Ion Selective Electrodes for Microalgal Biotechnology

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*This paper presents the results obtained in monitoring potassium, calcium, and magnesium ions content in Chorella homosphaera 424 culture media using in-house manufactured potassium, calcium and magnesium ion selective electrodes. The sensors were prepared with poly(vinyl chloride) plasticized with o-nitrophenyl octyl ether. Valinomycin(**I**), N,N,N',N'- tetra[cyclohexyl]diglycolic acid diamine, and bis-N,N-dicyclohexyl-malonamide were chosen as ionophores. The membranes worked well in the 10^{-4}- 10^{-1} mol/L concentration range, displaying Nernstian slopes of 57.0 ± 1.0, 27.0 ± 1.0, and 27.0 ± 0.5 mV per decade of K^+, Ca^{2+}, and Mg^{2+} ions activity. The electrodes responses showed pH dependence at pH < 4.0 and at pH > 11.0. The selectivity coefficients, measured by the mixed solutions method, ranged from $1.0 . 10^{-1}$ to $3.2 . 10^{-9}$. The newly prepared sensors gave good results in monitoring potassium, calcium, and magnesium levels in Chlorella homosphaera 424 culture media, as cross-checked by AAS.*

Keywords: ion selective electrode, selectivity coefficients, algal culture media

Microalgal culturing techniques represent a fast growing branch of biotechnology. It is successfully used in producing fine chemicals [1,2], as well as growing algae as source of renewable fuels [3,4]. The media ionic composition is a crucial factor in microalgal culturing techniques, influencing the yield. Therefore continuous *in situ* measurement of concentration and control of different ionic species is highly beneficial. Usage of ion selective electrodes as transducers for the analysis is an obvious choice, as they display good selectivity and deliver specific and sensitive results in short time. They can be manufactured rapidly, at a very low cost, and can be used without complex pre-treatments and can be applied for *in situ* analysis in many types of aquatic environments. There are several reports on monitoring K^+, Mg^{2+}, and Ca^{2+} ions concentrations with the aid of ion selective electrodes, applications ranging from clinical analyses to environmental projects [6-9]. Gupta et al. [8] developed magnesium ion selective electrodes using benzo-15crown-5 as ionophore. Best performance was exhibited by the membrane containing crown: PVC: NaTPB: DOP in the 10 : 150 : 3 : 150 ratio. The device worked well in the 10^{-5} - 10^{-1} mol/L concentration range, being used for determination of magnesium by potentiometric titration with EDTA in aqueous and non-aqueous media. No significant changes of slope or working range concentration were noticed when solutions contained up to 20% (v/v) non-aqueous fraction. ETH 5282 [(N',N",N"'-iminodi-6,l-hexanediyl)-tris(N-heptyl-N-methyl-malonamide)] was tested as magnesium selective carrier [10] in undiluted blood serum samples. The membrane contained ETH 5282 / PVC/ o-nitrophenyloctyl ether (1/33/65 by weight), and potassium tetrakis(p-chlorophenyl) borate (KTpClPB) in a molar ratio of 1.5 relative to the ionophore. However, the ionophore used suffered from calcium interference at 1% concentration. Şendil et al [11] proposed a potassium ion selective electrode based on benzo-15-crown-5 as neutral carrier, which worked well in the 10^{-4} - 10^{-2} mol/L range, with a slope of 56.0 ± 3.0 mV/decade of potassium ion concentration. This electrode gave good results in determining K^+ in orange juice, vitamin, and edible salt. Bedlechowicz et al. [7] studied the plasticizer effect on the response and selectivity of a calcium selective device containing [(—)-(R,R)-N,N'-bis-[11-(ethoxycarbonyl) undecyl]-N,N'-4,5-tetramethyl-3,6-dioxaoctanediamide] (ETH 1001). (2-ethylhexyl)sebacate (DOS) and o-nitrophenyl octyl ether (o-NPOE) were used as plasticizers. o-NPOE extended the linear domain of the calibration curve and lowered the detection limit when compared to the DOS-containing device. The optimal activity of free Ca^{2+} and Na^+ ions in the internal reference solution varied from 10^{-4} to 10^{-1} mol/L for DOS, and 10^{-6} to 10^{-1} mol/L for o-NPOE respectively. Other two calcium ion selective electrodes based on ETH 1001 and N,N,N',N'-tetracyclohexyl-3-oxapentanediamide (ETH 129) were reported [12] and compared using a similar procedure. The detection limit decreased to $10^{-8.8}$ mol/L when an internal filling solution buffered for the primary ion was employed. ETH 129 worked well for monitoring low Ca^{2+} ions concentration in the presence of a significant K^+, Na^+, and Mg^{2+} background.

