up to control the amount of fertiliser to be added to the irrigation system. That approach is quite innovative regarding the analysis of nutrients in soil but it is using a 'on-line' method, therefore the results are not in real time.

In this work, the use of ion selective field effect transistors (ISFETs) for 'in-soil' determination of chemical parameters of interest in agricultural production is proposed. These devices are the result of the integration of two technologies: ISEs and microelectronics technology (Bergveld, 1970). The application of ISFETs as potentiometric sensors has great advantages over conventional ISEs. These advantages are derived from key ISFET features. Small size and a solid state nature; low output impedance that reduces interference from external electromagnetic fields;

mass fabrication and low cost; the possibility of integrating compensation and data processing circuits in the same chip with the sensor; and a short response time.

The response mechanism of ISFETs is based on their semiconductor nature and on the electrochemical phenomena that occur in the chemically sensitive membrane placed on the gate of the transistor. ISFETs have a gate layer that is sensitive to pH variations. The materials making these layers include SiO_2 (Bergveld, 1972), Si_3N_4 (Matsuo and Wise, 1974) and Al_2O_3 (Abe et al., 1979). Further progress has been introduced by the deposition of ion recognition membranes on top of the gate area of ISFETs in order to modify the selectivity of the device. Membranes based on PVC, similar to those developed for ISEs, have been deposited on ISFETs to produce devices sensitive to K^+ (Moss et al., 1975), Ca^{2+} (Moss et al., 1978), NH_4^+ and Na^+ (Oesch et al., 1981). However, these PVC-based membranes have several disadvantages such as a poor adherence to the ISFET surface and a difficulty to control the thickness of the deposited membrane because of the manual technique used.

A viable solution to these problems is the use of polymers other than PVC that are compatible with ISFET fabrication technology. Photocurable polymers meet this requirement as they are cured with UV radiation making them compatible with the photolithographic techniques used for the fabrication of ISFETs. Additionally, these polymers show an enhanced adherence to the gate materials compared to PVC, especially when the gate surface has been chemically modified with silanol groups. These technological improvements allow the fabrication of multisensor arrays because the deposition and definition of each membrane is performed at the wafer level. These advantages are very important since they permit the use of automated fabrication techniques that are capable of yielding a great quantity of reliable devices. Several polymers have been studied including polysiloxanes (Van der Wal et al., 1994), polyurethanes (Cattrall and Iles, 1985; Bratov et al., 1995a; Puig-Lleixà et al., 1998) and polymethylmetacrylates (Tietje-Girault et al., 1990) to develop ion selective membranes.

The purpose of the present work is the development of a 'in-soil' analysis system applying ISFET based sensors directly in soil. The ISFETs have been fabricated using standard microelectronic technology applied to integrated circuits (Alegret et al., 1991). For nitrate, calcium and potassium measurement, the surface of the ISFET has been modified with an ion selective membrane. Since a more robust membrane is required for such application, the membrane matrix is based on a photocurable polymer. The response of the ISFETs has been evaluated previously in aqueous solutions to test their performance – sensitivity, selectivity, linear range, etc. Further studies by inserting ISFETs directly into the soil were performed in different types of soil for horticultural crops. Measurements of response to different ion concentrations and comparison with standard methods were also performed to establish the viability of ISFET application to soils.

2. Materials and methods

2.1. Fundamentals of ion sensitive field effect transistor response mechanisms

The response mechanism of ISFETs is based on its behaviour as an electronic field effect device and on the phenomena that takes place in the electrolyte–insulator interface. The presence of ions in the solution results in a gate voltage (V_{GS}) shift and consequently a drain current shift (I_D). This current, which is flowing between the drain and the source of the transistor, can be modulated by the gate voltage so that the voltage between drain and source (V_{DS}) is amplified (Fig. 1).

The Site Binding theory (Bousse et al., 1983) explains the process occurring in the electrolyte–insulator interface. This model considers Si–OH groups in the oxide layer as active centres whose charge varies according to the ions (protons in this case) present in the surface of the ISFET. These active centres are responsible of the electrical double layer formation described by the Gouy–Chapman–Stern theory (Bockris et al., 1970) and therefore for the potential at the oxide–electrolyte interface.

Using the model described above, an equation relating the potential at the oxide–electrolyte interface and the proton concentration can be established (Bousse et al., 1983):

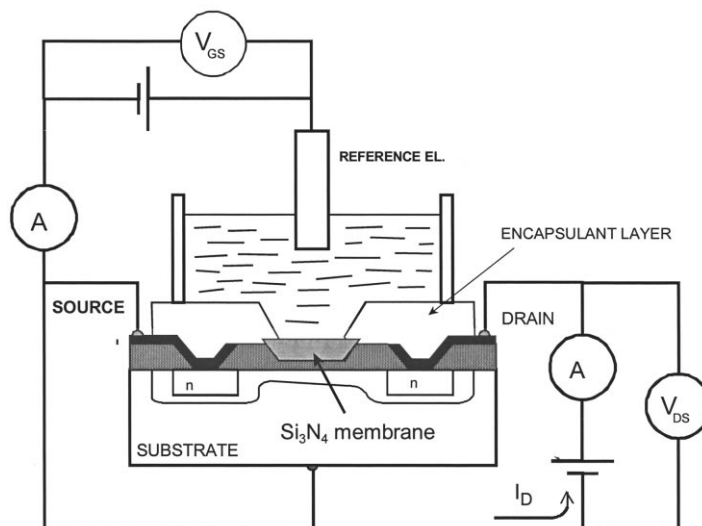$$E = E_0 + g\left(\frac{\beta}{\beta + 1}\right)\log[H^+]$$

Fig. 1. Scheme of the ISFET mechanism of response and the electronic circuit associated with it.

This equation is similar to that described by Nernst for potentiometric sensors. The parameter g corresponds to the theoretical Nernstian value of sensitivity (59.15 mV/decade at 25°C), E_o correspond to the standard potential and β depends on the nature of the gate material. Since β is always positive, the sensitivity of a pH ISFET is usually sub-Nernstian. However, for oxides such as Al_2O_3 where β has a high value, the slope is very close to Nernstian value (Abe et al., 1979).

When an ion sensitive membrane is cast on the gate of an ISFET the response mechanism is determined by the charge distribution phenomena in the membrane–electrolyte interface already described for ISEs with a mobile carrier membrane (Buck et al., 1978). The literature on ISFETs with membranes based on PVC is wide and it has shown that the response of ISFETs featuring these membranes is equivalent to those of ISEs (Janata, 1994). However, new polymers are under study to improve the characteristics of these devices (Cattrall and Iles, 1985; Bratov et al., 1995a).

2.2. Reagents and solutions

Sensor components: bis-(4-1,1,3,3-(tetramethylbutyl)phenyl) phosphate (TMB-PhPP), valinomycin, tetraoctyl-ammonium nitrate (TOA), di-n-octylphenylphosphate (DOPP), dioctylsebacate (DOS), trioctyl phosphonate (TOP) and potassium tetrakis (4-clorophenyl)borate (KTpClPhB) were from Fluka. Monomers and oligomers: aliphatic urethane diacrylate Ebecryl 270 and hexanediol diacrylate (HDDA) were donated by UCB Chemicals and 2-2′-dimethoxy phenylacetophenone (Irgacure 651) was from Ciba-Geigy.

Fertiliser solution for ISFET evaluation in soils contained salts based on nitrate, sulphate, phosphate and chloride anions and calcium, magnesium, ammonium, iron and zinc cations in the usual concentrations for horticultural applications (Ansorena, 1994; Soil and plant analysis Council, 1992).

Sample soils were obtained from the Guadalquivir basin in the south of Spain. Two types of soils were studied, loam and peat for hydroponic cultures used for rose cultivation in greenhouses. All aqueous solutions were prepared with de-ionised water.

2.3. Ion sensitive field effect transistor fabrication

The chip has a size of 2950×2050 μm^2 (Fig. 2). The ISFET chip contained an associated MOSFET, which was used to test the encapsulating layer lifetime and the technological viability of the device. The ISFET n-channel is 20 μm long and 500 μm wide. The structure of the Si_3N_4 pH-sensitive membrane layer is 130×610 μm^2 and 180 nm thick. The ISFET was fabricated on (100) p-type silicon wafers with a boron concentration of 10^{16} atoms/cm^3. More technological details of the ISFET fabrication are described in the reference (Alegret et al., 1991). Each chip was fixed on a standard printed circuit board and wire bonded to the connection paths. To protect all the electrical parts of the chip, an encapsulating layer of a photocurable polymer was applied (Bratov et al., 1995b).
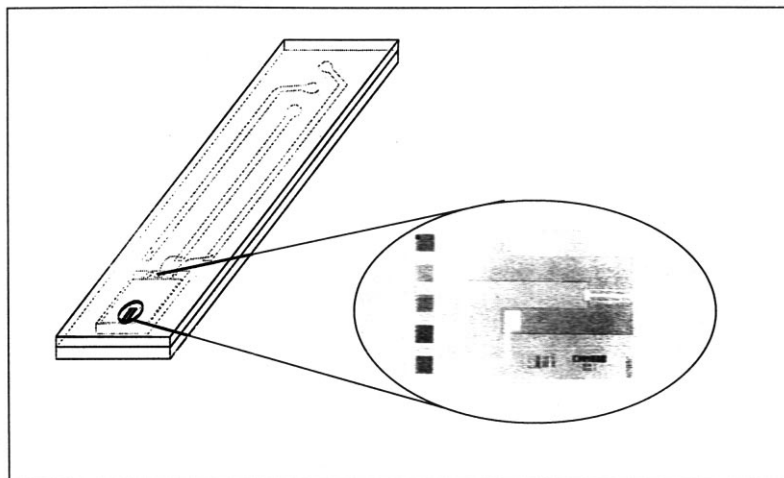
Fig. 2. Scheme of the ISFET probe and photograph of the chip.

2.4. Membrane preparation and deposition

Photocurable membranes for K^+, Ca^{2+} and NO_3^- ISFETs were prepared by mixing the pre-polymer components: the oligomer Ebecryl 270 and the crosslinker HDDA. Afterwards, this viscous solution was mixed with the photoinitiator and the sensor components: the plasticiser, the ionophore and the additives. The composition of each membrane is shown in Table 1. This mixture was dissolved in 150 µl of tetrahydrofuran and stirred in an ultrasonic bath until it became homogeneous. Two micro litres of the membrane cocktail was deposited to the ISFET gate and was exposed to UV. The un-polymerised membrane was removed with ethanol. The ISFETs were conditioned for 1 h in a 0.1 M stock solution and were kept between calibrations in a 10^{-3} M solution.

Table 1
Membrane composition (% weight) for Ca^{2+}, K^+ and NO_3^- ISFETs

Components	Ca^{2+}	% weight	K^+	% weight	NO_3^-	% weight
Polymer	Eb270+HDDA	38.4	Eb270+HDDA	48.4	Eb270+HDDA	34.7
Photoinitiator	Irgacure 651	2.4	Irgacure 651	2.3	Irgacure 651	2.0
Plasticizer	DOPP	55.1	DOS	46.4	TOP	59.4
Ionophore	TMBPhPP	4.1	Valynomicin	2.3	TOAN	3.9
Additives	–	–	KTpClPhB	0.6	–	–

2.5. Apparatus

For the measurement of ISFET characteristics, an amplifier ISFETmeter based on the source and drain follower was used. The drain voltage (V_{DS}) and the drain current (I_D) were fixed to 0.5 V and 100 μA respectively. A glass electrode Ingold U455-S7 connected to a Crison 2002 potentiometer was used to test the pH of the solutions. For the evaluation of ISFETs in aqueous solutions, a Ag/AgCl double junction Orion electrode was used as a reference electrode.

For measurements in soil, a copper wire was used as the reference electrode. Considering the type of sample, a reference electrode that can maintain an intimate contact with the soil moisture and can be placed close to the ISFET to achieve a good polarization is required. Furthermore, if the reference solution can be excluded, problems of maintenance are avoided. For these reasons, only the conductive part of the reference electrode was chosen. Among the materials used as reference, copper was chosen due to its stability in soil (this material is used as ground) and its low cost.

Experimental data obtained with the copper wire electrode against a double junction reference electrode, both inserted in soil, was performed. The results demonstrated that there is a drift of 0.4 mV/h – value comparable and opposite to that of the ISFET – and a signal stability of ± 0.1 mV.

To insert the ISFET in soil a special cell was designed. This cell allowed the introduction of the four ISFETs and the reference electrode in the same point.

2.6. Evaluation procedure of ion sensitive field effect transistors

The evaluation of ISFET sensors in aqueous solutions was performed by adding a known volume of a concentrated stock solution of the corresponding ion in 25 ml of a background electrolyte solution under constant stirring. Calibration curves were obtained for a range of 10^{-7} to 10^{-1} M of the ion being measured. To obtain selectivity coefficients, the mixed solution method was used for different concentrations of the interfering ion.

Measurements in soil were done by inserting the ISFET cell in a set of rose pots with loam or peat soil as follows: every day, ISFETs were calibrated using three standard solutions, washed with de-ionised water and introduced into the soil (7–10 cm deep) using the cell. Soil was irrigated with 30 ml of a commercial fertiliser solution. When a steady state was achieved (after 30–40 min) the signal was recorded.

3. Results and discussion

The ISFETs developed were evaluated in aqueous solutions to establish the response characteristics and their feasibility for soil application. The results are summarised in Table 2. The sensitivity shown is quasi-Nernstian in all cases and the linear range is wide enough for soil application. A dynamic response and a

Table 2
Response parameters of ISFET based sensors in aqueous solutions

	Ca^{2+}	K^+	pH	NO_3^-
Sensitivity (mV/decade)	26.5	55.7	54.1	62.6
Linear range (M)	5×10^{-6}–0.1	7×10^{-5}–0.1	2–12	2.3×10^{-5} -6×10^{-2}
Detection limit (M)	10^{-6}	4×10^{-5}		1×10^{-5}
Working pH range	3.0–8.5	3.5–8.0		2.0–11.0
Lifetime (months)	>7	7	9	>7

calibration plot for NO_3^--ISFET is shown in Fig. 3. These results are comparable to those obtained with PVC membranes deposited onto ISFET sensors (Jiménez et al., 1996). This demonstrates the good performance of the photocurable membranes developed.

The selectivity of each ISFET can be evaluated from the selectivity coefficient value. For Ca^{2+} and NO_3^--ISFETs the interference from Mg^{2+} and Cl^- ions respectively was the only significant data ($K^{pot} = 10^{-2}$). Considering usual concentrations in soil of these interfering ions, errors less than 1% in the measurement were observed. This value is low and permits us to conclude that the sensor has good selectivity for this application.
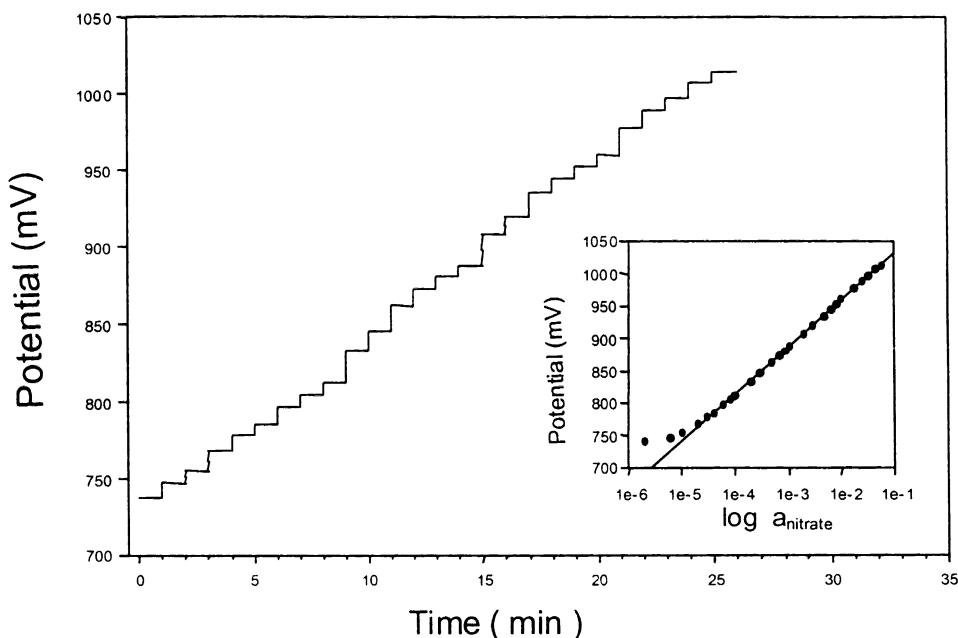

Fig. 3. Recording of the NO_3^- ISFET dynamic response during a calibration run and the calibration plot obtained.

The sensor response was stable and fast as shown in Fig. 3. No membrane damage was observed during the time they were tested in aqueous solutions – over nine months – and no decrease of sensitivity was observed in that period.

When ISFETs were introduced in soil samples, and due to the measurement procedure, an initial period of 30–40 min was needed for achieving the equilibrium in soil. Adding nutrient solutions with different concentrations of K^+, Ca^{2+} and NO_3^- ions and pH values were used to study the response of the ISFETs in soil. For the latter parameter, the signal was stable after additions of different acid and base solutions thus indicating the buffer capacity of soils (Fig. 4(a)). For the other ions, the ISFET response decreased slightly or was maintained (Fig. 4(b) and (c)) for concentrations below 10^{-2} M, which corresponds to usual values of these ions in soils. This effect can be explained by the buffering effect of soil at these low ion concentrations. When 0.1 M concentration of calcium, potassium or nitrate was added, the fast ISFET response observed indicated a sizeable increment of the ion concentration in soil and a good dynamic response of ISFETs.

The reversibility of the sensor response was indicated by the signal change when adding water. For calcium measurement (Fig. 4(b)), it is supposed that the amount of water added is not high enough to runoff all the excess of calcium from soil and the signal does not return to its initial value. For the nitrate sensor, complete runoff of nitrate solution is achieved, perhaps due to its higher lixiviating capability.

During these experiments, ISFETs were calibrated each day with synthetic pH buffer solutions. The drift observed (obtained from the calibration curve intercept) was around 2–4 mV/day. This value can be considered satisfactory when compared to the usual drift of ISFETs in aqueous solutions that is around 1 mV/h. Also, this potential drift is constant and can be compensated by software or electronic means.

The sensitivity of all sensors remained constant during the two months this study was performed: 53 ± 1 mV/decade for pH; -32 ± 4 mV/decade for potassium; and -52 ± 2 mV/decade for calcium. Only for the nitrate ISFET, with a sensitivity of 62 ± 4 mV/decade, after the first month of measuring in soil an important decrease in sensitivity was observed. It is supposed that leaching of sensor components out of the membrane was occurring.

A study comparing the data obtained with ISFETs and that from standard methods was carried out for the two kinds of soil substrates and samples taken at different times. Sampling for calcium, potassium and nitrates measurement with standard methods was performed by aspiration of the aqueous solution from soil. For pH measurement 1 g of soil was taken and mixed with 5 ml of distilled water.

From results shown in Table 3, it was observed a slight correlation between two methods (see *t*-paired test results). The differences can be attributed to the sampling process and furthermore to the concentration data, expressed in terms of activity for ISFETs measurements. These aspects are more notable for pH measurements, were the correlation between two methods is poor. However, taking into account these limitations, the similar order of magnitude obtained with both methods can be interpreted as demonstrating the feasibility of ISFET responses in soil.

