J Solid State Electrochem (2000) 4: 325–329

ORIGINAL PAPER

Giorgio Papeschi · Stefano Mancuso
Anna Maria Marras

Electrochemical behaviour of a Cu/CuSe microelectrode and its application in detecting temporal and spatial localisation of copper(II) fluxes along *Olea europaea* roots

Received: 29 September 1999 / Accepted: 11 January 2000

Abstract The electrochemical behaviour of a Cu/CuSe electrode was studied in order to define its selectivity towards cupric ions, Nerstian response, limit of detection and response time. The chalcogenide electrode was prepared by cathodic deposition of Se and subsequent formation of a thin layer of CuSe on a copper substrate. A Cu/CuSe microelectrode was prepared using copper wire 75 μm in diameter. The dimensions and response time (<0.5 s) allowed use of this electrode in the "vibrating probe method" with the aim of measuring net influxes as well as effluxes of copper(II) ions in *Olea europaea* roots. The electrode potential was measured along the root at a distance of 5 μm from the surface for 5 s, and then again for 5 s at a distance of 55 μm, moving the microelectrode with respect to the root surface by steps with a frequency of 0.1 Hz. The potentials measured at the two extremes of vibration were then converted to copper(II) concentrations. Substitution of these values in Fick's law yields the flux, assuming the diffusion constant D for copper ions in aqueous solutions. The results enabled us to detect copper(II) fluxes as small as 0.05 pmol cm^{-2} s^{-1}. Copper(II) influx showed marked spatial and temporal features: it was highest at about 1.5 mm from the root apex and exhibited an oscillatory pattern in time.

Key words Copper selenide · Copper(II) uptake · Microelectrode · *Olea europaea* · Vibrating probe

Presented at the international conference "Modern Electroanalytical Methods", 19th to 23rd September, 1999, in Seč, Czech Republic

G. Papeschi (✉) · A.M. Marras
Department of Pharmaceutical Sciences,
University of Florence, Via Gino Capponi 9,
50122 Florence, Italy
e-mail: papeschi@farmfi.scifarm.unifi.it

S. Mancuso
Department of Horticulture,
University of Florence,
Via Gaetano Donizetti 6, 50144 Florence, Italy

Introduction

The electrochemical approach to thin chalcogenide film preparation offers exceptional possibilities for ion-selective electrodes. The electrolytic co-deposition of copper selenide has been a well-known fact for some time [1–3]. According to this procedure, copper-selective electrodes [4, 5] are prepared by electroplating copper selenide onto a conducting platinum base from a solution of $CuSO_4$ and Na_2SeO_3 dissolved in 0.5 M H_2SO_4.

In previous work [6, 7] we have shown that chalcogenide coatings on copper substrates as well as on silver ones are obtained by cathodic deposition of selenium onto the metal base, where it reacts to give the corresponding selenide. The process occurs in 0.1 M Na_2SeO_3 solution whose pH is adjusted to about 6 with sulfuric or hydrochloric acid. Following this procedure, we have been able to prepare Cu/CuSe disk microelectrodes with diameters ranging from 25 to 75 μm.

The present work deals with the preparation and also the performance characteristics of the copper-selective microelectrode, taking advantage of its reduced dimensions along with the short response time, with its application using the vibrating-electrode [8] technology and in detecting temporal and spatial localisation of Cu^{2+} fluxes in *Olea europaea* roots.

Experimental

Construction of Cu^{2+}-selective microelectrodes

Copper wires 25, 50 and 75 μm in diameter and insulated with a thin layer of lacquer were used to prepare the microelectrodes. A sharp surgical knife was utilised to cut the tip of the wire in order to expose a copper disk flush with the insulator surface. A layer of CuSe was formed on the copper surface by cathodic deposition of selenium from 0.1 M sodium selenite solution whose pH was adjusted to about 6 with sulfuric acid.