However, adaptation of ion selective electrodes for biotechnological applications is rather challenging, because of the significant concentrations of interfering chemical species. Also the aquatic environment needed for *Chlorella homosphaera 424* growth as biofuel material undergoes *p*H variations during cultivation [5].

We present here the preparation of three ion selective electrodes based on poly(vinyl chloride) plasticized with o-NPOE for in situ determination of potassium, calcium, and magnesium ions in *Chlorella homosphaera 424* growth media. Valinomycin (**I**), N,N,N',N'- tetra[cyclohexyl] diglycolic acid diamine, and bis-N,N-dicyclohexyl-malonamide were used as carriers for the selective monitoring of potassium, calcium, and magnesium ions both in synthetic solutions and algal culture media.

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ISE	Membranes composition			
	Ionophore (mg)	*o*-NPOE (mg)	PVC (mg)	PTCB
K^+	2.5	172.51	87.5	3.3 mg
Mg^{2+}	4.0	120.0	64.0	70 mol%
Ca^{2+}	1.48	173.03	89.88	7.49 mg

Table 1
ELECTRODE MEMBRANE
COMPOSITIONS

Analyte	Wavelength (nm)	Flame gases	Dilution factor (times)
K^+	769.9	Air-acetylene	100
Mg^{2+}	285.2	Air-acetylene	100
Ca^{2+}	422.7	Air-acetylene	3

Table 2
ATOMIC ABSORPTION SPECTROMETRY
CONDITIONS USED FOR DETERMINATION
OF K^+, Mg^{2+}, Ca^{2+} IN THE *CHLORELLA
HOMOSPHAERA 424* CULTURE MEDIA

Experimental part
Reagent and materials

The N,N,N', N' -tetra[cyclohexyl]diglycolic acid diamide, valinomycin, *o*-NPOE ($\geq$ 99.0%), poly(vinyl chloride) high molecular weight (PVC), and potassium tetrakis(4-chloro-phenyl)borate (PTCB) ($\geq$ 98.0%) were obtained from Fluka (Germany). The bis-N,N-dicyclohexyl-malonamide magnesium ionophore was prepared and kindly donated by Istvan Bitter from the Technical University of Budapest, Department of Organic Chemical Technology, Hungary [1]. Tetrahydrofuran (THF) from Inter Kemia (Hungary) was used as solvent. All reagents were used without further purification.

Magnesium chloride hexahydrate (Fluka-Germany), potassium chloride (Panreac - Spain), calcium chloride dihydrate, and dibasic phosphate (Sigma-Aldrich - Germany) with an assay of 99 % were used without further purification. Sodium nitrate, magnesium sulphate heptahydrate (Merck - Germany), potassium sulphate (Riedel-deHaën-Germany) were ACS reagents, with a 99.5 % assay.

All solutions were prepared with freshly deionized water. Stock solutions were kept in polypropylene bottles. 'A grade' glassware was used for all experiments.

Chorella homosphaera 424 growth media

The algal culture media composition consisted of 16.80 g/L $NaHCO_3$, 0.5 g/L K_2HPO_4, 2.5 g/L $NaNO_3$, 1 g/L K_2SO_4, 1 g/L NaCl, 0.2 g/L $MgSO_4$. $7H_2O$, 0.04 g/L $CaCl_2$. $2H_2O$, 1 mL transition metals solution and 5 mL chelated iron solution. The transition metals mix contained H_3BO_3 (2.860 g/L), $MnSO_4$. $4H_2O$ (2.03 g/L), $ZnSO_4$. $7H_2O$ (0.222 g/L), MoO_3 (0.018 g/L), $CuSO_4$. $5H_2O$ (0.079 g/L), $Co(NO_3)_2$. $6H_2O)$ (0.494 g/L). The chelated iron stock solution was prepared by dissolving 0.69 g $FeSO_4$. $7H_2O$ and 0.93 g Na_2EDTA in 80 mL with deionized water, and boiling for a short time. After cooling at room temperature, the solution was made up to 100 mL [5].
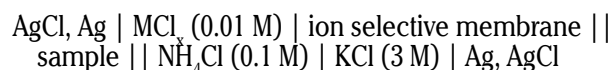
Membrane preparation and electrodes