Table 3
Results obtained with ISFETs and standard methods for Ca^{2+}, K^+, NO_3^- and pH for samples of loam type soil and peat taken at different times. Results of t-paired test are shown below each substrate type and ion data column ($P = 0.05$)[a]

| | CALCIUM | | POTASSIUM | | NITRATE | | pH | |
	ISFET	Titration with EDTA	ISFET	Atomic absorption	ISFET	Espectrophotometry	ISFET	Glass electrode
SOIL	**$1.2\,10^{-2}$**	**$1.5\,10^{-2}$**	$1.4\,10^{-2}$	$3.9\,10^{-3}$	$1.5\,10^{-2}$	$2.4\,10^{-2}$	6.1	6.7
	$3.9\,10^{-3}$	$6.3\,10^{-3}$	$1.0\,10^{-2}$	$5.6\,10^{-3}$	$3.0\,10^{-2}$	$3.1\,10^{-2}$	5.8	6.9
	$3.3\,10^{-3}$	$7.4\,10^{-3}$	$3.8\,10^{-2}$	$6.5\,10^{-3}$	$1.0\,10^{-2}$	$3.0\,10^{-2}$	**7.0**	**7.3**
	$1.3\,10^{-3}$	$1.7\,10^{-3}$	$1.4\,10^{-2}$	$5.6\,10^{-3}$	$2.7\,10^{-2}$	$1.8\,10^{-2}$	**6.8**	7.4
	-	-	**$4.0\,10^{-3}$**	$4.7\,10^{-3}$	-	-	**6.3**	7.3
Results of t-paired test	$t_{cal}=3.16$ $t_{tab}=3.18$		$t_{cal}=1.95$ $t_{tab}=2.78$		$t_{cal}=0.85$ $t_{tab}=3.18$		$t_{cal}=5.14$ $t_{tab}=2.77$	
PEAT	**$14.0\,10^{-3}$**	**$13.3\,10^{-3}$**	**$9.0\,10^{-3}$**	$6.6\,10^{-3}$	$4.1\,10^{-2}$	$1.8\,10^{-2}$	5.3	5.0
	$1.4\,10^{-3}$	**$8.2\,10^{-3}$**	$1.4\,10^{-2}$	$5.9\,10^{-3}$	$2.7\,10^{-2}$	$1.8\,10^{-2}$	**6.5**	5.1
	$2.5\,10^{-3}$	$6.4\,10^{-3}$	$3.3\,10^{-2}$	$6.1\,10^{-3}$	$3.2\,10^{-2}$	$1.8\,10^{-2}$	6.5	**5.0**
	$3.2\,10^{-3}$	$5.6\,10^{-3}$	$1.6\,10^{-2}$	$5.9\,10^{-3}$			**6.5**	**4.8**
			$8.0\,10^{-3}$	$6.0\,10^{-3}$			5.2	4.9
Results of t-paired test	$t_{cal}=2.35$ $t_{tab}=3.18$		$t_{cal}=2.19$ $t_{tab}=2.78$		$t_{cal}=3.74$ $t_{tab}=\mathbf{4.30}$		$t_{cal}=3.35$ $t_{tab}=2.78$	

[a] Sampling for measurement with standard methods was performed as follows, (a) For calcium, potassium and nitrates, 1 ml of aqueous solution was aspirated from soil with a peristaltic pump and (b) For pH, 1 g of soil was mixed with 5 ml of distilled water and stirred.
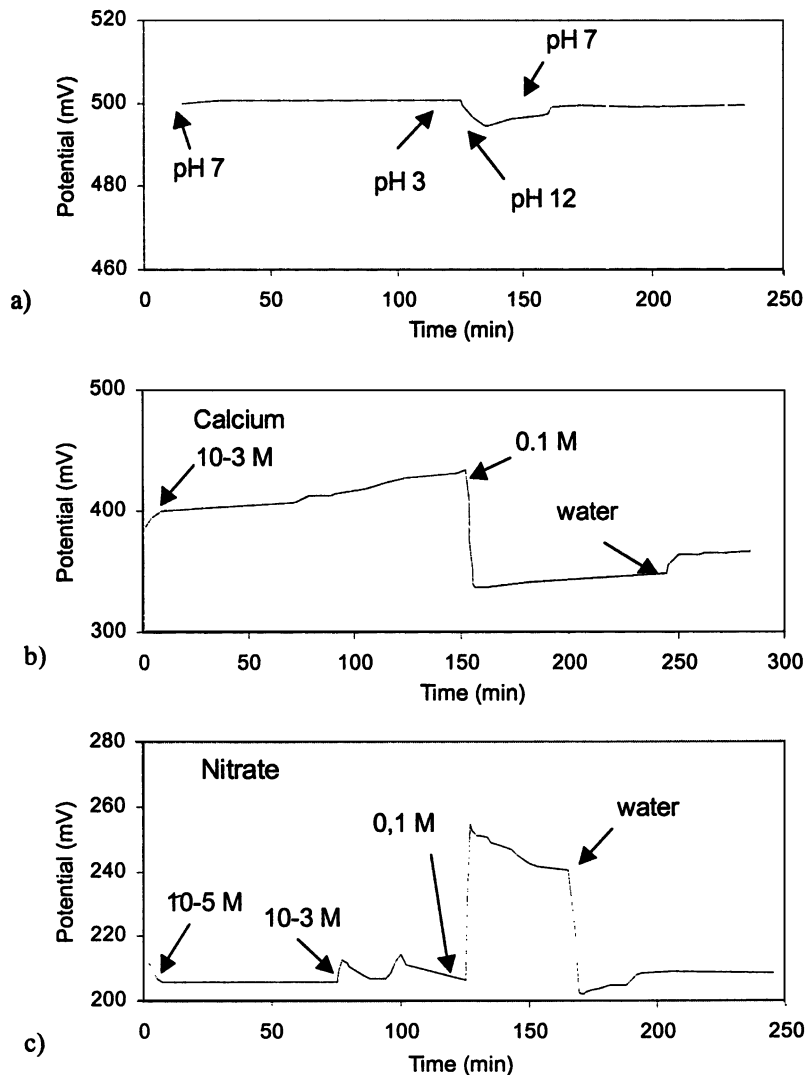
Fig. 4. Response of pH, Ca^{2+} and NO_3^--ISFETs in soil along the addition of fertiliser solutions (a) with different Ph, (b) with different calcium concentrations and (c) with different nitrate concentrations.

4. Conclusions

The results obtained from the evaluation of the ISFET sensors demonstrate the feasibility of this technology for sensor construction and the robustness of the photocurable membrane. The polymer used provides a membrane with similar chemical characteristics to those made with PVC but with some advantages. These include an easy and fast preparation procedure, the possibility of automation and

a better adhesion to the surface of the ISFET. This last consideration is very important for 'in-soil' applications since the insertion of the device could be the main cause of membrane damage. Additionally, a long sensor lifetime may be expected and hence long periods of unattended operation in the field.

The signal obtained during the time when ISFETs were inserted in soil was relatively stable. The dynamic response observed after the addition of several fertiliser solutions was fast and reproducible demonstrating proper sensor action in the designed cell. Sensor response characteristics were stable for two months, being continuously inserted in soil. During that time no membrane damage and no peel off was observed.

The results obtained from the comparative studies with standard methods demonstrate that, although high accuracy was not obtained, a correlation is obtained between both methods. It is important to point out that the main objective of this work was to demonstrate the feasibility of ISFET measurement directly in soil. In that sense, the similar order of magnitude obtained with both methods demonstrate that soil humidity is adequate to obtain a correct polarisation between ISFETs and reference electrode and that ISFET response mechanisms are correct. The small dimensions of the sensors contribute to this result.

The usefulness of this present application has focused on the rapid measurement of variations of nutrient levels in soil to control their excess and to provide intelligent irrigation and fertilisation management systems for crop production. ISFETs could be useful for environmental control purposes (i.e. to avoid lixiviation of nitrates into ground waters). All these applications require the placement of the measurement system in field to get results in real time. ISFETs can be used for this purpose as it is demonstrated in this work.

Acknowledgements

References

Abe, H., Eshashi, M., Matsuo, T., 1979. ISFETs using inorganic gate thin films. IEEE Trans. Electron Dev. ED-26, 1939.

Alegret, S., Bartrolí, J., Jiménez, C., Del Valle, M., Dominguez, C., Cabruja, E., Merlos, A., 1991. pH-ISFET with NMOS technology. Electroanalysis 3, 355.

Ansorena J., 1994. Substratos: propiedades y caracterización. In: Mundi-Prensa Libros S.A., (Eds.), Madrid.

Bergveld, P., 1970. Development of an ion-sensitive solid-state device for neurophysiological measurements. IEEE Trans. Biomed. Eng. BME-17, 70.

Bergveld, P., 1972. Development, operation and application of ion-selective field-effect transistor as a tool for electrophysiology. IEEE Trans. Biomed. Eng. BME-19, 42.

Bockris, J.O'M., Reddy, A.K.N., 1970. Modern Electrochemistry, vol. 1 and 2. Plenum Press, New York.

Bousse, L., De Rooij, N.F., Bergveld, P., 1983. Operation of chemically sensitive field-effect sensors as a function of the insulator electrolyte interface. IEEE Trans. Electron Dev. ED-30, 1263.

Bratov, A., Abramova, N., Muñoz, J., Dominguez, C., Alegret, S., Bartrolí, J., 1995a. Photocurable polymers matrices for potassium-sensitive ion-selective electrodes membranes. Anal. Chem. 67, 3589.

Bratov, A., Muñoz, J., Dominguez, C., Bartrolí, J., 1995b. Photocurable polymers applied as encapsulating materials for ISFET production. Sensors and Actuators B24–25, 823.

Brenes, N., Quigley, M.N., Reid, W.S., 1995. Determination of soil pH using a laboratory robot. Anal. Chim. Acta 310, 319.

Buck, R.P., Pungor, E., Buzas, I., 1978. Ion-Selective Electrodes. Elsevier, Amsterdam, pp. 21–55.

Cattrall, R.W., Iles, P.J., 1985. Photocurable polymers in ion-selective electrode membranes. Anal. Chim. Acta 169, 403.

Ferreira, A.M.R., Lima, J.L.C., Rangel, A.O.S.S., 1996. Potentiometric determination of total nitrogen in soils by flow injection analysis with a gas-diffusion unit. Aust. J. Soil Res. 34, 503.

Hesse, P.R., 1972. A textbook of soil chemical analysis. Chemical Publishing, New York.

Hongbo, C., Hansen, E.H., Ruzicka, J., 1985. Evaluation of critical parameters for measurement of pH by flow injection analysis. Anal. Chim. Acta 169, 209.

Janata, J., 1994. Twenty years of ion-selective field transistors. Analyst 119, 2275.

Jiménez, C., Marqués, I., Bartrolí, J., 1996. Continuous-flow system for on-line water monitoring using back-side contact ISFET-based sensor. Anal. Chem. 68, 3801.

Matsuo, T., Wise, K.D., 1974. An integrated field-effect electrode for biopotential recording. IEEE Trans. Biomed. Eng. BME-21, 485.

Moss, S.D., Janata, J., Johnson, C.C., 1975. Potassium, ion-selective field effect transistor. Anal. Chem. 47, 2238.

Moss, S.D., Johnson, C.C., Janata, J., 1978. Hydrogen, calcium and potassium ion-sensitive FET transducers: a preliminary report. IEEE Trans. Biomed. Eng. BME-25, 49.

Oesch, U., Caras, S., Janata, J., 1981. Field effect transistor sensitive to sodium and ammonium ions. Anal. Chem. 53, 1983.

Puig-Lleixà, C, Jimenez, C., Fabregas, E., Bartrolí, J., 1998. Potentiometric pH sensors based on urethane-acrylate photocurable membranes. Sensors and Actuators B49, 211.

Ruzicka, J., Hansen, E.H., Zagatto, E.A., 1977. Flow injection analysis. Part II: use of ion-selective electrodes for rapid analysis of soil extracts and blood serum. Determination of potassium sodium and nitrate. Anal. Chim. Acta 88, 1.

Soil and plant analysis Council, 1992. Handbook of reference methods for soil analysis. Athens.

Torres, P., Garcia-Mesa, J.A., Luque de Castro, M.D., Valcarcel, M., 1993. Determination of soil pH by use of a robotic station. Fresenius J. Anal. Chem. 346, 704.

Tietje-Girault, J., MacInnes, I., Schröder, M., Tennant, G., Girault, H.H., 1990. Photopolymerization of ion-selective membranes onto silicon nitride surfaces for ISFET fabrication. Electrochim. Acta 35 (4), 777.

Van den Vlekkert, H.H., Verkerk, U.H., Van der Wal, P.D., Van Wingerden, A., Reinhoudt, D.N., Haak, J.R., Honig, G.W.N., Holterman, H.A.J., 1992. Multi-ion sensing device for horticultural application based upon chemical modification and special packing of ISFETs. Sensors and Actuators B6, 34.

Van der Wal, P.D., Van der Berg, A., De Rooij, N.F., 1994. Universal approach for the fabrication of Ca^{2+}, K^+ and NO_3^- sensitive membranes ISFETs. Sensors and Actuators B18-19, 200.

DEVELOPMENT OF A MULTIPARAMETRIC ANALYSER BASED ON ISFET SENSORS APPLIED TO PROCESS CONTROL IN THE WINE INDUSTRY

J. Artigas, C. Jiménez, C. Domínguez, S. Mínguez, A. Gonzalo, J. Alonso

Development of a multiparametric analyser based on ISFET sensors applied to process control in the wine industry

Judit Artigas[a], Cecilia Jiménez[b,*], Carlos Domínguez[b],
Santiago Mínguez[c], Albert Gonzalo[c], Julian Alonso[a]

[a]*Grup de Sensors i Biosensors, Departament de Química, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain*
[b]*Institut de Microelectrònica de Barcelona (IMB-CNM) CSIC, Campus U.A.B., 08193 Bellaterra, Barcelona, Spain*
[c]*Institut Català de la Vinya i el Vi (INCAVI), c) Amàlia Soler 29, 08720 Vilafranca del Penedès, Barcelona, Spain*

Abstract

The continuous monitoring of processes in the food industry, and in particular in wine production, holds great interest as it can help to increase the quality of the goods produced and also the efficiency of the production process. One process that is feasible to control is the stabilisation of wine by means of ion-exchange resins. The use of this technique is of great promise since it allows continuous wine treatment and also on-line monitoring systems can be applied to determine the saturation of the resin in addition to the fraction of wine requiring stabilisation. The development of a pH, calcium and potassium analyser for on-line monitoring of wine in a continuous flow system is reported here. The potentiometric sensors used are ion-selective field-effect transistors (ISFETs). The stability of these sensors in hydro-alcoholic media was studied obtaining a sensitivity of 56 mV per decade for the pH ISFET and sensitivities of −26 mV per decade and −50 mV per decade for calcium and potassium sensors, respectively. The long-term stability of pH-ISFETs was 7 and 4 months for cation sensors. Results obtained in the continuous flow system with wine samples demonstrated that the proposed system is capable to detect the saturation point of the ion-exchange resin in wine stabilisation process and also to determine the ratio of wine required to stabilise it.
© 2002 Elsevier Science B.V. All rights reserved.

Keywords: Wine monitoring; Ion-exchange resin; Tartaric stabilisation; ISFET sensors; Continuous flow system

1. Introduction

Tartaric stabilisation is one of the key stages in wine treatment [1–3]. This stabilisation process minimises the effect of potassium bitartrate and calcium tartrate precipitation [4] in the bottle. Although the appearance of these crystals in wine bottles is a natural process, which does not affect either flavour or odour, it is considered undesirable by both consumers and producers. Physical techniques used to prevent the precipitation of potassium bitartrate or calcium tartrate involve cooling the wine and using separation membranes. Chemical techniques used to address this problem include the addition of metartaric acid [5]. The most widely used technique for the stabilisation of wine is artificial refrigeration [5,6]. However, this technique is expensive, as it requires two temperature changes: one to refrigerate and the other in the last filtration step where the precipitated crystals are separated. Precipitation is sometimes incomplete, as it is a slow process. Also, some enologists think that the organoleptic properties of the wine are affected by refrigeration, especially for wines with high polyphenol content.

Electrodyalisis and ion-exchange resins [3,4] are two novel techniques used for the efficient tartaric stabilisation of wine and are advantageous under the technical and economical point of view. Ion-exchange resins have been used since the 1960s in Australia, Canada and the United States as an attractive and reliable alternative process in wine making. The ion-exchange resin is capable of retaining calcium and potassium ions thus reducing their concentration in the wine and minimising the precipitation of tartrates. The use of ion-exchange resins offers advantages compared with traditional wine refrigeration methods such as the chance of continuous use and lower energy consumption.

The aim of the present work is to develop an industrial multiparametric analyser based on ion-selective field-effect transistors (ISFETs) coupled to a flow system. This analyser will be used to control the wine stabilisation process with ion-exchange resins by monitoring pH, calcium and potassium of the treated wine.

2. Experimental

2.1. Reagents and solutions

For the preparation of the membrane valinomycin, dioctylsebacate (DOS), bis-di(4-1,1,3,3-(tetra-methylbutyl)phenyl)phosphate (TMBPhPP), di-n-octylphenylphosphonate (DOPP) and potassium tetrakis (4-clorophenyl)borate (KTpClPhB), were used and supplied from Fluka. An aliphatic diacrylated urethane oligomer (Ebecryl 270) and a hexandiol diacrylate (HDDA) crosslinker were used as the polymer for membrane matrix. Both were donated by UCB Chemicals. The photo initiator 2,2'-dimethoxy phenylacetophenone (Irgacure 651) was obtained from Ciba-Ceigy.

Calibration of calcium and potassium ISFETs was carried out using $CaCl_2$ and KNO_3 0.01, 0.1 and 1 M stock solutions. Different volumes of these solutions were added to a background solution to change ion concentration in a range of 3.10^{-6} to 10^{-2} M. A universal buffer containing 0.1 M KNO_3 as a background was used for pH ISFETs calibration. Adding small amounts of 0.1 KOH changed the pH. A saturated solution of 15% ethyl alcohol with 1400 ppm of potassium bitartrate was used to study the effect of ethanol content in the sample. All reagents were analytical grade. Aqueous solutions were prepared with deionised water.

Wine samples were red and white wines of the Penedés (Barcelona, Spain) domain that had been previously clarified and filtrated prior to filling the column. To saturate the resin of the column, from 15 to 20 l of wine were used in laboratory experiments and more than 250 l of wine were used in the pilot plant.

2.2. Apparatus and devices

The signal was measured using an ISFETmeter supplied by the IMB. The drain voltage and the drain current were fixed at 0.5 V and 100 μA, respectively. The gate voltage was measured relative to the reference electrode, an Ag/AgCl double junction electrode (ORION 900200). The response was controlled by a PC computer connected to the ISFETmeter and a control programme based on the LabView graphic language (National Instruments, Austin, TX) was used to record sensor measurements. ISFETs with a pH-sensitive Si_3N_4 gate were fabricated in the IMB following standard NMOS technology [7]. They were fixed in a printed circuit board and encapsulated with a photopolymer using photolithographic technics [8].