Equipments and chemicals

A high-impedance millivoltmeter (E.C.D., model 905-4IN) was employed to measure the potentials of the microelectrode. The output of the instrument was connected to a potentiometric strip-chart recorder (Kipp & Zonen, model BD 111). The electrode potentials were measured against a double-junction reference half cell with a Ag/AgCl electrode in 0.08 M NaCl + 0.1 M KNO_3 inner solution, and with the bridge filled with 0.1 M KNO_3 solution. All measurements were taken at room temperature.

0.1 M stock solution of $Cu(NO_3)_2$ was prepared by dissolving a weighed amount of analytical reagent grade $Cu(NO_3)_2 \cdot 2H_2O$ in water. Cu^{2+} standard solutions for pCu from 2 to 8 were prepared in seven polyethylene cells by successive stepwise ten-fold dilutions, starting from the stock solution. All the solutions contained a background of 50 μM $Ca(NO_3)_2$, and each of them was prepared every time in the same cell.

All chemicals used were of analytical reagent grade, and MilliQ quality water was used throughout.

Vibrating-microelectrode system

The design and mode of the vibrating-microelectrode system were similar to those originally used by Shabala et al. [9] and are shown schematically in Fig. 1.

The Cu/CuSe microelectrodes were mounted on a manual micromanipulator, providing three-dimensional positioning. During measurements the distance between the root surface and the electrodes was changed by fixing the measuring chamber on a three-way hydraulic micromanipulator (WR-88, Narishige, Japan) driven by a computer-controlled step motor (type I 5PM-K004-01, Minebea, Japan). The electrodes were connected by screened cables to a high input impedance (10^{14} Ω) electrometer (homebuilt, based on an AD 645 JN operational amplifier). The output signals from the electrometer were low-pass filtered, amplified and connected via a multichannel A–D convertor card (Lab-PC-1200 National Instrument, USA) to a P133 personal computer.

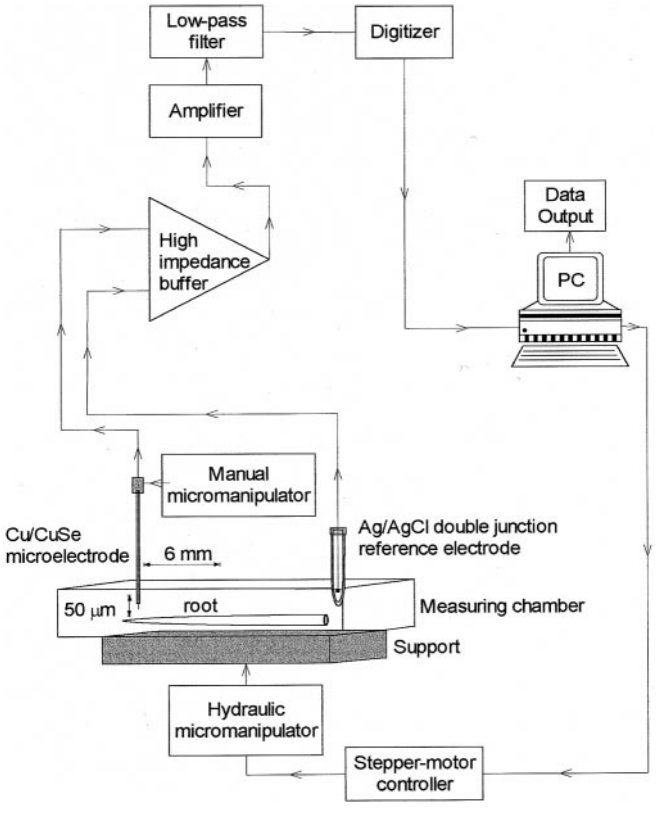

Fig. 1 Schematic diagram of the system and mode of operation for the "vibrating microelectrode technique"

Measurements of the difference in electrode voltage at the two extremes of vibration were achieved by digitising the electrode signals and computing the potential difference. Data were collected at a rate of 1000 data points per second.