The potassium ion selective membrane were prepared similar to the Fluka recommendations [13], using *o*-NPOE instead of bis(1-butylpentyl) decane-1,10-diyl diglutarate (ETH 469). The calcium ion selective membranes were prepared similar to the Fluka recommendations ratio [14]. The magnesium ion selective membrane was obtained as described in [6]. The typical membrane compositions are presented in table 1.

All components were dissolved in 4 mL THF. The resulting solution was poured into a 26 mm inner diameter glass ring placed on a glass plate, covered with another glass plate, and left for 2 days in the hood for solvent evaporation. A 7 mm disc was cut from the membrane material and fixed into the electrode body. The device was filled with solutions containing 10^{-2} mol/L KCl, 10^{-2} mol/L $MgCl_2$, and 10^{-2} mol/L $CaCl_2$ - 5.0 x 10^{-2} mol/L EDTA at *p*H

9.3, respectively. The *p*H of the calcium electrode inner filling solution was adjusted with NaOH, 10^{-1} mol/L solution. All membranes were conditioned for two days, before use in solutions containing the analyte of interest, 10^{-2} mol/L KCl, 10^{-2} mol/L $MgCl_2$, and 10^{-2} mol/L $CaCl_2$ respectively. The membranes were stored dry when not in use.

Potential measurements

Potentiometric measurements were performed with the aid of a pH meter Thermo Orion at 25 $\pm$ 2^{0}C, using an electrochemical cell set-up as follows:

AgCl, Ag | MCl_x (0.01 M) | ion selective membrane ||
sample || NH_4Cl (0.1 M) | KCl (3 M) | Ag, AgCl

NH_4Cl, 10^{-1} mol/L solution, was chosen as salt bridge filling, as preliminary tests proved that usage of KCl, NaCl, CsCl or $Al_2(SO_4)_3$ did not provide a Nernstian response from the prepared sensors (table 2).

The electrodes were washed with water and dried carefully between measurements.

The selectivity coefficients were determined by the *fixed interference method* (FIM) [15], using a 10^{-1} mol/L and 10^{-2} mol/L interfering ion concentration.

The electrodes pH dependence was monitored by measuring the potential response in solutions containing 10^{-3} mol/L potassium, calcium, and magnesium ions respectively, in the 3.0-12.0 *p*H range. The *p*H was varied using 10^{-1} mol/L HCl or 10^{-1} mol/L NaOH.

Potassium and magnesium concentrations were determined with the aid of the corresponding selective electrodes, using the standard addition method, while calcium content was evaluated indirectly, by potentiometric titration with ethylenediamine tetraacetic acid (EDTA). A 50 mL sample was titrated with EDTA, 10^{-2} mol/L solution, using the calcium electrode. The solution pH was set to 1.8 by addition of HCl, 1 mol/L solution, to decompose the acidic carbonate, the mixture was then boiled, cooled, and neutralized with NH_3/NH_4Cl buffer to *p*H 8.8. The calcium ions content was available by difference between the total titrated calcium and magnesium ions and the magnesium ions obtained with the ISE. The procedure was similar for algal growth media.