2.3. Membrane preparation and deposition

To obtain calcium [9]and potassium [10] sensors, membranes based on photocurable polymers were prepared. These polymers have been studied previously and have demonstrated to be compatible with sensor components and highly adherent to ISFET surface [11,12]. The prepolymer solution was prepared by mixing Ebecryl 270 and HDDA in an 87:13 ratio. This prepolymer solution was mixed with the plasticiser, the ionophore and the photoinitiator, all dissolved with tetrahydrofuran and stirred in an ultrasonic bath for homogenisation. The membrane cocktail was then deposited on the ISFET gate and exposed for 30 s to UV light (365 nm and 22 mN cm^{-2}). The unpolymerised membrane was removed by washing with ethanol. Both types of sensors were conditioned for 1 h in 0.1 M $CaCl_2$ and KNO_3 and they were stored between calibrations in a 10^{-3} M solution.

The calcium membrane composition was (% in weight) 38.4% Ebecryl 270 and HDDA, 55.1% di-n-octylphenylphosphate, 2.4% Irgacure 651 and 4.1% bis-(4-1,1,3,3-(tetra-methylbutyl)phenyl) phosphate. The composition of the potassium membrane was 48.4% Ebecryl 270 and HDDA, 46.4% dioctylsebacate, 2.3% Irgacure 651, 2.3% valinomycin and 0.6% potassium tetrakis (4-chlorophenyl)borate.

2.4. Cationic exchange resins

In order to optimise the functioning of the flow system in the laboratory a chromatography glass column that contained two sampling points and had 1 l capacity was used. The column was 50 cm in length with an internal diameter of 2.3 cm. For the pilot plant study, a stainless steel column was used. It had two sampling points, one at the top to control wine before treatment and another at the bottom to monitor saturation of resin. The column had a capacity of 24 l and a length of 70 cm with an internal diameter of 10.5 cm.

The resin used in the glass column was a cationic exchange resin made of polyester copolymerised with divinylbenzene and sulphonic acid as a functional group. The physicochemical features of the resin were the following: a density of 1.278 Kg/l, an exchange capacity of 3.56 meq./g and an average particle radius of 0.69 mm.

Prior to filling the column, the resin was wetted with deionised water for 12 h. Regeneration of the column resin was achieved using a 5% solution of hydrochloric acid which was passed through in a reverse direction at a flow rate of 4 l/h. Finally, it was cleaned using water at a flow rate of 4.5 l/h until the conductivity of the eluent was below 100 μS/cm or the pH was close to neutral.

2.5. Flow system manifold

Two channels formed the flow system: one channel carrying the sample and other channel used to adjust the ionic strength and the pH of the sample with a 0.05 M Tris
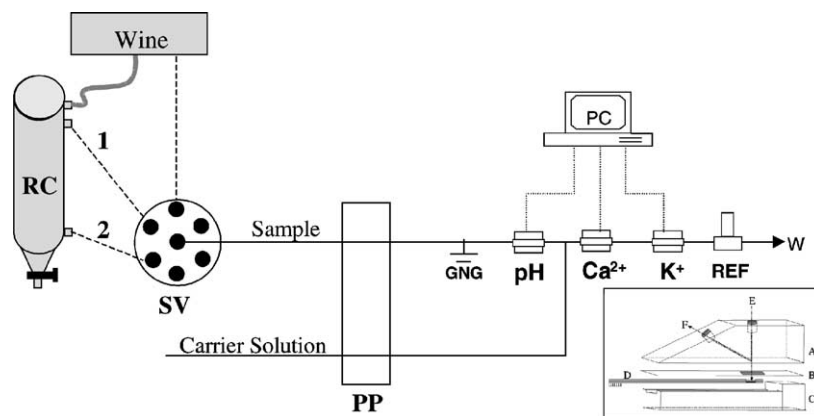
Fig. 1. Schematic representation of the flow injection system. RC: resin column with two sampling points, (1) wine before stabilisation and (2) treated wine; SV: solenoid valve; PP: peristaltic pump; GNG: grounding electrode. The enclosed box contains a scheme of the assembly to integrate the ISFET in the flow system: (A, C) methacrylate block showing (D) an entry channel perpendicular to the sensitive surface of the ISFET and (F) an exit channel at a 45° angle; (B) silicone sheet.

pH 7.5 buffer. A four channel peristaltic pump (Gilson, Minipuls3) and a six-way valve (Hamilton, MVP) were used. The flow system manifold is shown in Fig. 1. The ISFETs were integrated into the flow system using a home-made methacrylate assembly [13].

3. Results and discussion

3.1. Effect of the sample matrix on sensors

As it is known that polymers are susceptible to degradation from organic solvents, the effect of ethanol on the polymer chosen to encapsulate and to build the membranes of ISFET was studied. ISFETs devices sensitive to pH,

calcium and potassium were placed in a 15% ethanol solution saturated with potassium bitartrate. To investigate the lifetime and the response of these ISFETs periodic calibrations were carried out. In Table 1, it is shown the response characteristics of sensors over time. This data indicates that ethanol does not affect on the polymer used for encapsulation and membrane production for at least 8 months. The lost in sensitivity associated with calcium and potassium ISFETs after the fourth month suggest leaching of the ionophore from the membrane.

Leakage currents from the ISFET gate to the solution were studied also to appraise on the integrity of the encapsulation in the ethanol solution. The entrance of liquid between the device surface and the encapsulating layer interface was the cause of the increase of current on the
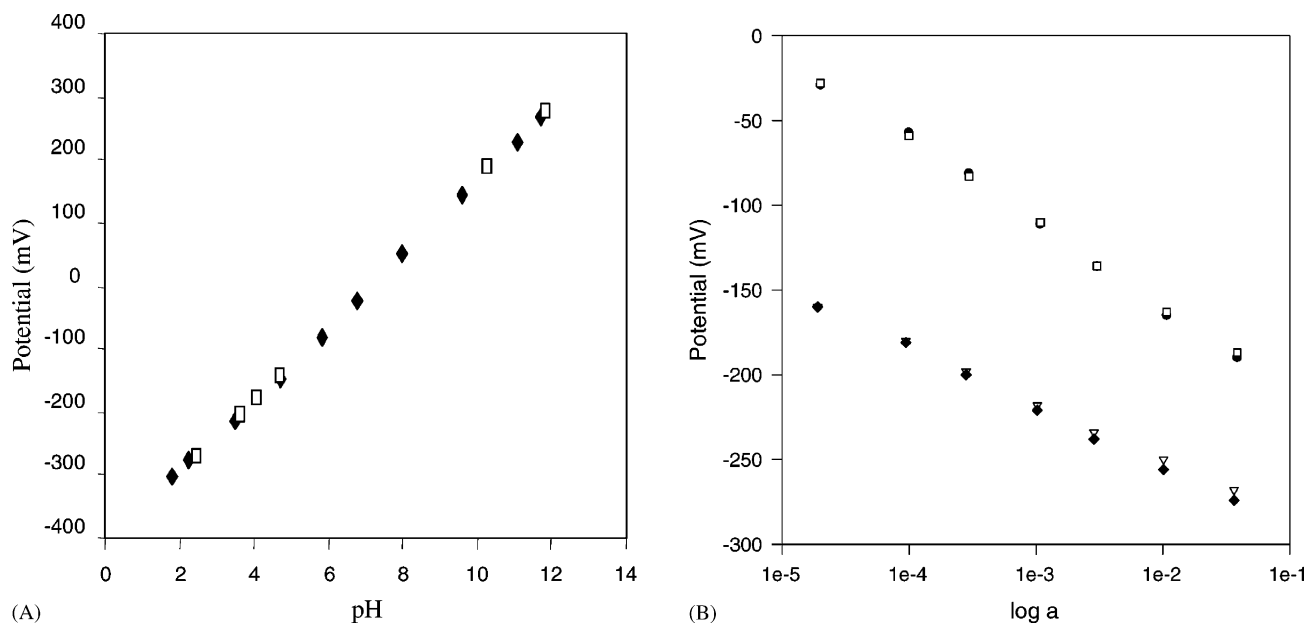

Fig. 2. Calibration curves of (A) pH ISFET in an aqueous solution (◆) and in an ethanol solution (□). (B) Calcium and potassium ISFET in an aqueous solution (□, ▽) and in a ethanol solution (◆, ▼), respectively.

Table 1
Response characteristics for calcium, potassium (up to 4 months) and pH (up to 8 months) ISFETs kept in an ethanol solution

	Ca^{2+}	K^+	pH
Sensitivity[a]	-26 ± 4	-50 ± 4	55.9 ± 0.2
(mV per decade)	($n = 60$)	($n = 70$)	($n = 75$)
Linear range	5×10^{-6} to	7×10^{-5} to	2–12
	8×10^{-2} M	8×10^{-2} M	

[a] Sensitivities are given as the mean of n calibrations and the uncertainly intervals are calculated at 95% confidence level.

Table 2
pH values obtained with the pH ISFET and a glass electrode for white (W) and red (R) wine samples

Samples	pH-ISFET	Glass electrode
W1	3.30	3.29
W2	3.23	3.15
W3	3.24	3.16
W4	2.94	2.99
R5	3.66	3.69
R6	4.21	4.09
R7	3.67	3.71
R8	3.88	3.91
R9	3.59	3.57

Results of t-paired test is shown below ($P = 0.05$). t-paired test: $t_{cal} = 0.875$; $t_{tab} = 2.31$.

ISFET gate, causing the disruption of the sensor. pH ISFETs were stable and no leakage currents appeared until the 8 month of the study.

To test the reliability of ISFETs response in wine, a calibration in a solution containing ethanol and saturated with potassium bitartrate was performed. This was compared with one performed in a standard aqueous solution. As seen in Fig. 2, there are no significant differences between the two sets of measurements.

Finally, the pH of white and red wines was measured using ISFETs and glass electrodes. Results are shown in Table 2. Values obtained using t-paired test show no significant differences between both sensors.

3.2. Study of the flow system

The flow system manifold described in Fig. 1 was used to control the process of wine stabilisation using exchange resins. Every 3 min a sequence of two wine samples was inserted in the system. These samples were of wine before treatment (point 1, Fig. 1) and eluent wine exiting the column (point 2, Fig. 1). The response of the wine prior to the treatment was used as a baseline against the response of the treated wine. The eluent wine is referred to determine when the resin has reached saturation. It is known that the pH, calcium and potassium concentrations will vary in the wine while passing through the column due to the ion exchange. Wine cations will be retained in the column liberating protons and, hence, the eluent wine becomes more acidic while the calcium and potassium concentrations will decrease. At the saturation point of the column, the wine exiting it will have the same qualities as the wine that has not been treated.

To make the set-up as simple as possible, a single channel flow system was used initially, passing the sample directly through the sensors. In Fig. 3, it is shown the response of the three sensors in the flow system. From all them a saturation time of 150 min was observed.

After this previous study, it was observed that the change of pH in the eluent wine affected on the calcium and potassium sensor response. The concentration of both cations drops suddenly and largely in the treated wine and under these conditions the response of the sensors is greatly affected by the pH of the sample (approximately 2.5). As seen in Fig. 4A, the signal from calcium and potassium sensors of the eluent wine is not constant due to this pH effect. For this reason, a second conditioning channel of 0.05 M Tris at pH 7.5 was added downstream the pH ISFET to minimise the effect of the pH on the calcium and potassium sensors. The effect of buffering the sample is shown in Fig. 4B, where the response of the calcium sensor in the eluent wine is stable. This response was similar for potassium sensor.

The pH sensor was calibrated after the column was saturated to measure the pH of the wine exiting the column.
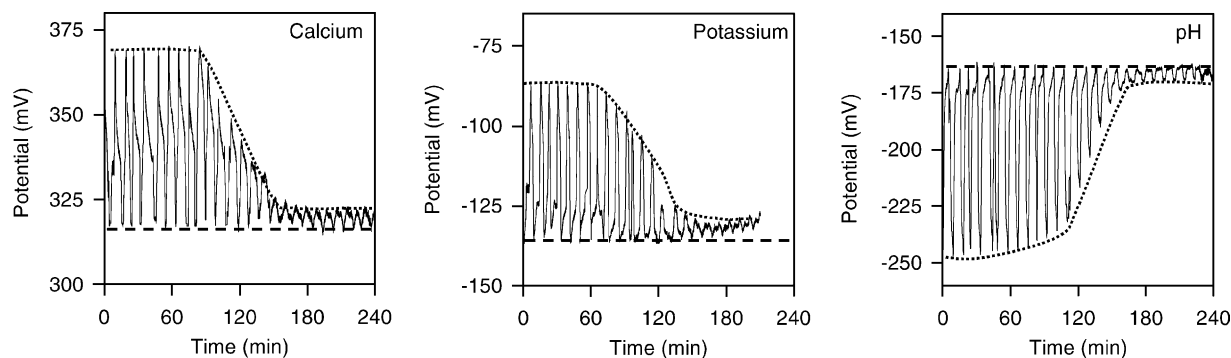

Fig. 3. Response of pH, calcium and potassium ISFETs in the flow system for untreated wine (- - -) and treated wine exiting the column (···). When the response of the two samples is almost equal corresponds to the saturation point of the resin.
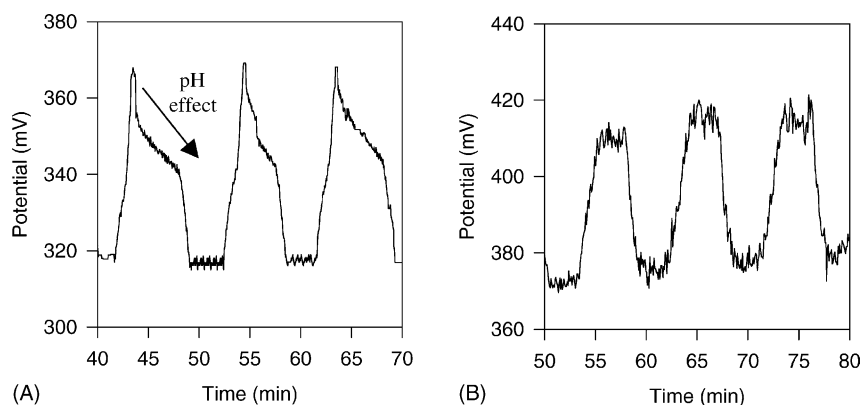
Fig. 4. Response of calcium ISFET to treated wine showing: (A) the pH effect; (B) response using an adjusting channel with 0.05 M Tris at pH 7.5. It can be observed that pH adjustment has a positive influence on the response.

Fig. 5 shows a recording of the pH ISFET response for the wine entering and exiting the column until it arrives to the saturation point and the calibration of the pH ISFET using two standards of pH 7.02 and 4.00. From this data, it has been obtained the pH of the treated wine (pH 2.67) and that of the untreated wine (pH 3.61). These values are in agreement with those obtained using a glass electrode.

3.3. Pilot plant

A pilot plant was installed in a warehouse of INCAVI (Vilafranca del Penedès, Barcelona, Spain) to study the performance of the system in an industrial setting. An electric pump was used to maintain a constant wine flow rate of 48–50 l/h. Samples were taken continuously at the top and the bottom of the column using a Teflon tube

and a filter to prevent blocking of the channel by resin particles.

Calcium, potassium and pH ISFETs were calibrated before being placed in the measuring cell following a batch approach. For that two solutions were used: one of pH 4 with 0.05 M calcium and potassium concentration and another solution of pH 7 and 0.005 M calcium and potassium concentration. The sensitivities obtained were −28, −40 and 50 mV per decade, respectively.

Samples corresponding to untreated and treated wine were passed sequentially every 2 min. In Fig. 6, it is shown the response of sensors through a wine stabilization process and the point when the column saturates after treating more than 250 l of wine. Once the resin was saturated, sensors were calibrated using the two solutions mentioned above. The sensitivities shown by the calcium, potassium and pH
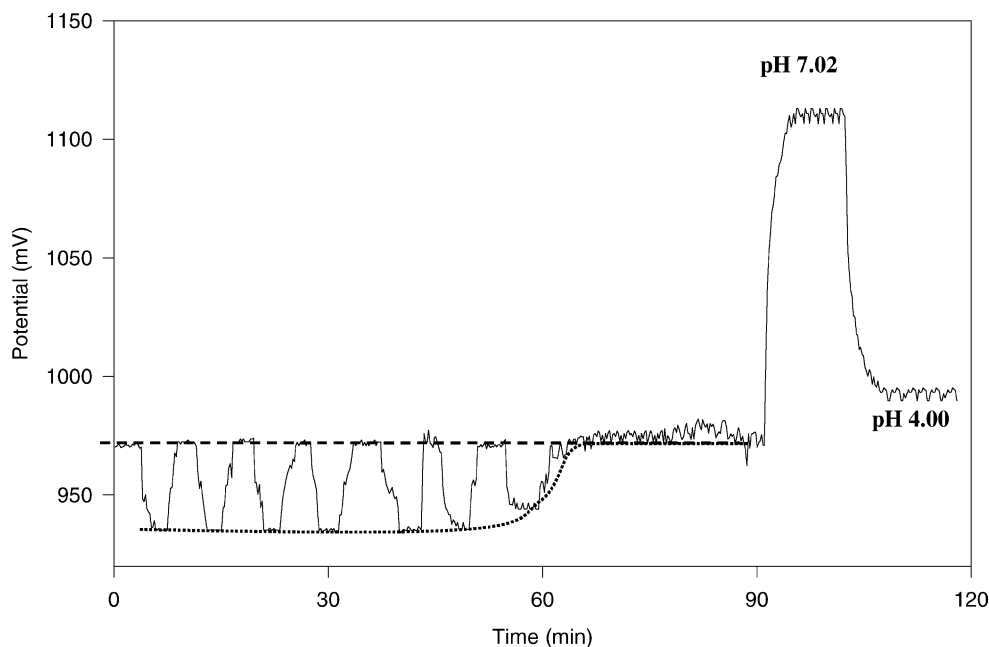

Fig. 5. Response of the pH ISFET in the flow system to untreated wine (- - -), to wine exiting the columns (···) and recording of a calibration with pH 7.02 and 4.00 buffers.
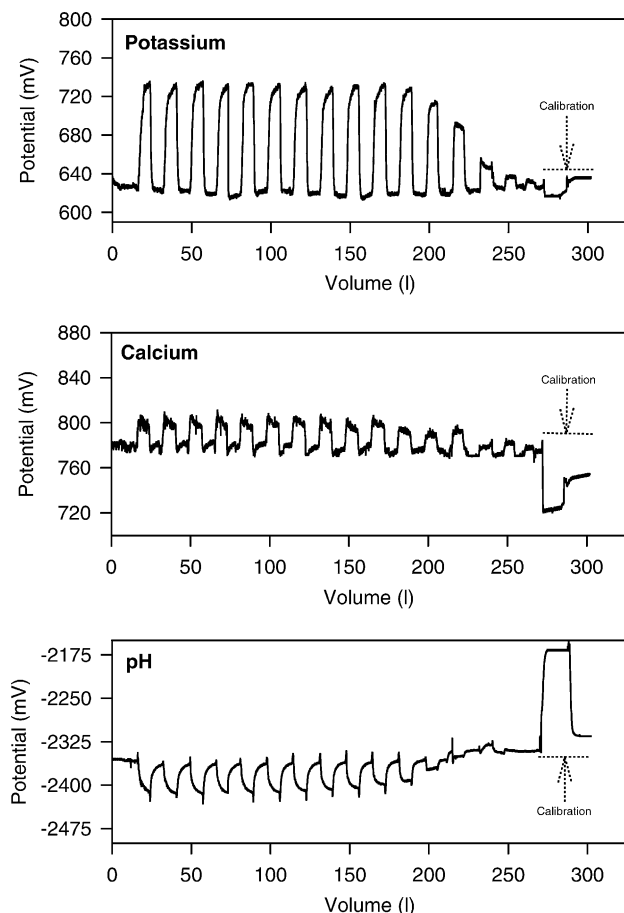
exchange resins for this process has demonstrated to be a viable technique reinforced by the chance of being continuously monitored. In addition to this, the pH, calcium and potassium sensors based on ISFET are capable of measuring the point the resin saturates in real time, hence, enabling the automation of the process and the use of sequential columns for the chain stabilisation of wine. The developed system is robust and simple to be used in a production setting.