For each electrode position, the first 2 s after the movement began were automatically discarded to eliminate movement artefacts and to allow the electrochemical settling of the electrodes; the remaining part of the signal was then averaged. The computer calculated the difference between this average and the previous one at the other extreme position and finally calculated a moving average of these differences over any desired time period.

Measurement of ion fluxes

Copper(II) fluxes were calculated using Fick's first law of diffusion:

$$J = -\frac{D(C_1 - C_2)}{\Delta x} \quad (1)$$

were J is the flux rate (mol cm^{-2} s^{-1}), D is the diffusion coefficient for Cu^{2+} (7.2×10^{-6} cm^2 s^{-1}), C_1 and C_2 are the ionic activities at the two measurement positions and Δx is the distance of measurement (cm).

For the transport studies, a single root from the root system of *Olea europaea* plants was anchored to the bottom of the measuring chamber, containing 50 μM $Ca(NO_3)_2$ solution, using a Plexiglas block fixed to the chamber bottom with silicone grease. One hour before starting flux measurements, the roots were exposed to a solution containing 50 μM $Ca(NO_3)_2$ + $Cu(NO_3)_2$ in concentrations between 5 and 100 μM according to the different measurements. A settling time of 1 h prior to the start of the experiment was necessary to detect significant copper(II) fluxes.

Experiments were performed at 25 ± 1 °C. Microelectrodes were set perpendicularly to the root and a differential signal was calculated from data obtained while oscillating the electrode as a square wave at 0.1 Hz between two points, 50 μm apart, such that the extremes of the vibration were between 5 and 55 μm from the root surface.

Plant material

Cuttings from one-year-old shoots, 12–15 cm long and with six leaves, were taken from seven-year-old stock plants of *Olea europaea* L. cv Leccino. In order to favour rooting, the lower portion of each cutting was immersed, to a depth of 5 mm, in an aqueous KIBA (3-indolebutyric acid, potassium salt) solution at 3600 μg mL^{-1} for 5 s. The cuttings were then placed in an inert medium of perlite and kept in a greenhouse under conditions of high relative humidity (mist propagation), daylight for 12 h/d and average diurnal and nocturnal temperatures of 22 °C and 15 °C, respectively. Rooting took 8 weeks. At the end of the rooting period, plants presenting uniform roots for length (2–3 cm), thickness (1–2 mm), and colour (white) were chosen. After removing the perlite residue, the root systems of entire plants were immersed, under weak illumination, in an aerated nutritive solution composed of (in mM): KCl, 1.0; NaH_2PO_4, 0.905; Na_2HPO_4, 0.048; $Ca(NO_3)_2$, 1.0; $MgSO_4$, 0.25. The solution temperature was maintained at 25 ± 1 °C for the immersion period. The plants were then utilised for the experiments.

Results

Microelectrode calibration

The calibration of the microelectrodes was carried out in unbuffered copper solutions made by serial dilution

from copper nitrate solution in water with a background of 50 μM calcium nitrate. After the electrode potential had reached a steady-state value in the calcium nitrate solution, the calibration run was started in the solution with the lowest cupric ion activity and proceeded towards higher activity.

When the electrodes were transferred from one solution to the next they were blotted dry with tissue paper. Potentials were allowed to stabilise within 0.1 mV before readings were taken; at the lower concentrations, stabilisation took up to 1–2 s. The calibration curve obtained for the microelectrode is shown in Fig. 2.

No differences were noted between the microelectrodes prepared and tested with respect to the slope of the Nernstian response, nor for the response time.

Response time

After the microelectrode had reached a steady potential in 50 mL of well-stirred 50 μM $Ca(NO_3)_2$ solution, a small aliquot of Cu^{2+} was added to the solution, producing a small step copper ion concentration increase. The electrode potential change was followed on the strip chart recorder. A typical trace of potential changes following the addition of copper solution is shown in Fig. 3.