Atomic absorption spectrometry measurements

Working parameters used for cross-checking K^+, Mg^{2+}, Ca^{2+} ions concentrations in the *Chlorella homosphaera 424* culture media are presented in table 3. Calibration curves were obtained in the 0-0.5 ppm concentration range for Mg^{2+}, 0 - 5 ppm concentration range for Ca^{2+}, and 0-10 ppm concentration range for K^+ ions, using stock standard solutions for atomic absorbtion (Panreac, Spain), a typical calibration curve for potassium being presented in figure 1. Algae growth media samples were prepared adding 1 mL aliqout into 100 mL freshly deionized water,

Salt bridge	Slope (mV)		
	K^+–ISE	Mg^{2+}–ISE	Ca^{2+}–ISE
$Al_2(SO_4)_3$, 0.9 mol/L	48	-	-
$AlCl_3$, 0.1 mol/L	72	-	-
CsCl, 0.1 mol/L	-	24	21
KCl, 3 mol/L	-	18	23
NH_4Cl, 0.1 mol/L	57	27	27

Table 3
RESPONSE OF THE *IN-HOUSE*
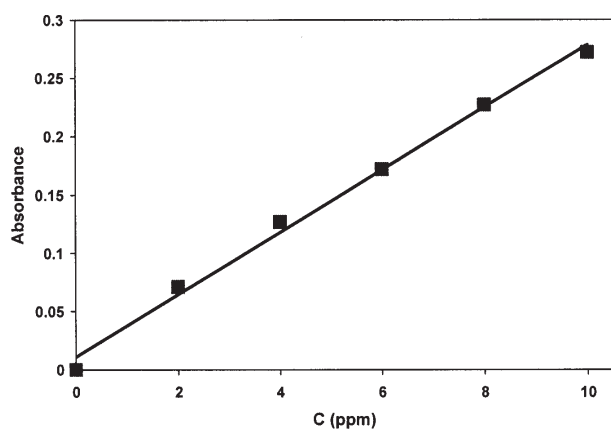PREPARED ION SELECTIVE
ELECTRODES FOR DIFFERENT
SALT BRIDGES

Fig. 1. Calibration curve for potassium as obtained by AAS, using
the experimental conditions in table 1

Fig. 2 Calibration curves for the *in-house* prepared ISEs in the
$1.0 \cdot 10^{-7} - 5.0 \cdot 10^{-1}$ mol/L domain, at 25 ± 2^0C

for K^+ and Mg^{2+}. For Ca^{2+} the samples were diluted 3 times. $SrCl_2$, 1% solution, was used to eliminate phosphorus interferences.

Resistance measurements

The voltage divider method was used for measuring the internal resistance (R_i) of an electrochemical cell containing the corresponding ion selective electrode [17, 19]. A precision resistor (R_k, 4.6 MΩ) was included in the experimental set-up. The cell contained 10^{-3} mol/L MCl_x solutions [17] (where M = K^+, Mg^{2+} or Ca^{2+} and x = 1 or 2). The corresponding indicating and reference electrodes were connected to the voltage follower and cell voltage was recorded with a Thermo Orion pH meter. When steady reading was achieved, the R_k precision resistor load was interconnected between the inputs of the voltage follower. ΔU value measured at steady reading was used in the R_i calculation from the relationship:

$$R_i = \frac{\Delta U \times R_k}{U_k}$$

where U_k was the steady voltage value after switching on R_k.

Results and discussions

Potassium, calcium and magnesium ion selective electrodes were calibrated against a double junction Ag/AgCl reference electrode, at 25 ± 2^0C, using a $10^{-6} - 10^{-1}$ mol/L analyte concentration range. Response time did not exceed 30 s, even for the more diluted solutions. As the curves in figure 2 show, the membranes worked well in the $10^{-4}-10^{-1}$ mol/L concentration range, displaying Nernstian slopes of 57.0 ± 1.0, 27.0 ± 1.0, and 27.0 ± 0.5 mV per decade of K^+, Ca^{2+}, and Mg^{2+} ions activity (table 4).
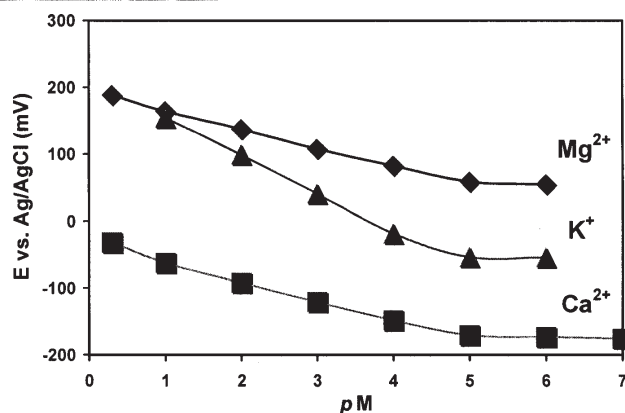