Besides, the stability of ISFETs sensors working in such an extreme conditions demonstrate the reliability of technology and materials used for sensor fabrication.

Acknowledgements

Fig. 6. Response of sensors in the flow system implemented in the pilot plant. The saturation of the resin is produced after 250 l of wine pass through the column. Last two peaks correspond to the calibration of sensors using two buffers of pH 7 with 0.05 M calcium and potassium and pH 4 with 0.005 M calcium and potassium.

sensors were -29, -30 and 49 mV per decade, respectively. Ion concentrations and pH of wine samples were calculated from these calibrations. The pH of wine before and after treatment was 3.27 and 2.18, respectively. The potassium concentration measured before and after wine treatment was 9.1×10^{-3} and 7.0×10^{-5} M, respectively, indicating a high rate of potassium retained in the resin. For calcium, the concentration measured before the treatment was 9.3×10^{-4} M and after the treatment was 1.4×10^{-4} M. Results obtained from calibration parameters indicate that for the time of analysis (4–5 h) ISFETs are quite stable despite the extreme conditions that are endured.

4. Conclusions

The results produced above the possibility of developing a multiparametric analysis system for use in the wine industry to control the tartaric stabilisation of wine. The use of ion-

References

[1] H.F. Linskens, J.F. Jackson (Eds.), Wine Analysis, Springer, Berlin, 1988.

[2] C. Flanzy, Enología Fundamentos Científicos y Tecnológicos, AMV Ediciones Mundi Prensa, Madrid, 2000.

[3] A. Niederbacher, Wine in the European Community, 2nd ed., Luxembourg Office for Official Publications of the European, Luxembourg, 1988.

[4] Kirby Moulton, Research Needs and Expectations in the Grape and Grape Products Sector, World Conference on Horticultural Research, Rome, Italy, 17–20 June, 1988.

[5] G.J. Mulder, in: H.B. Jones (Ed.), The Chemistry of Wine, London, 1974.

[6] A. Brugirard, J. Rochard, Aspects practiques des traitements thermiques des vins, Bourgogne publications, Paris, 1991.

[7] S. Alegret, J. Bartrolí, C. Jiménez, M. Del Valle, C. Domínguez, E. Cabruja, A. Merlos, pH ISFET with NMOS technology, Electroanalysis 3 (1991) 355–360.

[8] A. Bratov, J. Muñoz, C. Domínguez, J. Bartrolí, Photocurable polymers matrices applied do encapsulating materials for ISFETs productions, Sens. Actuators B 24–25 (1995) 823–825.

[9] J. Artigas, A. Beltran, C. Jiménez, J. Bartrolí, J. Alonso, Development of a photopolymerisable membrane for calcium ion sensors: applications to soil drainage waters, Anal. Chim. Acta 426 (2001) 3–10.

[10] A. Bratov, J. Muñoz, C. Domínguez, J. Bartrolí, Photocurable polymer matrices for potassium-sensitive ion-selective electrode membranes, Anal. Chem. 67 (1995) 3589–3595.

[11] A. Beltran, J. Artigas, C. Jiménez, R. Mas, J. Bartrolí, J. Alonso, Development of a durable nitrate-selective membranes for all-solid state ISE and ISFETs sensors based on photocurable composition, Electroanalysis 14 (3) (2002) 213–219.

[12] R.W. Cattrall, P.J. Iles, Photocured polymers in ion-selective electrode membranes, Anal. Chim. Acta 169 (1985) 403–406.

[13] S. Alegret, J. Bartrolí, C. Jiménez, M. Del Valle, C. Domínguez, E. Cabruja, A. Merlos, Flow-through pH-ISFET as detector in automated determinations, Electroanalysis 3 (1991) 349–354.

ANALYSIS AND IDENTIFICATION OF SEVERAL APPLE VARIETIES USING ISFETS SENSORS

J. Artigas, C. Jiménez, J. Alonso

ELSEVIER

Analysis and identification of several apple varieties using ISFETs sensors

J. Alonso [a,*], J. Artigas [a], C. Jimenez [b]

[a] *Grup de Sensors i Biosensors, Departament de Química, Universitat Autònoma de Barcelona, 08193 Bellatera, Spain*
[b] *Institut de Microelectrònica de Barcelona (IMB-CNM), CSIC, Campus Universitat Autònoma de Barcelona, 08193 Bellatera, Spain*

Abstract

There are a number of variables that are useful to determine the optimal moment for the fruit collection such as starch content, sugar content, acidity and firmness. Other variables, including calcium and potassium concentrations, may establish better the state of ripeness of fruit and can help to optimise the collection process as well as augmenting the nutritional value of fruits. At present, these novel parameters cannot be used for the control of fruit collection due to the slow standard methods required. The need for in situ and in real-time ion measurements calls for fast response sensors and simpler and portable instrumentation. Solid-state sensors respond to these requirements. This work describes the application of ISFET sensors to analyse calcium, potassium and nitrates in several apple varieties, both in juice and in situ fruit. Results show that the analysis of potassium, calcium and nitrate permits to distinguish among apple varieties and can also be used to determine correctly the concentrations of these ions.
© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Calcium, potassium and nitrate ISFETs; Photocurable membranes; Fruit analysis

1. Introduction

Fruit are an excellent food for our diet. Their quality is defined by physical (such as texture, size, colour and odour) and chemical parameters (such as sugar content, starch content, carbohydrates, lipids or vitamins). These factors are affected by the variety of the fruit, the ripeness state, the cultivation conditions and the climate. The analy-

sis carried out to evaluate the fruit quality will determine the optimal collection time and will also be useful for control and inspection purposes [1–3].

Collection time is identified mainly by different parameters that define the fruit composition. There are physiological, physical, climatologic and chemical ripeness factors. Physiological indicators involve the use of sensorial assays to determine colour, flavour and the easiness with which the fruit is detached from the tree. Physical indicators involve assays that determine the texture and the colour of the flesh. Climate indicators consider the age of the fruit and the time elapsed

* Corresponding author. Present address: Universitat Autonoma Barcelona, Quimica Analitica, Edifici Cn, 08228 Bellaterra, Spain. Tel.: +34-93-5812149; fax: +34-93-5812127.
E-mail address: julian.alonso@uab.es (J. Alonso).

since the moment of maximal floration of the plant. Finally, chemical indicators determine total acidity, sugar index and starch content.

With the exception of chemical and texture analysis, the rest of the assays are done directly by fruit collectors in the field. But the rest of the analyses are the tests that determine basically the ripeness-state of the fruit and its nutritional value [4–6]. Actually, only acidity, sugar and starch content are measured quickly to relate it with the fruit collection. Other key parameters (such as calcium, potassium, sodium, nitrogen and phosphor) are measured in specialised laboratories, obtaining high-precision results but days or months later. The development of new analytical tools for field-use would make available more control parameters for optimisation of fruit collection. Table 1 shows a set of standard analysis carried out to determine the nutritional value of fruit.

This work proposes the use of solid-state sensors as a complementary analytical technique for a better determination of collection time. Analyses are done together with the standard ripeness assays. For this reason, measurements with IS-

FETs were carried out in apple juice and directly in situ, using three varieties of apples involved in different fertilisation procedures. Photocurable polymers based on polyurethanes were used to obtain calcium, potassium and nitrate membranes.

2. Experimental

2.1. Reagents and solutions

For the preparation of the membrane, valinomycin, dioctylsebacate (DOS), bis-di(4-1,1,3,3-(tetra-methylbutyl)phenyl)phosphate (TMBPhPP), di-n-octylphenylphosphonate (DOPP), trioctylphosphonate (TOP) and potassium tetrakis(4-chlorophenyl)borate (KTpClPhB) were used and supplied from Fluka. Tetraoctylammonium nitrate (TOAN) was synthesised in our research group. An aliphatic diacrylated urethane oligomer (Ebecryl 270) and a hexanediol diacrylate (HDDA) cross-linker were used as polymer for membrane matrix, and UCB Chemicals donated both. The photo initiator 2-2′-dimethoxyphenylacetophenone (Irgacure 651) was obtained from Ciba-Ceigy.

All reagents were of analytical grade. Aqueous solutions were prepared with deionised water.

2.2. Methodology

Calibrations of calcium, potassium and nitrate ISFETs were performed by adding known volumes of 0.01, 0.1 and 1 M $CaCl_2$ and KNO_3 standard solutions to 25 ml of a buffer 0.01 M Tris of pH 7.5.

Two methods were used to determine the concentration of the sample: standard addition for juice analysis and direct potentiometric for in situ measurements. To minimise the effect of the matrix on the measurement, juice analysis was done using the standard addition method. The ISFET and the double junction Ag/AgI reference electrode (ORION 90-00-02) were placed in 10 ml of juice and known volumes of 0.1 M $CaCl_2$ and 1 M KNO_3 were added. The measurements in situ were obtained inserting the ISFET and the refer-

Table 1
Brief description of some parameters and methods used in the control and inspection of fruits

Parameter	Method description
pH	Potentiometric measurement with glass electrode (20 °C), after elimination of CO_2 (waving)
Total acidity	Potentiometric titration with NaOH, to pH 8.1
Sugar	Tabulated value for refraction index in °Brix (refractometers)
Firmness or texture	Penetrometer (kg or N)
Ascorbic acid	HPLC and detection of 268 nm
Sorbic acid	HPLC and detection of 230 nm
Density	Pycnometer measurement
Soluble-solid	Tabulated value (g l^{-1}) according to density
Total nitrogen	Kjeldahl method (wt.%)
Ashes	Dehydration and incineration (wt.%)
Phosphor	Calorimetric measurement (650 nm) with molybdate ammonium
Calcium	Analysis of ashes using FES
Potassium	Analysis of ashes using AAS

Table 2
Products and doses used to fertilise the apple orchards

Treatment	Product	Applications and frequency	%CaO p/v	ml/hl for application
2	Calibitt	Seven applications in 15 days	17.36	1000
3	Calibitt	13 applications in 10 days	17.36	1000
4	Nutropit	Seven applications in 15 days	43.00	500
5	Nutropit	13 applications in 10 days	43.00	500
6	Nutropit	Seven applications in 15 days	43.00	1000
7	Nutropit	13 applications in 10 days	43.00	1000

Treatment 1 was blank.

ence electrode (copper wire) to a depth of 1–1.2 cm in two apples of each variety.

2.3. Instrumentation

The signal was measured using an ISFETmeter supplied by the IMB-CNM. The drain voltage and the drain current were fixed at 0.5 V and 100 μA, respectively. The gate voltage was measured relative to the reference electrode, a Ag/AgCl double junction electrode (ORION 900200) and a copper wire. ISFETs with a pH-sensitive Si_3N_4 gate were fabricated in the IMB following standard NMOS technology [7]. They were previously encapsulated with a photopolymer using photolithographic techniques, as described in Ref. [8].

2.4. Membrane preparation

To obtain calcium [9], potassium [10] and nitrate [11] sensors, membranes based on photocurable polymers were prepared. These polymers have been studied previously and have demonstrated a good compatibility with sensor components and a high adhesion to ISFET surface [12,13]. The prepolymer solution was prepared by mixing Ebecryl 270 and HDDA in an 87:13 ratio. This prepolymer solution was mixed with the plasticiser, the ionophore and the photo initiator, all dissolved with tetrahydrofuran and stirred in an ultrasonic bath for homogenisation. The membrane cocktail was then deposited on the ISFET gate and exposed for 30 s to UV light (365 nm and 22 mN cm^{-2}). The unpolymerised membrane was removed with ethanol. Both types of sensors were conditioned for 1 h in 0.1 M $CaCl_2$ and KNO_3 solutions and stored between calibrations in 10^{-3} M solution.

The composition of the calcium [9] membrane was (in wt.%) 38.4% Ebecryl 270 and HDDA, 55.1% di-*n*-octylphenylphosphate, 2.4% Irgacure 651 and 4.1% bis-(4-1,1,3,3-(tetramethylbutyl)phenyl) phosphate. The composition of the potassium [10] membrane was 48.4% Ebecryl 270 and HDDA, 46.4% dioctylsebacate, 2.3% Irgacure 651, 2.3% valinomycin and 0.6% potassium tetrakis(4-chlorophenyl)borate. The nitrate [11] membrane composition was 34.7% Ebecryl 270 and HDDA, 59.4% trioctylphosphonate, 2.0% Irgacure 651 and 3.9% tetraoctyl-ammonium nitrate.

2.5. Ripeness analysis: sugar index, starch index, texture and total acidity

Texture and starch measurements were done from pure fruit. To determine starch content, the fruit was sectioned horizontally. One-half was left in contact with a solution of iodide–iodate solution, for 5 min. The parts of the fruit with high starch content are coloured. The starch index results from comparing the colour fruit with starch colour tables. Texture measurements are made with a penetrometer and are proportional to the force needed to stick it to a depth of 5 cm.

To produce the juice, two axials of 20 fruits (all of same variety and treated) were blended and filtered. A part of the juice was used for the sugar measurements, total acidity and the calcium, potassium and nitrate concentrations. The rest was dehydrated and sent for analysis to the Laboratori Agroalimentari de Cabrils (Cabrils, Barcelona, Spain).

Total acidity was measured by a potentiometric titration of 10 ml of juice with 10^{-3} M NaOH, until a pH of 8.1 was attained. Sugar content is determined by measuring the refraction index (°Brix) of the juice using a refractometer.

2.6. Description of the samples and the fertilising process

Measurements were done from September to October 1999 at La Tallada (Mas Badia, Girona, Spain) using three apple varieties (Granny Smith, Smoothee and Fuji Chofu No. 2). To obtain a higher firmness of the product and also to prevent the onset of Bitter Pit and other diseases, the composition of the fertiliser was altered (products and doses, as seen in Table 2). A total of 28 cultivated lots with Smoothee apples (seven treatments with four repetitions each) were studied.

3. Results

3.1. Response characteristics for calcium, potassium and nitrate sensors

The ISFETs developed were previously evaluated in the aqueous solution to establish the response characteristics and their feasibility for fruit analysis application. The results for calcium, potassium and nitrate sensors are detailed in [9–11], respectively.

The sensitivity in all cases was quasi-Nernstian and the linear range was wide enough for fruit analysis application. The sensor response was stable and fast, and good selectivity coefficients were obtained.

3.2. Application of sensors to identify varieties of apples

To test if the concentration of several ions, measured in situ, permits to identify the apple variety previously calibrated nitrate and potassium ISFETs were inserted in a depth of 1–1.2 cm in each analysed apples. Series of 10 apples each of the variety Granny Smith, Smoothee and Fuji

Table 3
Values of sugar and starch levels for 10 fruit of the Granny Smith, Smoothee and Fuji Chofu No. 2 varieties

Granny Smith					Smoothee					Fuji Chofu No. 2		
No.	Sugar	Starch	NO_3^- (M)	K^+ (M)	No.	Sugar	Starch	NO_3^- (M)	K^+ (M)	No.	Sugar	Starch	NO_3^- (M)
1	13.6	5	$2.7\times10^{-3}\pm3\times10^{-4}$	0.01 ± 0.008	1	17	6	$1.01\times10^{-3}\pm3\times10^{-5}$	0.011 ± 0.008	1	16.4	9	$3.7\times10^{-4}\pm4\times10^{-5}$
2	13	6	$1.48\times10^{-3}\pm3\times10^{-5}$	0.03 ± 0.001	2	17.6	7	$1.01\times10^{-3}\pm3\times10^{-5}$	0.014 ± 0.009	2	17	9	$4.3\times10^{-4}\pm3\times10^{-5}$
3	12.4	5	$1.48\times10^{-3}\pm4\times10^{-5}$	0.03 ± 0.001	3	16.2	9	$7.1\times10^{-4}\pm7\times10^{-5}$	0.014 ± 0.008	3	15.8	10	$3.8\times10^{-4}\pm5\times10^{-5}$
4	12.4	6	$1.78\times10^{-3}\pm3\times10^{-5}$	0.025 ± 0.008	4	15.2	10	$5.8\times10^{-4}\pm3\times10^{-5}$	0.013 ± 0.008	4	17.2	10	$2.9\times10^{-4}\pm3\times10^{-5}$
5	12	6	$2.61\times10^{-3}\pm3\times10^{-5}$	0.028 ± 0.008	5	14	10	$5.8\times10^{-4}\pm3\times10^{-5}$	0.015 ± 0.008	5	18.2	9	$4.3\times10^{-4}\pm3\times10^{-5}$
6	12.8	5	$2.2\times10^{-3}\pm2\times10^{-4}$	0.026 ± 0.008	6	16.8	9	$4.2\times10^{-4}\pm3\times10^{-5}$	0.024 ± 0.009	6	16.2	10	$3.4\times10^{-4}\pm3\times10^{-5}$
7	12.2	5	$1.5\times10^{-3}\pm3\times10^{-4}$	–	7	17	8	$4.6\times10^{-4}\pm3\times10^{-5}$	0.019 ± 0.008	7	15.4	9	$2.5\times10^{-4}\pm4\times10^{-5}$
8	11.8	6	$1.55\times10^{-3}\pm3\times10^{-5}$	–	8	15	10	$4.6\times10^{-4}\pm3\times10^{-5}$	0.019 ± 0.008	8	18.4	10	$4.5\times10^{-4}\pm3\times10^{-5}$
9	13	6	$1.77\times10^{-3}\pm3\times10^{-5}$	0.039 ± 0.008	9	15	8	$6.0\times10^{-4}\pm3\times10^{-5}$	0.011 ± 0.008	9	17.4	9	$5.3\times10^{-4}\pm3\times10^{-5}$
10	11	6	$1.90\times10^{-3}\pm3\times10^{-5}$	0.039 ± 0.008	10	16	9	$5.0\times10^{-4}\pm3\times10^{-5}$	0.018 ± 0.008	10	16	9	$2.3\times10^{-4}\pm3\times10^{-5}$