Selectivity

The selectivity of the microelectrode with respect to five cations (Na^+, K^+, NH_4^+, Ca^{2+}, Mg^{2+}), usually contained in nutritive solutions used in plant physiology, was verified by the mixed solution method.

A solution of constant ionic strength, containing an interferent ion Me at a fixed concentration of 10^{-2} M, was used as a background solution to which portions of copper(II) standard were added to span the concentration range between 10^{-7} and 10^{-3} M. The activity of the primary ion was determined by extrapolating the linear part of the calibration plot as far as the limit of

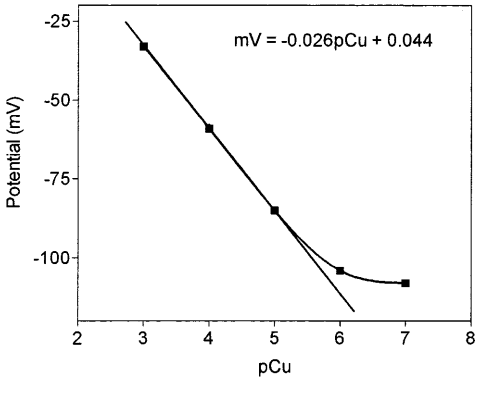

Fig. 2 Calibration curve for the Cu^{2+} microelectrode in $Cu(NO_3)_2$ solutions containing a background of 50 μM $Ca(NO_3)_2$

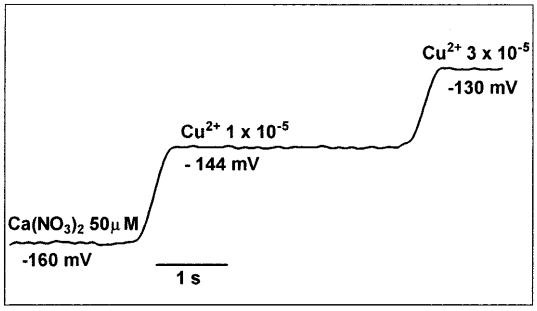

Fig. 3 Response time for the Cu/CuSe microelectrode. The electrode was subjected to changes in the Cu^{2+} concentration of 50 mL of base electrolyte solution by the addition of appropriate aliquots of 1×10^{-3} M Cu^{2+} solution

Nernstian response. The results are summarised in Table 1.

Local copper(II) concentration measurement

As the procedure for the flux measurement is based on the assumption that both the extreme positions of the electrode tip are inside the Nernstian diffusion layer, local copper(II) ion concentration was measured as a function of the distance from the root surface. For this purpose the electrode vibration amplitude was increased in steps of 10 μm. The electrode was first positioned in direct contact with the cellular wall of a single cell (average diameter larger than 80 μm) located at the root surface. Then, the electrode was moved back 5 μm and this point was the zero starting point of the vibration. The amplitude of the first set of oscillations was 10 μm, beginning from the zero starting point and that of the following one was increased in steps of 10 μm. The maximum amplitude reached was 250 μm.

The results plotted in Fig. 6 below show that with the oscillation amplitude selected for the flux measurements throughout this work, the upper position of the electrode is inside the Nernstian diffusion layer. Therefore, Fick's first law can be applied to calculate the flux.

Cu^{2+} fluxes in intact roots of *Olea europaea*

The application of a Cu/CuSe microelectrode furnished results which show the utility of such electrodes as a valuable research tool for copper transport studies in

Table 1 Selectivity coefficients for the Cu/CuSe microelectrode

Interfering ion (Me)	Log $K_{Cu,Me}$
Ca^{2+}	−3.5
Mg^{2+}	−3.6
NH_4^+	−7
Na^+	−5.7
K^+	−7.4

biological systems. To our knowledge, this is the first time that a selective microelectrode for Cu^{2+} has been used in a biological application.