Fig. 3 Effect of *p*H on cell potential measured in 10^{-3} mol/L MCl_x solutions (where M = K^+, Mg^{2+}, Ca^{2+} and x = 1 or 2) in the 3.0 - 12.0 *p*H range

The ISEs responses towards solution *p*H changes were also investigated in order to optimize the measuring conditions for the selected analytes. As experimental results presented in figure 3 show, the potential is unaffected by *p*H changes in the 6.5 – 11.0 domain for the potassium ion selective electrode, 4.0 - 11.0 *p*H range for the magnesium ion selective electrode, and 4.5-11.0 for the calcium ion selective electrode.

In more basic conditions (*p*H > 11.0) all three devices present a 5 - 6 mV potential drift. The observed drifts at higher *p*H values could be connected to the formation of magnesium and calcium hydroxocomplexes, thus decreasing the free ions concentrations. As for the potassium, there is a high chance of sodium interference. The behaviour at low *p*H values might be attributed to the lower ability to form metal complexes.

ISE characteristic	K^+–ISE	Mg^{2+}–ISE	Ca^{2+}–ISE
Slope (mV/decade)	57.0 ± 1.0	27.0 ± 0.5	27.0 ± 0.5
Linear working range (mol/L)	$10^{-4} - 10^{-1}$	$10^{-5} - 5.0 \times 10^{-1}$	$10^{-4} - 10^{-1}$
pH working range	4.0 – 11.0	6.0 – 11.0	4.5 – 11.0
Membrane resistance (MΩ)	5.8	4.6	5.5
Response time (s)	< 30	< 30	< 30
Life time (months)	4	4.5	4.5

Table 4
CHARACTERISTICS OF THE *IN-HOUSE* PREPARED ION SELECTIVE ELECTRODES
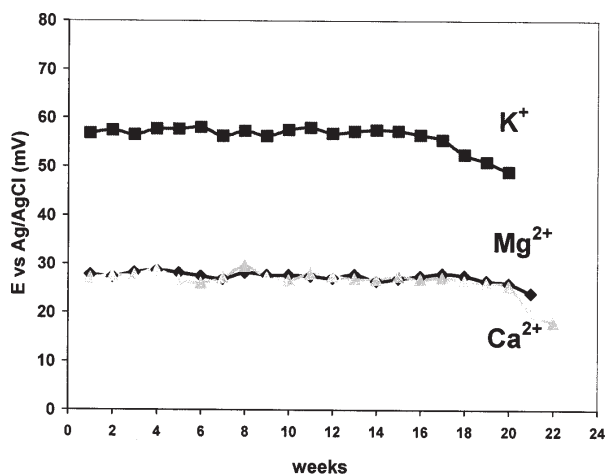
Fig. 4. Nernstian factor value changes of K+, Mg²⁺ and Ca²⁺ ISEs in time, in the $10^{-4} - 10^{-1}$ mol/L domain, at 25 ± 2^0C

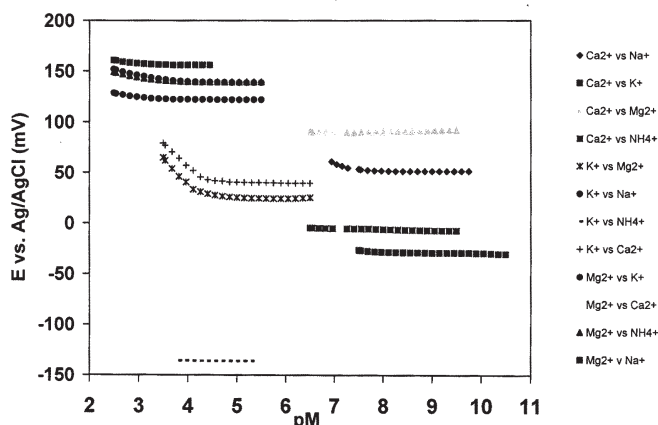Fig. 5b. Calibration curves for the *in-house* prepared ISEs at fixed interferent level (10^{-1}mol/L Mg²⁺, 10^{-2} mol/L K+ or Ca²⁺) at 25 ± 2^0C

membranes were re-equilibrated with 10^{-2} mol/L MCl$_x$ (where M = K+, Mg²⁺, Ca²⁺ and x = 1 or 2) for 1-2 day.