Concentrations (M) and the interpolation error (95% confidence level) obtained with nitrate and potassium ISFETs in situ placed at a depth of 1–1.2 cm. Sugar, refraction index (°Brix); starch, starch regression index.
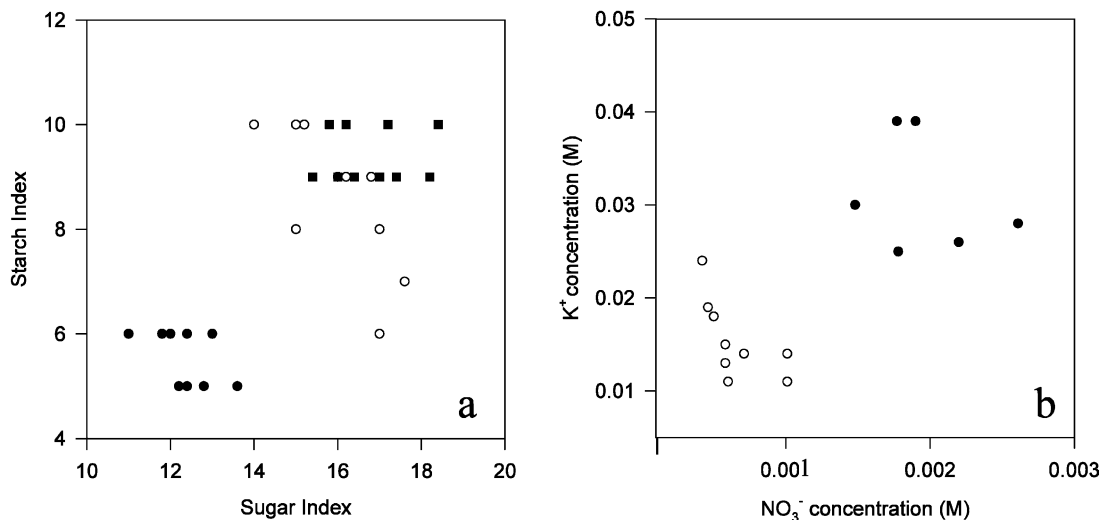
Fig. 1. Plot combining data of (a) sugar and starch indexes for the varieties (●) Granny Smith, (○) Smoothee and (■) Fuji Chofu No. 2 and (b) potassium and nitrate concentration values obtained in situ using the ISFETs for the varieties (●) Granny Smith and (○) Smoothee.
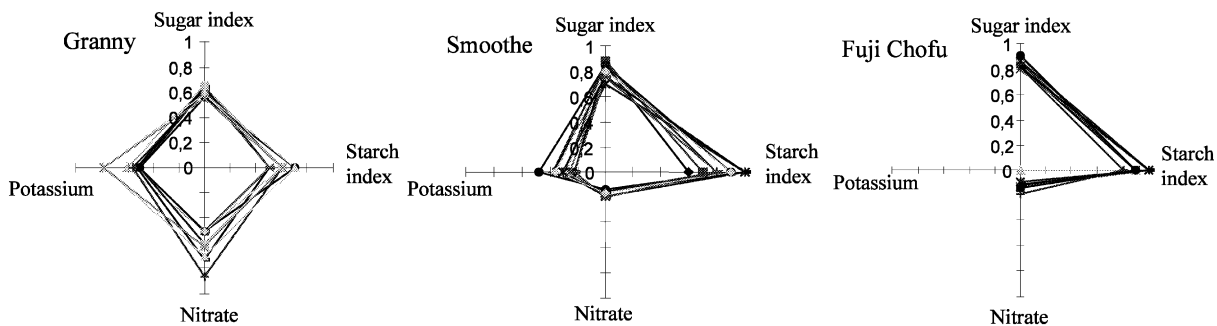

Fig. 2. Radar plot of the normalised values of sugar, starch, potassium and nitrates (obtained in situ with ISFETs) for the varieties Granny Smith, Smoothee and Fuji Chofu.

Chofu No. 2 were used. The interpolation of measured potentials with the calibration curve yields to the activity of free ions in the fruit. At the same time, assays to establish sugar and starch index of studied apples were carried out. Results are shown in Table 3.

Data obtained from the measurements of sugar and starch and nitrate and potassium obtained with ISFETs are presented in Figs. 1 and 2. In Fig. 1a, the relationship between both indexes and the three apple varieties is shown. It can be seen that there is a clear difference between Granny Smith and the other two varieties. This is also confirmed

in Fig. 1b, from the potassium and nitrate concentration data obtained for Granny Smith and Smoothee varieties. Fig. 2 shows the pattern obtained for normalised sugar, starch, potassium and calcium values represented in a radar plot. It can be seen again that there are significant differences between the Granny Smith variety and the Smoothee and Fuji varieties. Nitrate and potassium concentrations are clearly higher in the Granny Smith apples while sugar and starch are higher in Smoothee apples. Since there were no potassium measurements for Fuji Chofu apples, no significant differences are observed between
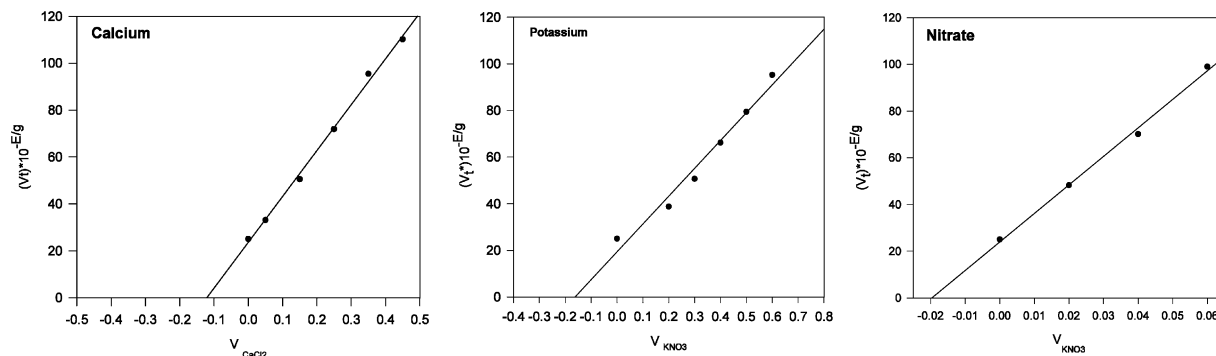
Fig. 3. Standard additions obtained with the calcium, potassium and nitrate ISFETs.

Table 4
Values corresponding to the averages of concentrations (M), standard deviations and errors (95% confidence level) for nitrate and potassium

	Granny Smith		Smoothee		Fuji Chofu No. 2
	---	---	---	---	---
	NO_3^- (M)	K^+ (M)	NO_3^- (M)	K^+ (M)	NO_3^- (M)
In situ	$1.9 \times 10^{-3} \pm 3 \times 10^{-4}$, $\sigma = 4.7 \times 10^{-4}$ ($n = 10$)	$3.2 \times 10^{-2} \pm 5 \times 10^{-3}$, $\sigma = 7.3 \times 10^{-3}$ ($n = 8$)	$5.1 \times 10^{-4} \pm 7 \times 10^{-5}$, $\sigma = 7.2 \times 10^{-5}$ ($n = 10$)	$1.6 \times 10^{-2} \pm 3 \times 10^{-3}$, $\sigma = 4.1 \times 10^{-3}$ ($n = 10$)	$3.7 \times 10^{-4} \pm 6 \times 10^{-5}$, $\sigma = 9.4 \times 10^{-5}$ ($n = 10$)
Standard addition	$1.2 \times 10^{-3} \pm 5 \times 10^{-4}$, $\sigma = 3.3 \times 10^{-4}$ ($n = 4$)	$2.6 \times 10^{-2} \pm 4 \times 10^{-3}$, $\sigma = 3.2 \times 10^{-3}$ ($n = 5$)	$5.0 \times 10^{-4} \pm 3 \times 10^{-4}$, $\sigma = 1 \times 10^{-4}$ ($n = 3$)	$1.7 \times 10^{-2} \pm 2 \times 10^{-3}$, $\sigma = 1.0 \times 10^{-3}$ ($n = 3$)	$5 \times 10^{-4} \pm 1 \times 10^{-4}$, $\sigma = 9 \times 10^{-5}$ ($n = 4$)
Statistic	F_{Calc}, 2.03; F_{Tab}, 14.47; T_{Calc}, 2.68; T_{Tab}, 2.18	F_{Calc}, 5.02; F_{Tab}, 8.90; T_{Calc}, 1.73; T_{Tab}, 2.14	F_{Calc}, 1.927; F_{Tab}, 5.715; T_{Calc}, 0.17; T_{Tab}, 2.18	F_{Calc}, 16.81; F_{Tab}, 39.40; T_{Calc}, 0.38; T_{Tab}, 2.18	F_{Calc}, 1.09; F_{Tab}, 14.47; T_{Calc}, 2.16; T_{Tab}, 2.18

　Measurements were done in situ fruit (interpolation) and in fruit juice (standard addition). Values correspond to F- and T-tests for n measurements.

this and Smoothee variety, although this difference is evident with Granny Smith apples. These results confirm the use of ISFETs sensors, combined with other techniques, to identify different varieties of apples.

3.3. Comparison between in situ and juice measurements

　The apple juice was analysed previously using ISFETs and the standard addition method to establish if there were any significant differences between the measurements in situ and in juice. Fig. 3 shows the typical standard addition curves obtained for calcium, potassium and nitrate ana-

lysis in juice samples. Averages measured for two kinds of methods are shown in Table 4.

　The application of the F criterion permits to conclude that there are no significant differences between the precision obtained for each of the two methods for any given variety. The application of the T criterion permits to conclude that there are no significant differences between the averages obtained in situ (with direct ISFET measurement) and in fruit juice (with standard addition measurement) for a 95% confidence and Smothee anf Fuji Chofu nª 2 varieties (except for the nitrate measurement for the Granny Smith variety). The conclusion is that the concentration of these ions in the fruit flesh and in the juice is not significantly different and therefore it is possible to use sensors

Table 5
Juice analysis of the Smoothee variety

	Ripeness analysis				Potentiometric measurements			Standard methods		
	Firmness	Starch	Sugar	T.A.	NO_3^- (mg l^{-1})	Ca (mg l^{-1})	K^+ (mg l^{-1})	N_T (%)	Ca_T (µg g^{-1})	K_T (µg g^{-1})
1	6.1±0.3	8.4±0.7	14.1±0.3	4.0±0.4	74±12	56±8	700±80	0.21±0.03	0.54(8)±0.08	189±33
2	6.0±0.2	8.2±0.7	14.3±0.3	4.2±0.6	74±12	52±8	660±80	0.25±0.06	0.58(8)±0.08	205±36
3	6.3±0.3	8.2±0.2	14.9±0.5	4.2±0.5	75±12	48±2	700±80	0.22±0.04	0.53(9)±0.08	222±70
4	5±1	8.3±0.3	14.9±0.6	4.3±0.4	81±12	40±2	800±400	0.22±0.08	0.5(3)±0.1	166±20
5	6.2±0.6	8.2±0.3	14.5±0.7	4.2±0.7	112±49	49±2	660±80	0.23±0.05	0.55(8)±0.04	205±19
6	6.2±0.9	8.3±0.8	14.4±0.9	4.0±0.6	93±12	64±8	700±80	0.24±0.1	0.53(9)±0.09	174±53
7	6.1±0.9	8.1±0.5	15±1	4.1±0.4	105±25	43±2	660±80	0.23±0.07	0.52(0)±0.04	197±17

For potentiometric measurements, the data of each treatment correspond to the average of four standard additions. The data from each treatment correspond to the average of the four replicates. The uncertainty error is expressed with a 95% confidence. Firmness, kg; starch, regression index for starch; sugar, refraction index (°Brix); T.A., total acidity (g malic acid/l); NO_3^-, mg NO_3^-/l juice; Ca^{2+}, mg Ca^{2+}/l juice; K^+, mg K^+/l juice; N_T%, wt.% of N in ashes; Ca_T, µg Ca/g ashes; K_T, µg K/g ashes.
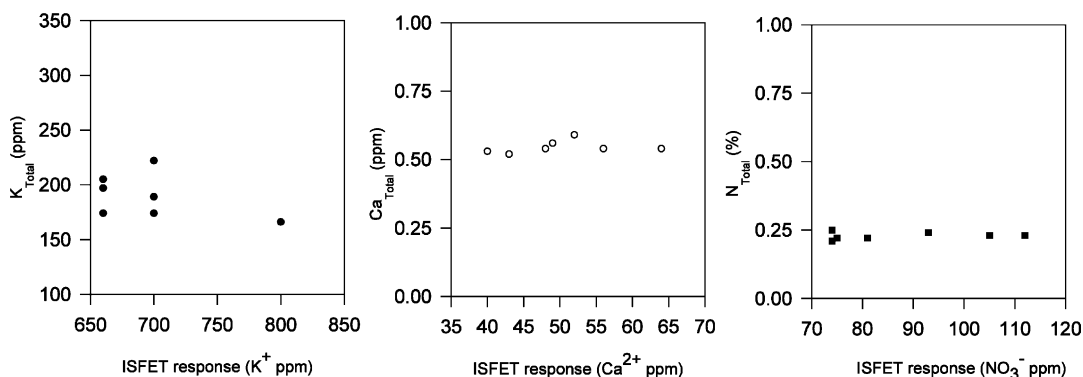

Fig. 4. Relationship between the (●) potassium, (○) calcium and (■) nitrate values obtained using the standard methods (in ashes samples) and using ISFETs (in juices samples) for the seven fertilising treatments.

to indicate the ripeness of the fruit before collection.

3.4. Study on the firmness of the fruit

The lack of firmness of a fruit is related to low concentration of essential nutrients, mainly calcium, and is also related to an inadequate fertiliser application [14,15]. Due to this, in 1988 the collection yielded fruits of low firmness; the agronomy station of La Tallada (Mas Badia, Girona, Spain) started a fertilising campaign in September 1998 to identify the relationship between fertilisation (considering both the type of fertiliser and the doses used) and the firmness of

the product. A total of 28 orchards were fertilised with seven different treatments.

Sugar, starch, hardness and acidity were measured after the collection using standard analytical methods and also measuring nitrates, potassium and calcium in juice using standard additions and ISFETs. After this, ion concentrations were analysed in dehydrated juice in the Laboratoris Agrícola de Cabrils. Results are shown in Table 5.

Although the objective of this work was the study of the firmness of the fruits, no relation was found between the parameters measured and firmness. In all cases, constant values of firmness, sugar, starch, acidity, nitrogen, calcium and potassium were observed even though different fertilising procedures were followed. On the other

hand, a good correlation was observed between the data obtained with the standard methods and those obtained with ISFETs as shown in Fig. 4. Although the data obtained with both methods are not easily comparable (standard methods measure total concentration in the ashes and the ISFET measure the activity of free ions in natural juice), the results demonstrate a good correlation between the total and the free ion concentration. This allows us to conclude that the concentration of calcium, potassium and nitrates in juice could be good parameters to define the fruit quality.

Furthermore, ISFETs can be applied directly on fruit to measure these parameters with a good precision and in short time.

4. Conclusions

At present, the food industry need to incorporate analytical methods to their processes for measuring quality components in shorter times and using automated systems. It is precisely in this industry, where there is a growing demand for sensors and biosensors, to monitor processes and to deep more in the study of those processes and other phenomena. Within the fruit industry, the process of collection and inspection call for new tools capable of measuring, simply and quickly, the essential ions that determine the degree of ripeness of fruits. Results presented here show the viability of using potentiometric sensors, namely ISFETs, as novel tools to recognise varieties of fruit and also to measure ion concentrations while the fruit is still in the tree or in juices to determine the degree of ripeness.

Acknowledgements

References

[1] E.P. Yúfera, Química Agrícola: Alimentos, Alhambra Ed., Madrid, 1979.
[2] Ministerio de Sanidad y Consumo, Control e Inspección de Frutas y Hortalizas, Centro de Publicaciones, Documentación y Biblioteca del Ministerio de Sanidad y Consumo, Madrid, 1986.
[3] I. Tomo (Ed.), Ministerio de Agricultura y Alimentación, Métodos Oficiales de Análisis, Secretaría General Técnica, Madrid, 1993.
[4] N.A. Turner, I.B. Ferguson, R.O. Sharples, J. Agric. Res. 20 (1977) 525.
[5] W.J. Bramlage, S.A. Weis, M. Drake, J. Am. Soc. Hort. Sci. 110 (4) (1985) 493.
[6] R.G. Riley, P.E. Kolattukudy, Hort. Sci. 11 (3) (1976) 249.
[7] S. Alegret, J. Bartrolí, C. Jiménez, M. Del Valle, C. Domínguez, E. Cabruja, A. Merlos, Electroanalysis 3 (1991) 355.
[8] A. Bratov, J. Muñoz, C. Domínguez, J. Bartrolí, Sens. Actuators B24–B25 (1995) 823.
[9] J. Artigas, A. Beltran, C. Jiménez, J. Bartrolí, J. Alonso, Anal. Chim. Acta 426 (2001) 3.
[10] A. Bratov, J. Muñoz, C. Domínguez, J. Bartrolí, Anal. Chem. 67 (1995) 3589.
[11] A. Beltran, J. Artigas, C. Jiménez, R. Mas, J. Bartrolí, J. Alonso, Electroanalysis 14 (3) (2002) 213.
[12] R.W. Catrall, P.J. Illes, Anal. Chim. Acta 169 (1985) 403.
[13] C. Puig-Lleixà, C. Jiménez, E. Fábregas, J. Bartrolí, Sens. Acuators B 49 (1998) 211.
[14] O. Vang-Petersen, Sci. Hort. 12 (1980) 1.
[15] W.J. Bramlage, M. Drake, J.H. Baker, J. Am. Soc. Hort. Sci. 99 (4) (1973) 376.

ANNEX 5

DEVELOPMENT OF A SCREEN-PRINTED THICK-FILM NITRATE SENSOR BASED ON A GRAPHITE-EPOXY COMPOSITE FOR AGRICULTURAL APPLICATIONS

J. Artigas, C. Jiménez, S.G. Lemos, A.R.A. Nogueira, A. Torre-Neto, J. Alonso

Development of a screen-printed thick-film nitrate sensor based on a graphite-epoxy composite for agricultural applications

J. Artigas[a], C. Jimenez[b], S.G. Lemos[c], A.R.A. Nogueira[c], A. Torre-Neto[d], J. Alonso[a,*]

[a]*Grup de Sensors i Biosensors, Departament de Química, Universitat Autònoma de Barcelona, Edifici Cn, 08228 Bellaterra, Spain*
[b]*Instituto de Microelectrónica de Barcelona (IMB-CNM) CSIC, Campus U.A.B., Barcelona, Spain*
[c]*EMBRAPA Pecuária do Sudeste, São Carlos, Brazil*
[d]*EMBRAPA Instrumentação Agropecuária, São Carlos, Brazil*

Abstract

The present report describes the development of a thick-film sensor with intended applications for soil analysis. A probe incorporates three sensors, each at a different depth and featuring a copper plate as the reference electrode. In addition, the probe also contains all necessary instrumentation required for processing the sensor signal and to transmit it via radio. The developed sensors were calibrated in situ using two different soil compositions by adding standard solutions. Results show a quasi-Nernstian response in soil, in response to the addition of fertilisers. Extracts of soil samples analysed by the Kjeldahl method were compared with results from the probe. This comparison confirms that the sensors are capable or measuring chemical parameters in situ in an automated fashion.
© 2002 Elsevier Science B.V. All rights reserved.

1. Introduction

The quest for an ever growing agricultural output has resulted in an uncontrolled use of fertilisers world-wide. Additionally, intensive agriculture methods has also generated environmental problems including soil acidification, an increasing presence of toxic elements, the eutrophication of surface waters and, in some instances, a reduction in biodiversity. The negative influence derived from an excessive use of fertilisers is not only an environmental problem as it also raises costs, diminishing the competitiveness of the farmer [1].

A number of innovative studies have been published recently [2] to minimise the problems produced by the excessive input of nutrients to the soil with the hope of developing a more efficient agriculture practice. From a chemical point of view, optimisation efforts demand a focus on both the input and the output of the nutrients used. At present, farmers do not fully control the fertilising process since, complex procedures are required including sample pre-treatment and analysis in specialised laboratories. These procedures are costly and do not yield information in real time to support meaningful and timely decisions. However, sizeable efforts are being developed at present to develop analytical techniques capable of monitoring chemical and physical parameters in real time.