The use of the selective microelectrodes with the "vibrating microelectrode system" allowed a considerable degree of resolution, both spatially and temporally, in the measurement of the copper fluxes in roots. In fact, the system was able to measure very small fluxes (as low as 0.05 pmol cm^{-2} s^{-1}) in specific locations along the root.

The Cu^{2+} flux profile showed a clear spatial organisation, with a significantly higher Cu^{2+} influx at positions 1.5–2 mm from the root apex than at positions further from the apex (Fig. 4). Moreover, the flux was negligible at the very close apex positions (0–100 μm from the apex); at the more distal positions, after the region of maximum uptake, the Cu^{2+} flux decreased very quickly and varied between Cu^{2+} influx and efflux at different positions and times.

Net Cu^{2+} uptake measured over a range of Cu^{2+} concentrations (5–100 μM Cu^{2+}) yielded concentration-dependent kinetics that could be described by the Michaelis-Menten equation (Fig. 5).

Figure 6 shows the copper(II) flux in relation to the distance from a root cell of *Olea europaea* when a steady-state Cu^{2+} gradient was reached in a 10 μM copper(II) solution.

Cu^{2+} uptake showed an oscillatory course over time. The results indicate the existence of at least two oscillatory components in the copper influx: one is slow with a period of around 80 min and the other is fast with a period of around 7 min (Fig. 7). Similar results have been reported for Ca^{2+} and H^+ fluxes [9].

Discussion

The technological progress of recent years has enabled the production of liquid membrane ion selective electrodes (ISEs) for a certain number of ions. These electrodes, despite some limitations, have made it possible to study ion activity in the proximity of plant roots (for example, mechanisms involved in transport). In the present study, the opportunity to use a Cu/CuSe microelectrode made it possible to overcome many of the limitations normally associated with the use of liquid membrane ISEs, especially in biological studies (elaborate preparation, dedicated instrumentation, significant noise, low efficiency, easy poisoning of the membrane).

The response time is a factor of crucial importance in the use of microelectrodes with the "vibrating probe" technique. In fact, the electrode remains in the two measurement positions for times not longer than 5 s (more often 2–3 s). The small size of the Cu/CuSe microelectrode allows the electrode potential to change rapidly following a sharp Cu^{2+} concentration change, and permits measurements to be made on a subsecond time scale.

In recent years, ion flux profiles in roots of different plants have been reported. The spatial organisation of

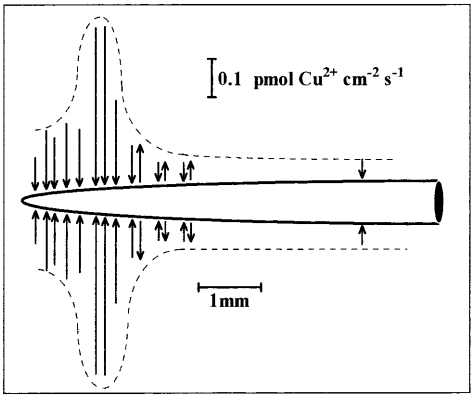

Fig. 4 Diagram illustrating the Cu^{2+} flux profile along the roots of *Olea europaea*. The position and magnitude of the fluxes are indicated by *arrows*: arrows directed toward the root indicate influx and arrows directed away from the root denote efflux

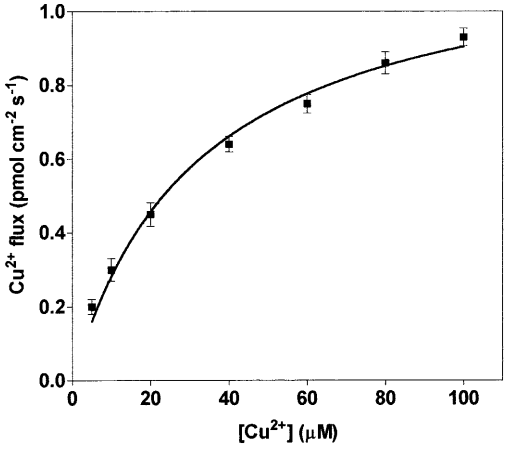

Fig. 5 Concentration-dependent kinetics of Cu^{2+} uptake by root cells of *Olea europaea*. Data were collected by oscillating the electrode over a 50 μm excursion at 0.1 Hz, 2 mm from the root apex