Potentiometric selectivity coefficients, defining the ability of the ISEs to differentiate a primary ion from the interfering ions [15,18] measured by *fixed interference method* as recommended by IUPAC [15] are presented in figures 5a and 5b.

The selectivity coefficients values, calculated using the responses recorded in figures 5a and 5b, are collected in table 5. The experimental values are in agreement with data reported by other groups [8,14,16,18,20]. The magnesium electrode does not exceed in terms of selectivity in the presence of potassium and ammonium ions, as it had also been reported for a magnesium electrode incorporating benzo-15-crown-5 ionophore [8].

The potassium electrode shows similar selectivity coefficients towards magnesium, and calcium as the device reported in [16], employing a valinomycin carrier; selectivity towards ammonium is one order of magnitude better, while the sodium selectivity is one order of magnitude smaller.

Selectivity coefficients against ammonium ions were determined to check for the influence of the NH$_4$⁺ ions present in salt bridge upon the electrode response. Results showed that the bridge filling does not affect the electrode behaviour.

The analytical potential of the prepared ion selective electrodes was checked on the *Chlorella homosphaera*

Fig. 5a. Calibration curves for the *in-house* prepared ISEs at fixed interferent level (10^{-1}mol/L K+ or Ca²⁺, 10^{-2} mol/L Mg²⁺) at 25 ± 2^0C

The membrane resistance values determined by the voltage divider method are collected in table 4. The experimental values are rather similar for all three membranes and comparable to those reported by Mettler Toledo for K+ ISE > 20 MΩ, Ca²⁺ ISE > 10 MΩ, Mg²⁺ ISE > 10 MΩ [20].

The life time of potassium, magnesium and calcium ion selective electrodes was determined by measuring the Nernstian factor value over 22 weeks. Nernstian factor values measured each week are shown in figure 4. The sensors provided reproducible results between 4 and 4.5 months. Whenever a drift in potential was observed,
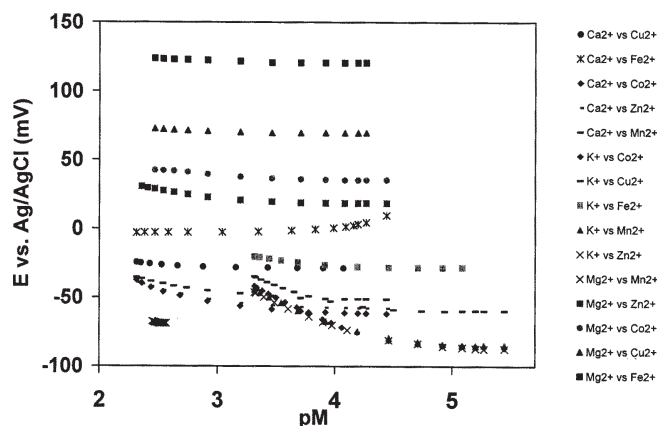
Interferent, B^{z+}	-log $K_{M, B^{z+}}$					
	K+–ISE		Mg²⁺–ISE		Ca²⁺–ISE	
	Present study	Literature [16, 20]	Present study	Literature [8]	Present study	Literature [14,18,16]
Mg²⁺	4.00	4.00	–	–	6.50	9.30
Ca²⁺	3.90	4.00	1.50	1.60	–	–
Na+	2.90	3.60	2.60	1.49	7.90	8.30
K+	–	–	0.90	0.30	8.60	10.10
NH$_4$⁺	3.91	2.90	0.84	1.90	8.50	2.10
Fe²⁺	2.92	3.40	1.67	1.70	2.26	0.10
Co²⁺	2.82	–	2.00	1.72	1.00	–
Cu²⁺	2.55	2.89	1.70	1.72	1.27	0.82
Zn²⁺	3.00	3.40	1.00	1.74	2.00	1.19
Mn²⁺	2.87	3.46	2.00	–	1.50	0.68