The term *precision farming* [1] was developed in the US at the start of the 1980s, and described a new philosophy that prioritises the optimisation of agricultural production processes by monitoring and controlling key agricultural parameters. These aims involve the physical and chemical characterisation of soils and also the monitoring of nutrient delivery measuring their influence on crop growth. The use of *precision farming* implies the development of new analytical procedures and new instrumentation capable of supplying the relevant information. This aim is attained by the integration of global positioning systems (GPS), geographic information systems (GIS), variable rate applicators (VRT) for nutrients and agrochemicals, remote sensing, field computer systems for monitoring and field sensors. One objective of precision farming is to measure the chemical and physical parameters that influence the growth of agricultural products. Knowledge of the chemical and physical conditions of soil enables to one optimise the addition of fertilisers based on the results of the monitoring process, while minimising their impact on the environment.

* Corresponding author. Tel.: +34-93-5811-581; fax: +34-93-5812-127.
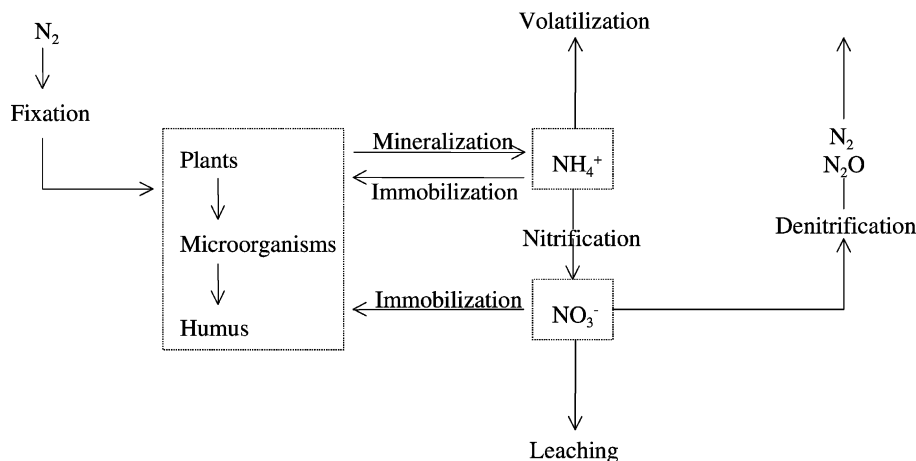*E-mail address:* julian.alonso@uab.es (J. Alonso).

Fig. 1. Cycle of nitrogen in soil.

Within this context, potentiometric systems hold great promise because of their robustness, rapid response and low cost. The use of sensors can help minimise problems posed by the lack of homogenicity in soils [3,4] as the monitoring process is followed by a modelling step yielding distribution maps. Numerous references exist in literature reporting the use of potentiometric sensors to analyse soil extracts in an automated fashion [5–14]. However, few reports describe sensors monitoring any given parameter in situ [15–17].

Nitrogen is an essential nutrient for the development of crops. It is present in soil in several forms, but, mainly as ammonium and nitrate naturally bonded (Fig. 1). To satisfy the requirements of current agricultural practices, farmers add fertilisers with a high nitrate content, causing important environmental problems such as the contamination and eutrophication of surface waters. As a result of this environmental concern, the monitoring of nitrates in soil is a key parameter in the context of *precision farming*.

A nitrate probe based on thick-film sensors was developed in the course of the present work. The sensors produced involved screen-printing of graphite-epoxy composites and PVC membranes [18–20]. The associated instrumentation was also developed and enabled automated measurements that were transmitted via radio. The response of the sensors was evaluated in several types of soil, calibrating them in situ.

2. Materials and methods

2.1. Thick-film sensors construction

A standard photolithographic process equipment (Black-Ray UVP, model B 100AP, 100 W, 360 nm) and a screen-printing apparatus Marprint 350 (Marbay) were used to build the sensor strips. Sensor supports were built using a positive printed circuit board (Ariston). The reagents and solutions used to produce the tracks were H_2O_2 (3%; Merck), NaOH (Panreac) and HCl (Panreac).

A paste using polymer Epotek H77 (Epotek Technology Inc.), graphite (Aldrich) and cyclohexanone (Merck) as a solvent was used to produce the transducer. Fig. 2 shows a thick-film sensor with a composite-epoxy transducer where a PVC membrane is deposited. The electrical connections of the sensor are located in the upper part. Ebecryl 600 (UCB Chemicals) was used to encapsulate the non-sensitive copper tracks.

The use of a metallic reference electrode with minimum maintenance is employed to obtain a probe. The in situ metal insertion in soil causes its superficial surface oxidation, originating a metal oxide constant potential. In order to obtain probes with a reasonable cost, an economic and relatively mechanisable metal was used. The experience of the Sensors and Biosensors Group [17], concerning the use of copper track as a reference metal, has allowed developing copper reference electrodes to obtain probes for in situ soil applications.

2.2. Probe construction

A PVC tube ($L = 1.2$ m, external diameter 0.75 in.) was used to build the probe. The tube was joined to a T on the top and the lower part was closed using a plug. The probe contained three thick-film sensors to measure nitrates at depths of 15, 30 and 60 cm, respectively. All connections were inside the tube to protect them from wetting and were isolated with silicone.

Fig. 3 shows the probe design. As can be observed a current follower circuit was connected to the sensors and placed inside the tube. The amplifier was placed in an airtight box located in the upper part of the probe. The PVC tube had slanting holes just above each one of the thick-film sensors. The purpose of these holes as to place a piece of silicone tubing that comes out of the T placed on top of the PVC tube. These silicone tubes will permit the calibration of the sensors once they are placed in the soil.

The composition of the PVC membrane was: 28.7% PVC (Fluka), 65.2% dibutylphthalate (DBP; Fluka) and 6.1% of
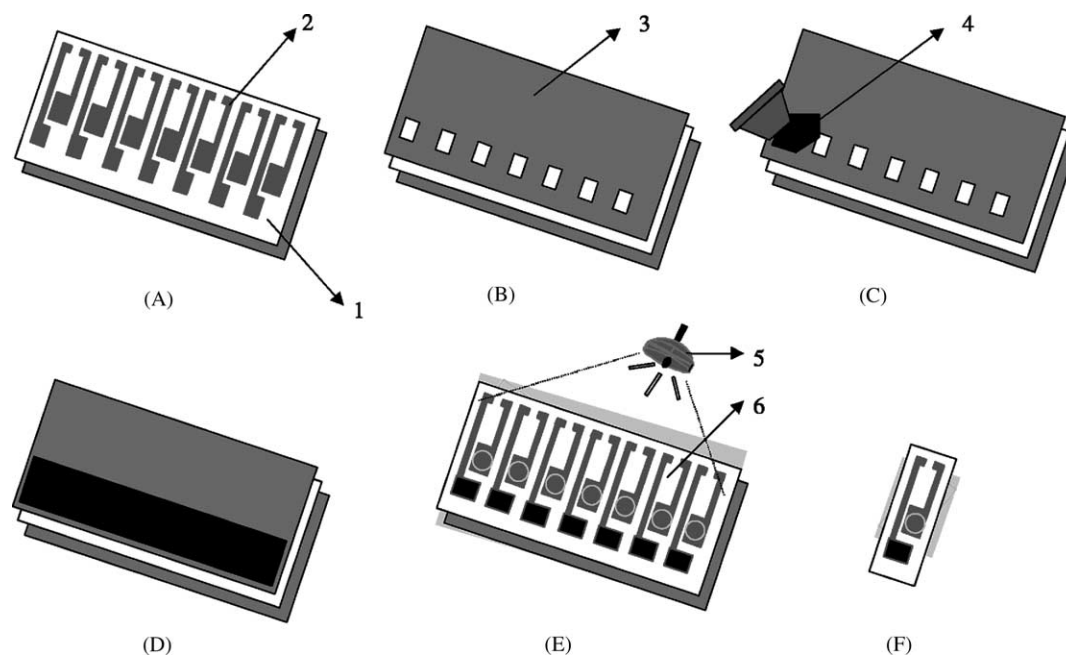
Fig. 2. Production process for the thick-film sensor: (1) support; (2) conducting tracks; (3) mesh; (4) paste and impression palette; (5) UV lamp; (6) photocurable polymer.

nitrate ionophore to quaternary ammonium salt (TOAN) synthesised by the Sensors and Biosensors Group.

2.3. Apparatus

A two-stage circuit was designed to incorporate with the developed sensors: a current follower and an amplifier with a
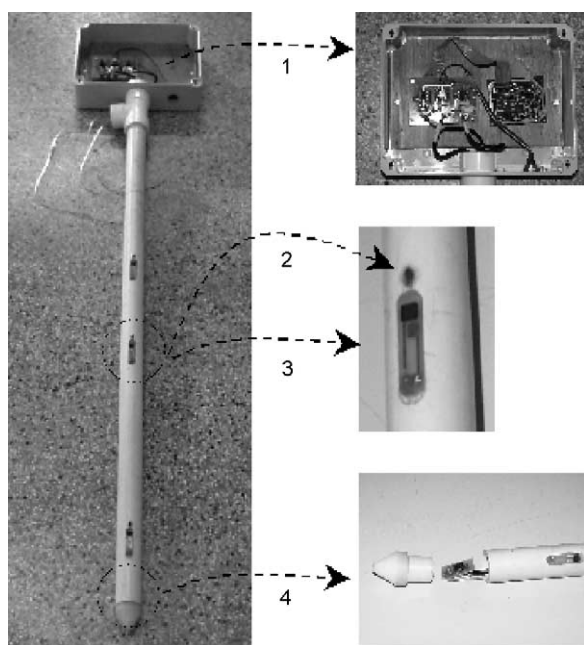

Fig. 3. Schematic representation of the nitrate probe: (1) instrumentation and data radio transmitter; (2) calibration tube; (3) screen-printed thick-film nitrate sensor and the reference electrode; (4) current follower.

gain of 5. The follower was connected to the sensor in the lower part of the PVC tube. The amplifier was mounted inside a box that was placed in the upper part of the tube and included an analogical to digital converter as well as the system for the transmission of data via radio. The current follower is used to protect the circuits from electrical noise and while permitting the use of regular cables to carry the signal to the amplifier.

EMBRAPA Instrumentação Agropecuária developed all necessary instrumentation to obtain results in a completely automated fashion. The system was designed to pick up the data of several sensors networked by a single wire. The transmission of the data was digital, using the communication standard RS-485. The acquisition programme was developed using LabView, a graphic programming language. Active X produced and stored data in the form of an Excel sheet. Two sets of data were obtained: the first set was obtained every 5 min (average of values obtained every 30 s) whiles the second set was the average of all data every hour.

2.4. Kjeldahl method

To analyse soil samples, 15 g of dry and sifted earth was taken and mixed with 50 ml of water under constant agitation of 150 rpm for 30 min. After the soil settled, the sample was filtered to obtain the extract. Due to addition of H_3SO_3, MgO and Zn [21] nitrite and nitrate content in the sample was lowered by reduction. Ammonia resulting was distilled and collected in 10 ml of a boric acid solution and an indicator. To obtain the indicator 2 volumes of 0.2% methyl red in 95% ethyl alcohol were mixed with 1 volume of 0.2%
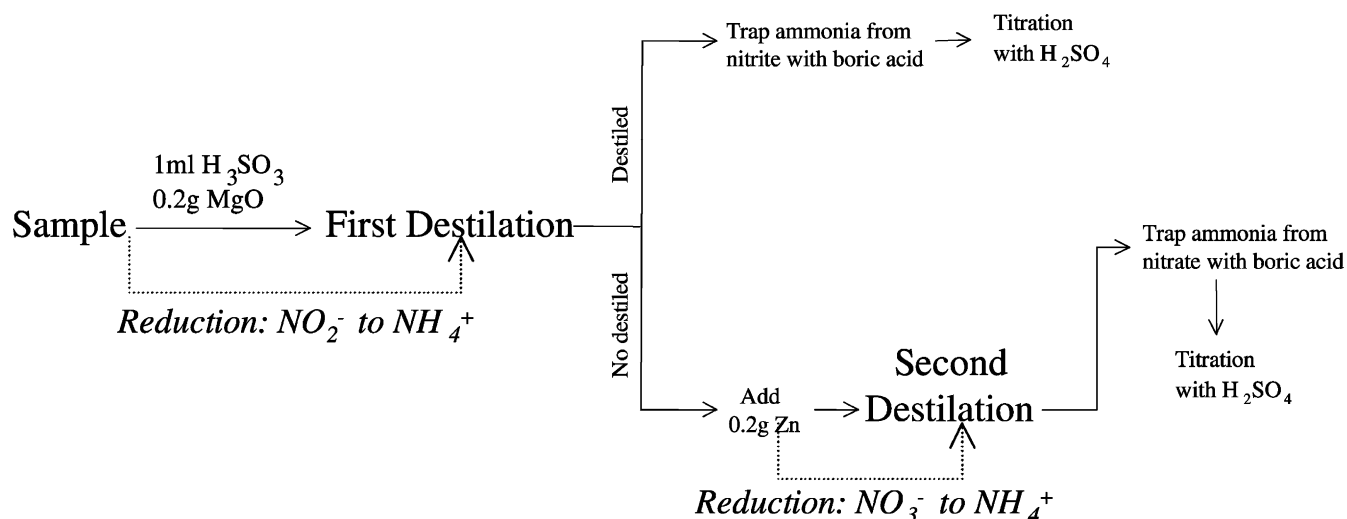
Fig. 4. Schematic of the measurement of soil samples using the Kjeldahl method. Samples were treated with H_3SO_3 and MgO to reduce nitrite to ammonium. The ammonium was in turn distilled and titrated. A further attack of the sample with Zn reduces the nitrate in the sample that was later distilled and titrated.

methylene blue in 95% ethyl alcohol. The solution was titrated with sulphuric acid 0.0025 M. The first distillation of ammonia corresponds to the reduction of nitrite and a second attack with Zn will reduce the nitrates. Twenty-five millilitres of each extract were analysed. The procedure is shown in Fig. 4. All reagents were analytical grade. All aqueous solutions were prepared with deionised water.

3. Results

3.1. In situ nitrate sensors calibration

A column filled with earth was used to simulate the conditions of the soil under laboratory conditions to evaluate the sensors prior to inserting them in soil. This column has lateral openings to obtain a soil sample at the height where the sensors were placed. The probe was placed inside the tube and was the packed with soil. The potential of the sensors in the soil was taken prior to addition of the calibration solutions. Once the potential was constant, 5 ml of the standard nitrate solution was added. Two calibration solutions were used with water being added as the last step to wash the zone around the sensors.

Fig. 5 shows the calibration of the 15 cm sensor in sandy soil. With the probe in place and allowing time for potential to stabilise, solutions of 0.001 and 0.01 M sodium nitrate and water were added. For this case a sandy soil with a low concentration of nitrates was used. The addition of the 0.001 M nitrate solution causes a large variation in potential. Two nitrate solutions, a decade in concentration apart, were added to calibrate the sensor. Fig. 5 (points 2 and 5) illustrates that the addition of these solutions results in a 60 mV potential decrease. Such results confirm quasi-Nernstian behaviour of the sensor inserted in soil. The final

addition of 10 ml of water washes the soil well recovering the potential to its initial value.

In a second calibration experiment, an organic soil sample with a high initial content of nitrates was used. Fig. 6 shows the calibration of the sensors placed at 15 and 30 cm of depth using a 0.1 M sodium nitrate solution. As can be seen from Fig. 6, the potential decreases and then becomes stable. At the end of the calibration, 5 ml of water were added to wash the soil. In this case, the addition of a highly concentrated nitrate solution (0.1 M) causes a variation of about 80 and
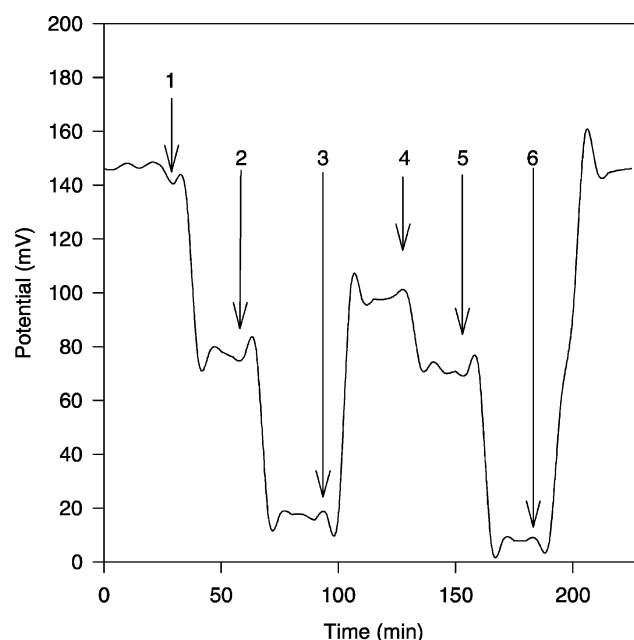

Fig. 5. Variation of the potential with the addition of different solutions delivered by the calibration pipes. The solutions added were: (1) 0.001 M sodium nitrate; (2) 0.01 M sodium nitrate; (3) water; (4) 0.001 M sodium nitrate; (5) 0.01 M sodium nitrate; (6) wash cycle using water.
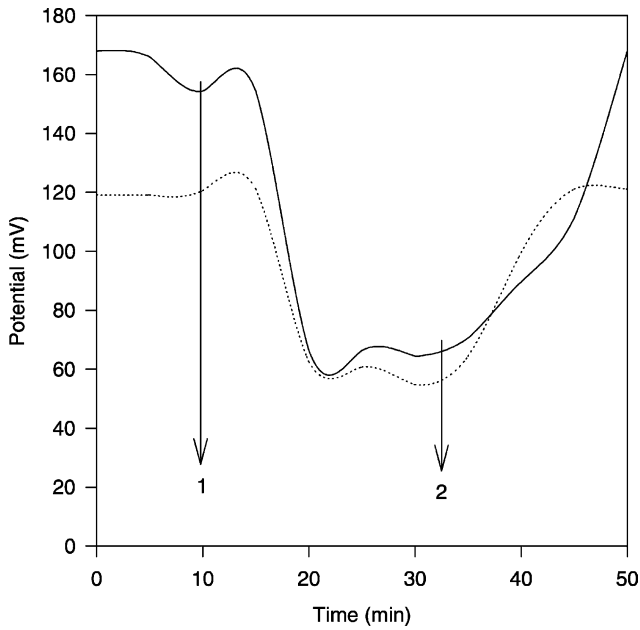
Fig. 6. Calibration of the sensors to 15 cm (—) and 30 cm (· · ·) in organic soil: (1) addition of sodium nitrate 0.1 M; (2) addition of water.

60 mV when sensors were placed at the depth of 15 and 30 cm, respectively. These variations were smaller than those observed in sandy soil, due to the different nature and composition of two types of soils.