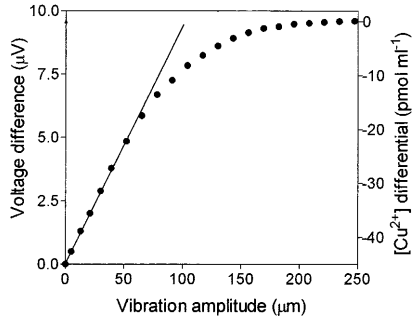

Fig. 6 Local copper(II) concentration as a function of the distance from a root cell of *Olea europaea*. Data were collected by changing the electrode vibration amplitude between 0 and 250 μm at 0.1 Hz, 2 mm from the root apex, in 50 μM $Ca(NO_3)_2$ background solution containing 10 μM $Cu(NO_3)_2$

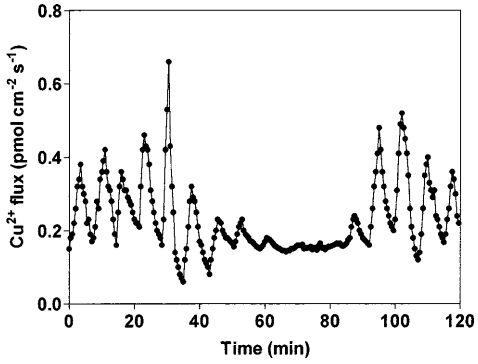

Fig. 7 Oscillatory behaviour of Cu^{2+} flux around the region 2 mm from the root apex. Fast and slow oscillatory components are clearly shown

uptake for Cd^{2+} [10] and Mg^{2+} [11] appears to be similar to the present findings for Cu^{2+}.

Figure 7 shows that the copper(II) uptake at the position of maximum flux (about 1.5 mm from the root apex) follows two clearly different oscillation periods. Such different oscillation periods suggest that it is unlikely that only one transport system is involved in the copper uptake. The hypothesis that the slower oscillations of the Cu^{2+} flux are due to oscillations in the behaviour of the passive Cu^{2+} transporters, and that the faster oscillations are due to regular fluctuation in the behaviour of energy-dependent mechanisms, would seem probable. Further studies are necessary to identify the mechanisms involved in the Cu^{2+} uptake. Recently, Rae et al. [12] found in *Saccharomyces cerevisiae* an extraordinary capacity for intracellular chelation of copper, leading to a substantial driving force for Cu^{2+} passage into the cell.

References

1. Müller E (1922) Z Phys Chem 100: 346
2. Ladriere J (1973) Bull Soc Chim Belge 82: 87
3. Norwitz J (1951) Anal Chim Acta 5: 109
4. Neshkova M, Sheytanov H (1979) J Electroanal Chem 102: 189
5. Neshkova M (1988) Cu^{2+} electrode function dependence on the membrane composition for selenide-based all solid-state copper ion-selective electrodes. In: Pungor E (ed) Proc Ion-selective electrodes 5. Matrafured, Hungary, p 503
6. Papeschi G, Pinzauti S, Gratteri P, Larini M (1992) Sensors Actuators B 7: 544
7. Papeschi G (1995) Sensors Actuators B 24–25: 336
8. Smith P J S (1995) Nature 378: 645
9. Shabala NS, Newman IA, Morris J (1997) Plant Physiol 113: 111
10. Piñeros MA, Shaff JE, Kochian LV (1998) Plant Physiol 116: 1393
11. Grunes DL, Ohno T, Huang JW, Kochian LV (1993) Planta 189: 213
12. Rae TD, Schmidt PJ, Pufahl RA, Culotta VC, O'Halloran TV (1999) Science 284: 805