Table 5
SELECTIVITY COEFFICIENTS MEASURED FOR THE *IN-HOUSE* PREPARED POTASSIUM, CALCIUM AND MAGNESIUM ION SELECTIVE ELECTRODES IN 10^{-2} AND 10^{-1} MOL/L INTERFERENT ION SOLUTIONS, AT 25 ± 2^0C

Ion	$10^{-2} \times C_{M^{x+}}$ (mol/L) in *Chlorella homosphaera 424* culture media			Recovery (%)
	Potentiometric titration with ISE*	ISE*	AAS*	ISE
K+	-	1.860±0.003	1.700±0.005	100.01
Mg²⁺	-	0.081±0.003	0.080±0.001	99.98
Ca²⁺	0.027±0.001	-	0.026±0.001	-

*Average of three determination ± standard deviation

Table 6
ANALYSIS OF IONIC CONTENT IN THE *CHLORELLA HOMOSPHAERA 424* CULTURE MEDIA AT 25 ± 2^0C

http://www.revistadechimie.ro

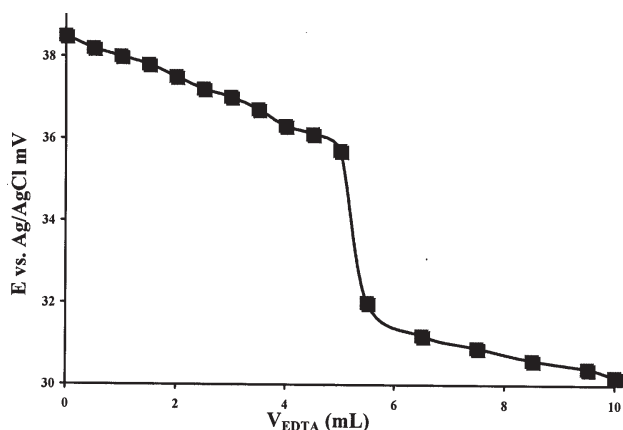Fig. 6. Potentiometric titration of total Ca^{2+} and Mg^{2+} concentration in the *Chlorella homosphaera 424* culture media with EDTA, 10^{-2} mol/L solution, at 25 ± 2^0C

424 culture media. The titration curve in figure 6 shows the potential variation with the total calcium and magnesium ions concentration. The calcium ions concentration was accessible by difference between total calcium and magnesium ion concentration value signaled by the equivalence point in the titration curve, and the magnesium ions concentration value determined directly, with the aid of the magnesium ion selective electrode. The experimental results were crossed-checked by atomic absorption spectrometry and results are available in table 6. The results obtained by these two techniques are similar, the recovery yield ranging form 99.8 to 100.0 %.

Conclusions

The electrodes prepared using PVC matrix and selective carriers worked well over a 10^{-4}- 10^{-1} mol/L concentration range, with a Nernstian slope of 57.0 ± 1.0, 27.0 ± 1.0 and 27.0 ± 0.5 mV per decade of K^+, Ca^{2+}, Mg^{2+} ions activity, convenient response time, and lifetime. Electrodes did not suffer from H^+ interference in the 6 - 11 pH range. Selectivity over other Na^+, K^+, NH_4^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+}, and Mn^{2+} was remarkably good, similar to most values already reported in the literature and the lifetime of all devices was between 4 and 4.5 months. Although the sensors presented good selectivity over Na^+, K^+, NH_4^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+}, and Mn^{2+}, the matrix complexity required evaluation of potassium and magnesium content by potentiometric standard addition method and indirect calcium quantification by potentiometric titration with EDTA. The three sensors were successful in assessing the Ca^{2+}, Mg^{2+}, and K^+ content in a typical *Chlorella homosphaera 424* culture media, fact also ascertained by atomic absorption spectrometry. Future work will require attentive evaluation of ISEs performances in terms of repeatability, accuracy, precision, limit of detection, and limit of quantification, and a comprehensive report on species concentration variations during a complete algae time cycle.

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