3.2. Nitrate measurements at 15 and 30 cm

Having established a correct sensor response, fertiliser with varying nitrate concentration was added. These solutions were 300 ml of 0.01 M and 300 ml of 0.1 M of sodium. Again water was used to wash the column. From Fig. 7 it can be seen that the potential of the sensors was constant until soil nitrate concentration was altered. The first addition of 300 ml of 0.01 M sodium nitrate corresponds to point 1 in Fig. 7 and causes a decrease of the about 50 mV, at both depths. Subsequent to their addition, nitrates can be lixiviated or retained. No significant differences were noticed between the responses of both sensors indicating that for the period of the analysis and at similar depths retention was minimal. The addition of a second standard solution of 0.1 M sodium nitrate caused a 60 mV potential change for the sensors also indicating a good sensor response.

It is important to note that a positive peak appears with the second potential change for the 15 cm sensor and for both potential changes for the 30 cm sensor. These peaks occur
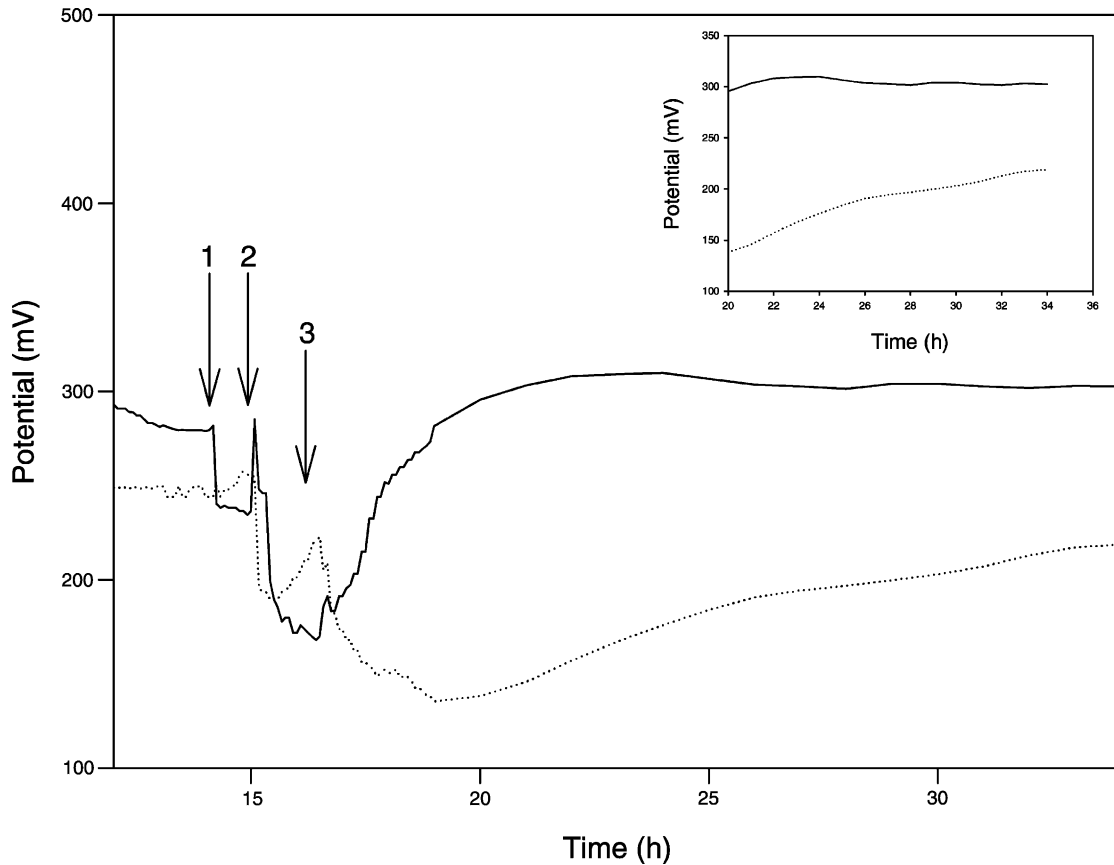

Fig. 7. Evolution of the potential measured continuously with the sensors at 15 cm (—) and 30 cm (· · ·) when fertilising solutions with variable nitrate content were added: (1) 0.01 M; (2) 0.1 M; (3) water. The stability of the potential is showed in the amplification.

prior to potential decrease following the addition of nitrate. It is possible to explain this due to the different mobility of the nitrate and sodium ions and water resulting in two fronts with different compositions where the last front is more concentrated.

The effect of dispersed front, due to the retention and dilution, was more evident at greater depths and, hence, the sensor placed at 30 cm was more sensitive. This confers a greater definition to the response variations of the 15 cm sensor than those registered by the 30 cm sensor. From Fig. 7 it can be seen that the response produced by the sensor place at 30 cm always 1 min after the 15 cm sensor had responded.

Finally, the addition of water washed the soil, causing an increase of the measured potential. Fig. 7 shows a rapid washing process for the 15 cm sensor, taking a total of 4 h to return to the initial experimental conditions. As can be expected, the washing process for the 30 cm sensor required more time to return to its initial values. The amplification of Fig. 7 confirms the stability of the measurement under laboratory conditions. This fact demonstrates the suitability of copper as a reference electrode.

3.3. Comparison of the potentiometric measurements and the standard method

Soil samples was taken for analysis in order to compare the results with those obtained following a standard method. The probe was placed in the column and after the potential had stabilised, a 0.05 M sodium nitrate solution was added. Fig. 8 shows that the sensor behaviour is similar to what was previously observed. The addition of a concentrated solution results in a decrease in sensor potential. Finally, water was added to wash the soil.

Different soil samples were taken at the depths where the sensors were placed. Samples were taken both before and some hours after the addition of the standard solution and, also, water. Following, soil extracted samples were analysed using the Kjeldahl method [20]. There are several extractants reported in the literature for soil samples, these extractants include potassium chloride and calcium sulphate. Additionally reports exist on the use of water as an extractant in the analysis of assimilate nitrate by plants [15,21]. In the present work, water was used as the extractant.

Nitrate measurements were expressed as mg nitrates/g soil and are detailed in Fig. 9. The liberated H_2SO_4 volume used to titrate the ammonia resulted from nitrite reduction in all cases was lower or similar to the volume obtained when working with blanks. Such results, this indicate that the nitrite content in soil is negligible. A profile of the evolution of nitrates at different depths, the mg nitrate/g soil, was obtained and represented as a function of time.

Fig. 9 shows that the addition of the 0.05 M sodium nitrate solution raises nitrate concentration at 15 and 30 cm. Additionally, the nitrate concentration at 60 cm decrease. The addition of a concentrated solution resulted in two fronts with the first one appearing to be more diluted. This dilution
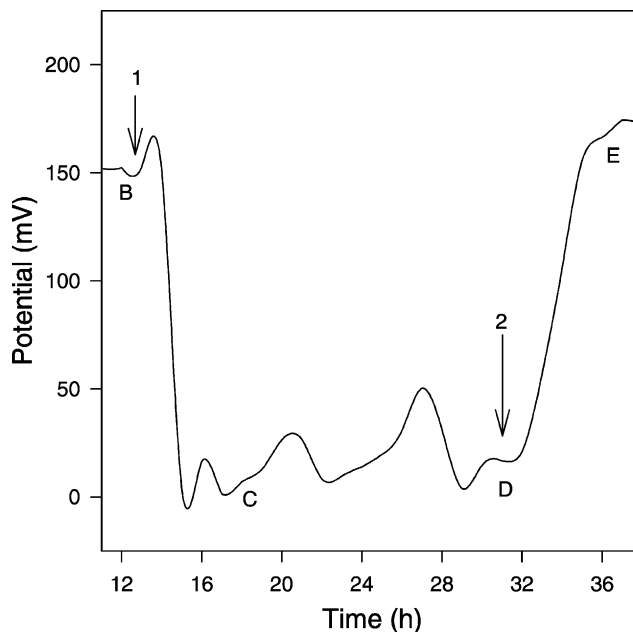

Fig. 8. Evolution of the potential measured continuously with the 15 cm sensor. Addition of different solutions: (1) 0.05 M of sodium nitrate; (2) water. Sampling points are shown.

effect increased with an increasing depth. Nitrates did not leech to a depth of 60 cm until after 10 h had elapsed from the moment of the addition of the standard solution. This process was slow and is seen by the curve at 60 cm depth (Fig. 9). The addition of water to wash the soil was more effective at shallow depths. At 15 cm a return to the initial concentrations was observed after adding water.

The evolution of the measured potential and the results obtained from the extractions were compared to the results obtained with the probe and those from the Kjeldahl method.
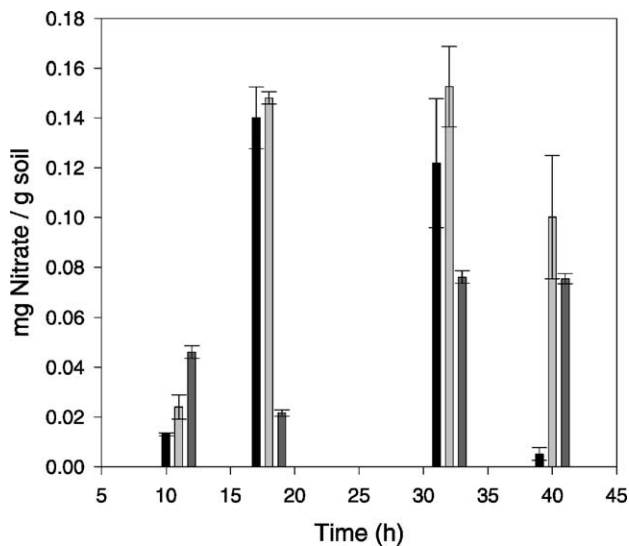

Fig. 9. Values of mg NO_3^-/g soil obtained using the Kjeldahl method at several depths (■, 15 cm; □, 30 cm; ▤, 40 cm) for different sampling points shown in Fig. 8.
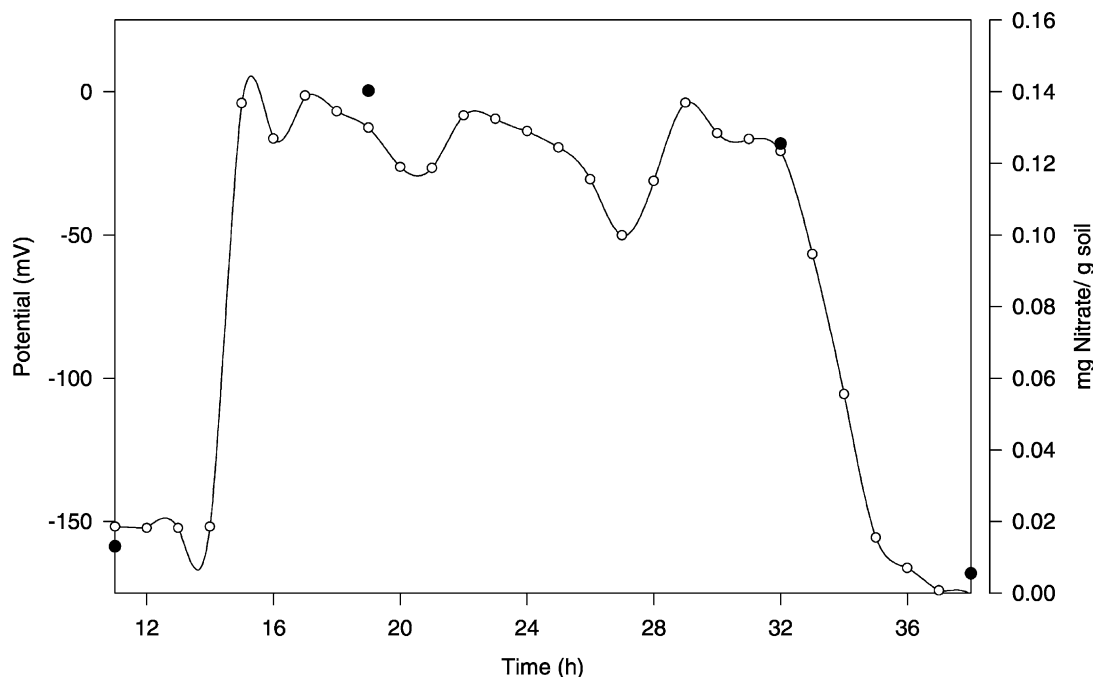
Fig. 10. Results obtained at a depth of 15 cm using the nitrate probe (—) and following the Kjeldahl method (●).

For the nitrate sensor, a concentration increase implies a decrease of the potential. Therefore, the measured potential is represented multiplied by a factor of -1 so that an increase of the potential corresponds to a concentration increase. Fig. 10 shows that the standard method and proposed method have similar trends. Such results, confirms that the sensors have good response characteristics and demonstrates the viability of using this probe to monitor the tendencies of soil nutrients in an automated manner.

4. Conclusion

Results demonstrate the viability of thick-film sensors for agricultural applications and validate the instrumentation developed to measure the potential, store the data and transmit them via radio. The designed calibration tubes permit the calibration of the sensors in situ and predict a Nernstian behaviour of these sensors. The analysis and comparison of the extracts using a standard method, confirms that the sensors have good response qualities. The use of field sensors in the context of an automated analytical system will allow for future the construction of expert systems capable of controlling the input of nutrients, minimising the negative environmental impact of fertiliser.

Acknowledgements

References

[1] P. Roblin, D.A. Barrow, Microsystems technology for remote monitoring and control in sustainable agricultural practices, J. Environ. Monit. 2 (2000) 385–392.

[2] J. Namiesnik, Trend in environmental analysis and monitoring, Crit. Rev. Anal. Chem. 30 (2000) 221–269.

[3] M.E. Crismer, Field sensor networks and automated monitoring of soil water samples, Soil Sci. 154 (6) (1992) 149–153.

[4] A. Torre-Neto, P.E. Cruvinel, J.F.W. Slaets, S. Crestana, Remote monitoring of environmental variables for modeling of pesticide transport in soil, Appl. Eng. Agric. 13 (1) (1997) 115–122.

[5] J. Ruzicka, E.H. Hansen, E.A. Zagatto, Flow injection analysis. Part VII. Use of ion-selective electrodes for rapid analysis of soil extracts and blood serum: determination of a potassium, Anal. Chim. Acta 88 (1977) 1–16.

[6] A.M.R. Ferreira, A.O.S.S. Rangel, J.L.F.C. Lima, Determination of chloride in soil by flow injection potentiometric pseudo-titration, Commun. Soil Sci. Plant Anal. 27 (5–8) (1996) 1437–1445.

[7] A.M.R. Ferreira, J.L.C. Lima, A.O.S.S. Rangel, Potentiometric determination of total nitrogen in soil by flow injection analysis with a gas diffusion unit, Aust. J. Soil Res. 34 (1996) 503–510.

[8] M.Y. Kamogawa, A.R.A. Nogueira, M. Miyazawa, J. Artigas, J. Alonso, Determination of soil calcareous efficiency using flow system with pervaporation separation, Anal. Chim. Acta 438 (2001) 273–281.

[9] N. Brenes, M.N. Quigley, W.S. Reid, Determination of soil pH using a laboratory robot, Anal. Chim. Acta 310 (1995) 319–327.

[10] G. Hienzmann, R. Czolk, Investigation of the stability of an optochemical sensor, Sens. Actuators B 18 (1994) 47–50.

[11] U. Bilitewski, A. Jäger, P. Reiger, W. Weise, Electrodes for the determination of carbohydrates in food, Sens. Actuators B 15 (1993) 113–118.

[12] M. Knoll, K. Cammann, C. Dumscahat, M. Borchardt, G. Hoegg, Microfibre matrix-supported ion selective PVC membrane, Sens. Actuators B 20 (1994) 1–5.

[13] O. Wassmus, K. Cammann, Nitrate sensor system for continuos flow monitoring, Sens. Actuators B 19 (1994) 362–364.

[14] H.H. van der Vlekkert, U.H. Verkerk, P.D. van der Wal, A. van Wingerden, D.N. Reinhoudt, J.R. Haak, G.W.N. Honing, A.J. Holterman, Multi-ion sensing device for horticultural applications based upon chemical modification and special packaging of ISFETs, Sens. Actuators B 6 (1992) 34–37.

[15] J. Thottan, J.F. Adsett, K.J. Sibley, C.M. MacLeod, Laboratory evaluation of the ion selective electrode for use in an automated soil nitrate monitoring system, Commun. Soil Sci. Plant Anal. 25 (17–18) (1994) 3025–3034.

[16] M.M. Wander, L.M. Shuman, R.B. Pitts, Monitoring nitrate in soil with a coated wire ion specific electrodes, Commun. Soil Sci. Plant Anal. 26 (15–16) (1995) 2391–2401.

[17] J. Artigas, A. Beltran, C. Jimenez, A. Baldi, R. Mas, C. Domínguez, J. Alonso, Application of ion sensitive field effect transistors based sensors to soil analysis, Comput. Electron. Agric. 31 (2001) 293–301.

[18] C.A. Galan-Vidal, J. Muñoz, C. Domínguez, S. Alegret, Configurations used in the design of screen-printed enzymatic biosensors: a review, Sens. Actuators B 69 (2000) 153–163.

[19] M. Albareda-Sirvent, A. Merkoçi, S. Alegret, Thick-film biosensors for pesticides produced by screen-printing of graphite-epoxy composite and biocomposite pastes, Sens. Actuators B 79 (2001) 48–57.

[20] Handbook on Reference Methods for Soil Analysis, Soil and Plant Analysis Council Inc., 1992, 157 pp.

[21] W.C. Dahnke, Testing soil for available nitrogen, Commun. Soil Sci. Plant Anal. 2 (2) (1971) 73–85.

ANNEX 6

ANÀLISI DE LA PÈRDUA DE UREA EN EL SÒL MITJANÇANT LA INTEGRACIÓ DE SENSORS TUBULARS D'AMONI EN UN SISTEMA D'INJECCIÓ EN FLUX

J. Artigas, A.R.A Nogueira, J. Alonso

Anàlisi de la pèrdua d'urea en sòl mitjançant la integració de sensors tubulars d'amoni en un sistema d'injecció en flux.

J. Artigas[1], A.R.A. Nogueira[2], J. Alonso[1]

[1]*Grup de Sensor i Biosensors, Departament de Química, Universitat Autònoma de Barcelona, Spain.*
[2]*Embrapa Pecuária Sudeste, São Carlos, SP, Brasil*

Resum

Per a determinar el contingut d'elements derivats del nitrogen, en mostres agroalimentàries, el mètode Kjeldahl és un dels mètodes de referència més emprat. Tot i que en l'actualitat la digestió i destil·lació de la mostra es realitza utilitzant equips automàtics, la determinació d'una sola mostra pot comportar al voltant d'un mínim de 10 minuts. El present treball proposa l'ús d'un sistema de flux continu amb elèctrodes tubulars d'amoni per tal de disminuir el temps d'anàlisi. Degut a la baixa selectivitat de l'elèctrode selectiu d'amoni, s'empren membranes difusores de gasos per a eliminar les interferències d'ions presents en la matriu de les mostres.

Introducció

El nitrogen és un dels nutrients essencial més importants en l'anàlisi de mostres de sòl degut a que intervé en molts dels processos que es verifiquen durant el creixement de plantes i cultius. El seu exhauriment en el sòl es pot suplir addicionant fertilitzants, essent la urea un dels fertilitzants de nitrogen més emprats. Com a raons del seu ampli ús, cal destacar que la urea és un producte relativament econòmic, amb un alt contingut de N (46% en pes correspon a N) i té una gran solubilitat en solucions aquoses.

Malgrat presentar nombroses avantatges, un dels problemes més important associats a l'ús d'urea en sòls és la seva pèrdua, en forma d'amoníac, per volatilització. La hidrolització que pateix la urea en presència d'aigua es veu agreujada per factors ambientals incontrolables, com la pluja i la calor. Aquests efectes produeixen que, a la llarga, el nitrogen addicionat al sòl sigui menor que el nitrogen addicionat. Això provoca que, en molts casos, el fertilitzant sigui addicionat en excés.

$$H_2N\text{-}CO\text{-}NH_{2\,(S)} + H_2O \longrightarrow 2\,NH_{3\,(g)} + CO_{2\,(g)}$$
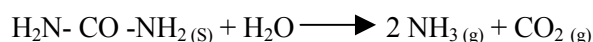
El control dels nivells de fertilitzant que cal addicionar per tal de garantir una bona collita, i ser a l'hora respectuosos amb el medi ambient, ha privilegiat la investigació dirigida aconseguir analitzar la pèrdua de nitrogen per volatilització. La *Empresa Estatal Brasilenya de Pesquisa Agropecuaria[1] (EMBRAPA)* situada en la ciutat de São Carlos (São Paulo) ha dissenyat un sistema trampa per a gasos alliberats des de el sòl. Aquestes trampes s'empren per relacionar la pèrdua d'urea en funció de la temperatura ambiental i la pluja. Diferents porcions de sòl van ser fertilitzades amb diferents dosis per posteriorment deposar-hi les trampes d'amoníac. El present treball proposa l'ús de trampes d'amoníac (Figura 1) per tal de determinar la pèrdua d'urea en les diferents porcions de sòls. Les mostres s'analitzaran emprant el mètode FIA-ISEs i mitjançant el mètode Kjeldahl.

Figura 1: Fotografia d'una finca fertilitzada amb urea com a font principal de nitrogen emprada per estudiar el grau de pèrdua de urea amb trampes d'amoníac. Finca Canchim (Embrapa Pecuária Sudeste, Brasil).

Experimental

Reactius

Per tal de preparar la membrana d'amoni es va emprar adipat de bis(1-butilpentil)) com a plastificant (BBPA), PVC, nonactina com a ionòfor d'amoni i tetrahidrofurà (THF) com a dissolvent, tot de Fluka. Per tal de preparar la pasta d'epoxy-grafit, es va emprar grafit (Aldrich), Araldit M i Enduridor HR (Cibageigy). Les solucions utilitzades en el sistema de flux, pel condicionament de la membrana i per l'anàlisi Kjeldahl van ser preparades emprant reactius de puresa analítica (Sigma o Fluka). Per tal d'ajustar el pH de la solució de Tris 0.01M es va emprar àcid clorhídric.

Trampes d'amoníac

Les trampes estan formades per un cos cilíndric obert per la part inferior (que es situa en el sòl) i tancat per la part superior. Dins del cos cilíndric de PVC (radi 10.5cm) es fan encaixar dues esponges empapades d'uns 100 – 120 ml d'una solució àcida.

L'interior del cos cilíndric esdevé una trampa per als gasos amb caràcter bàsic que emergeixen de la superfície del sòl, principalment amoníac. Transcorregut el temps de retenció (establert per als diferents assaigs des de alguns dies fins a varies setmanes) es recull l'amoníac retinguts en la esponja i s'analitza posteriorment emprant el mètode de Kjeldahl. La Figura 2 mostra les diferents parts de les trampes d'amoníac.

Si ens fixem en la Figura 2, podem observar com a l'interior de la trampa es situen dues esponges. L'objectiu de la esponja inferior és bàsicament la de retenir l'amoníac alliberat, mentre que la segona te la doble funció d'evitar contaminacions a l'interior de la trampa, ocasionats per gasos atmosfèrics aliens a l'experiment i retenir gasos amoniacals que no hagin quedat retinguts en la esponja inferior. Tal i com s'ha comentat, cal suposar que si la concentració d'àcid en la esponja és l'adequat, l'amoníac desprès es quedarà retingut en la esponja més propera al sòl, esperant retencions del 100% en la primera esponja i nul·la per a la segona.

El procés de rentat de les esponges, passat el temps de preconcentració fixat, consisteix en una esbandida de la esponja amb 300 – 350 ml, d'aigua o KCl 1M. Finalment el eluit s'enrassa a 500 ml emprant un matrau aforat. La dissolució resultant s'analitza per determinar el contingut d'amoni.
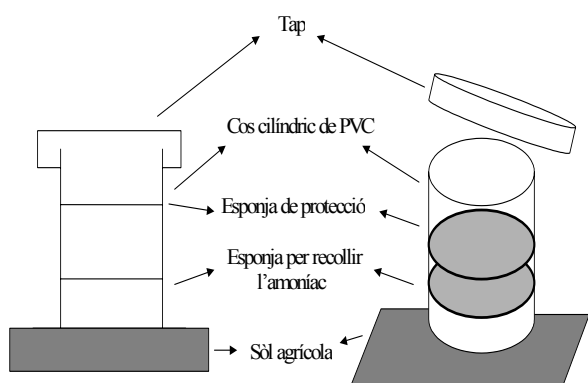

Figura 2: Trampes d'amoníac dissenyades per tal de ser instal·lades en camp.

Descripció de les mostres

El sòl emprat corresponia a la *Facenda Canchin* (Embrapa Pecuária Sudeste, São Carlos, SP, Brasil). Dotze plates, que contenien 1.50 Kg de sòl agrícola, es van regar amb 100 ml d'aigua i es van fertilitzar amb 1.26 g de Urea-200. Es va situar una trampa en cada plat. Després de tres dies, les mostres van ser analitzades.

Es van emprar tres plats per tal de situar-hi trampes amb esponges empapades d'àcid fosfòric i glicerina (50 ml d'àcid 85% (v/v) i 40 ml de glicerina dissolts en 1 litre d'aigua fosfòric), tres plats emprant esponges empapades de Tris 0.01 M a pH 7.5, tres plats més emprant dipòsits amb Tris 0.01 M a pH 7.5 com a trampes per el gas amoni i finalment 3 plats on l'amoníac alliberat era aspirat contínuament i atrapat en una solució de Tris 0.01 M a pH 7.5.

Anàlisi emprant mètode Kjeldahl

A un volum de 25 ml de mostra s'addicionen 50 ml de hidròxid potàssic 5M. L'amoni resultant es destil·la i es recull en 10 ml d'una solució d'àcid bòric que conté l'indicador de pH. La reacció de l'amoníac amb l'àcid bòric dóna borat d'amoni, que es valora amb àcid sulfúric 0.0025M. Per tal de preparar l'indicador[2,3] s'utilitzen solucions al 0.2% en pes de vermell d'etil dissolt en etanol (95% v/v) i del 0.2% en pes de blau de metilé dissolt en etanol (95% v/v). Dos volums de vermell d'etil es mesclen amb un volum de blau de metilé. La solució destil·lada es valora amb àcid sulfúric 0.0025 M. En tots els casos, els valors d'àcid alliberat per a analitzar el blanc van ser inferiors al error de la bureta.

Construcció dels elèctrodes tubulars

Per a la construcció dels elèctrodes de configuració tubular, s'empra la tècnica establerta en el Grup de Sensors i Biosensor[4,5] . A un cos circular de metacrilat (0.8 cm de longuitud) s'hi enganxa un connector i s'omple de pasta epoxi-grafit. Per tal de curar la pasta resultant es deixa, durant un mínim de 6 hores, en una estufa a 60º. Es poleixen les dues cares del cos cilíndric, ben bé arran. Per tal de protegir la connexió elèctrica, es deposa una fina capa d'epoxi no conductor damunt de la pasta i es deixa el cos altre cop a l'estufa, un mínim de 6 hores. Finalment, es perfora longitudinalment el mig del cos cilíndric amb una broca de 1.5 mm. La Figura 3 recull els diferents passos seguits per a construir els elèctrodes tubulars.

Per tal de deposar la membrana, es deixa caure gota a gota el còctel sensor. La evaporació del dissolvent deixarà una fina pel·lícula plàstica que recobreix l'interior del tub, reduint notablement el seu diàmetre. Cal deposar gotes fins observar una disminució considerable del diàmetre, però vigilant de no taponar el forat. Cal deixar un temps d'espera entre gota i gota per tal d'assegurar-nos que la última gota es deposa sobre una superfície lliure de dissolvent. Un cop deposada la última gota, l'elèctrode es condiciona submergint-lo durant un mínim d'una hora en una solució 10^{-3} M de clorur d'amoni.
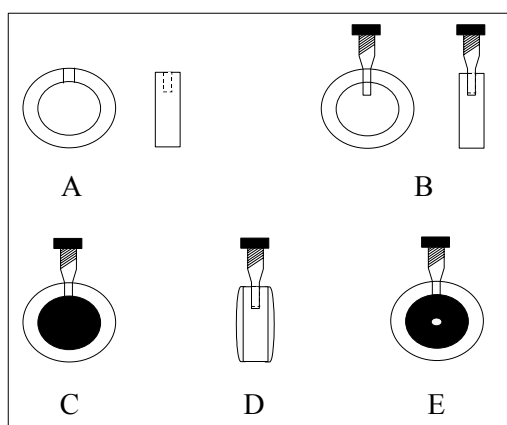
Figura 3: Etapes de construcció d'elèctrodes tubulars: A: Peça de metacrilat; B: Encaixar el connector en el cos cilíndric; C: Omplir d'epoxi-grafit i polir; D: Posar capa aïllant; E: Foradar part central (1.5 mm).

La composició de la membrana de PVC emprada[6] va ser: 65.5 % en pes de BBPA, 33.5 % en pes de PVC i un 1% en pes de nonactina. Com a dissolvent, per tal d'ajudar a homogeneïtzar la mescla, s'empra 0.05 ml de THF per cada 1 mg de membrana.

Anàlisi per injecció en flux continu emprant elèctrodes tubulars d'amoni

Per tal de calibrar els elèctrodes i analitzar les mostres, s'empra un sistema en flux continu que incorpora un canal portador de Tris 0.01M a pH 7.5, un canal de hidròxid sòdic 10^{-2} M condicionador del pH així com un tercer canal de Tris 0.01M a pH 7.5 (amb un fons de clorur

amònic 10^{-7} M, per tal d'estabilitzar el senyal de l'elèctrode) com a canal de recollida del amoníac gas difós a través de la membrana de difusió. Un esquema del sistema emprat es detalla en la Figura 4. Com a elèctrode de referència s'empra un Ag/AgCl de doble unió (ORION 900200). Per tal de calibrar els sensors, es van emprar set solucions de clorur d'amoni de concentracions que cobrien en rang de 10^{-4} a 0.1 M.

Resultats i discussió

Solució utilitzada per atrapar l'amoníac alliberat en sòl

Per tal de retenir l'amoníac alliberat durant tres dies, les esponges de les trampes es van empapar amb dues solucions força diferents. El pH d'aquestes ha de ser inferior a 9.25 (valor que correspon al pK_a del NH_4^+/NH_3) i quan més àcid sigui més alt serà el rendiment del procés d'atrapament. Per aquest motiu, es va emprar una solució fortament àcida, àcid fosfòric 0.63 M. L'ús d'aquesta solució, implica, a l'hora d'analitzar amb un sistema en flux, l'ús d'un canal ajustador de pH fortament bàsic. A més existeix la possibilitat de no obtenir una bona difusió de l'amoni obtingut.
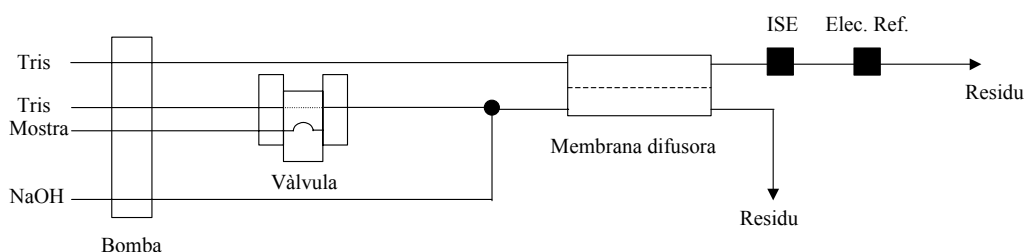

Figura 4 : Esquema emprat per a l'anàlisi d'amoni. La mostra que conté amoni s'alcalinitza. El gas amoníac alliberat i difós a través de la membrana difusora es recull en un canal portador, lleugerament àcid.

Per tal de minimitzar aquests efecte, es va emprar la mateixa solució utilitzada com a canal portador per tal d'empapar les esponges. L'ús de Tris 0.01M a pH 7.5 permetria, posteriorment, utilitzar solucions d'hidròxid sòdic menys concentrades. Els valors obtinguts per a les dues solucions i per l'anàlisi de les esponges superiors i inferiors es mostra en la Taula 1. Les mesures dels patrons i de les mostres es van realitzar per triplicat. La Figura 5 mostra un calibrat d'amoni emprant els elèctrodes tubulars.
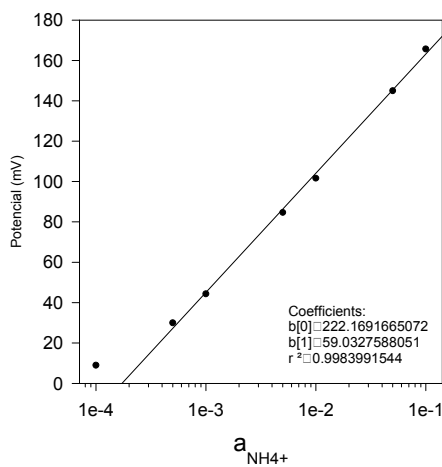

Figura 5: Calibratge d'un elèctrode tubular d'amoni

Els resultats ens permeten concloure que la solució per empapar les esponges ha de ser fortament àcid. El Tris 0.01M a pH 7.5 no permet atrapar adequadament l'amoníac alliberat. Això es veu reflexat en el fet que les esponges superiors tinguin nivells d'amoni similars als de les esponges inferiors. No succeeix el mateix amb

l'ús d'àcid fosfòric, on podem assegurar que tot l'amoníac alliberat queda retingut en les primeres esponges.

Tal i com és sabut, i com els valors també ens permeten concloure, el procés d'hidrolització depèn de diversos factors, fent-lo un procés poc reproduïble. Tant sols podem assegurar que els mg d'amoníac alliberats per cada experiment són del mateix ordre.

Alternatives a l'ús d'esponges com a atrapadores del gas amoníac

Per eluir el contingut de les esponges s'ha de realitzar un tractament posterior força llarg i no automatitzable, que augmenta el temps global d'anàlisi per mostra. Per tal de millorar aquesta part del procés, es van proposar dues alternatives. Es va aspirar l'aire de la trampa, mitjançant una bomba, i fer-lo bombollejar en una solució de Tris 0.01 M a pH 7.5. Per un altre banda, es van substituir les esponges d'atrapament per dipòsits reblerts de Tris 0.01M a pH 7.5.

En la primera opció, el gas alliberat de la hidròlisis de la urea hauria de ser retingut en la solució de Tris. No es va observar, però, presència del gas en cap de les tres solucions aspirades.

Pel que fa als dipòsits, es va observar que les mesures obtingudes d'amoníac retingut va ser inferiors al observat amb les esponges, però eren molt més reproduïbles. Els valors obtinguts per a les mostres analitzades pel mètode potenciomètric i pel mètode de Kjeldahl es mostren en la Taula 2.

Taula 1: Valors de l'amoníac hidrolitzat (mg d'amoni per cada 1.5 Kg de sòl fertilitzat amb 1.26 g d'urea-200) retingut en les esponges, superior i inferior, de les trampes. Valors obtinguts emprant el sistema de flux i els elèctrodes tubulars d'epoxi-grafit amb membrana d'amoni.

	Àcid fosfòric 0.63 M			Tris 0.01M a pH 7.5		
	Trampa 1	Trampa 2	Trampa 3	Trampa 4	Trampa 5	Trampa 6
Superior	Inferior L_D	Inferior L_D	Inferior L_D	21.97	Inferior L_D	19.04
Inferior	33.94	55.56	95.77	45.52	10.20	35.09

Taula 2: Valors de l'amoníac hidrolitzat (mg d'amoni) obtinguts pel mètode FIA-ISE i el mètode Kjeldahl

		Mètode potenciomètric	Mètode Kjeldahl
N° trampa	Esponja	*Àcid fosfòric 0.63M*	
1	Inferior	33.94	22.49
1	Superior	Inferor L_D	0.19
2	Inferior	55.56	62.36
2	Superior	Inferor L_D	0
3	Inferior	95.77	138.08
3	Superior	Inferor L_D	0
		Tris 0.01M a pH 7.5	
4	Inferior	45.52	51.67
4	Superior	21.97	26.00
5	Inferior	10.20	10.67
5	Superior	Inferor L_D	0.44
6	Inferior	35.09	40.10
6	Superior	20.04	21.09
N° Dipòsit		*Dipòsits amb Tris 0.01 M a pH 7.5*	
1		13.23	13.38
2		17.84	18
3		12.72	15.5

Comparació dels resultats obtinguts amb el mètode potenciomètric i el mètode Kjeldahl

Per tal de comparar els valors obtinguts amb els dos mètodes, Taula 2, es va realitzar el test de correlació. Exceptuant dos punts experimentals (corresponents al de l'esponja inferior de la trampa nº3 i el de l'esponja inferior de la trampa nº1), els valors dels test ens permeten concloure que no existeixen diferències significatives entres les dades obtingudes per el mètode Kjeldahl i per potenciometria. La recta obtinguda, Figura 6, té una pendent de 0.91 ± 0.08 i coordenada d'origen 1.4 ± 1.7.

Conclusions

Per tal de retenir l'amoníac alliberat, no és viable l'ús d'una solució Tris 0.01 M a pH 7.5, doncs els resultats indiquen una baixa capacitat de retenció. Per contra, l'ús d'àcid fosfòric 0.63 M permet retenir satisfactòriament el gas alliberat. Els temps d'anàlisi es simplifiquen notablement emprant una detecció potenciomètrica respecte els temps d'anàlisi consumits en el mètode Kjeldahl, reduint-se de 10 a 2 minuts. Per altre banda, l'ús d'un sistema de flux continu permet l'automatització de l'anàlisi reduint encara més el temps d'aquest. Degut a la bona correlació entre les dades obtingudes amb ambdós mètodes i les bones característiques de resposta observades pels sensors d'amoni, podem afirma la viabilitat de l'ús de sensors acoplats a l'anàlisi en flux per tal d'estudiar l'efecte de la hidròlisi de la urea així com per analitzar mostres de sòls.

Bibliografia

[1]   Empresa Brasileira de Pesquisa Agropecuária (http://www.embrapa.br)

[2]   Handbook on Reference Methods for Soil Analysis, Soil and Plant Analysis Council Inc., 1992, 157.

[3]   Danhke W.C., Testing soil for available nitrogen, Commun. Soil. Sci. Plant Anal., 2(2) (1971) 73.

[4] J. Alonso, J. Bartrolí, J.L.F.C. Lima, A.A.S.C. Machado, Anal. Chim. Acta, 179 (1986) 503.

[5] S. Alegret, J. Alonso, J. Bartrolí, J.M. Paulís, J.L.F.C. Lima, A.A.S.C. Machado, Anal. Lett., 18 (1985) 2291.

[6] E. Barquero. Disseny, construcció i avaluació d'analitzadors per a la monitorització mediambiental. Tesi doctoral. Universitat Autònoma de Barcelona, 2001.