The Manufacture and Characteristics of Magnesium Selective Macroelectrodes

W. Zhang[1], A.C. Truttmann[2], D. Lüthi, J.A.S. McGuigan

Zusammenfassung

Eine Methode zur Herstellung von magnesiumselektiven Makroelektroden wird hier beschrieben, bei welcher der neutrale Konduktor ETH 7025 für die Messung der ionisierten Magnesiumkonzentration ($[Mg^{2+}]$) in physiologischen Lösungen, mit oder ohne Proteine, verwendet wird. Während die Interferenz von pH, Na und K minimal ist, verursacht das Ca eine Reduzierung der Reaktion der Elektroden. Beim Vorhandensein von 1 mM Ca konnte sich der Meßwert der Elektroden des $[Mg^{2+}]$ bis hinunter auf 50 μM senken, und in nominal Ca-freien Lösungen war er fast auf Nernstian-Niveau unten bei dieser Konzentration.

Summary

A method is described to manufacture magnesium selective macroelectrodes using the neutral carrier ETH 7025 for the measurement of the ionized magnesium concentration ($[Mg^{2+}]$) in physiological solutions, with or without protein. While interference from pH, Na and K is minimal, Ca reduces the response of the electrodes. In the presence of 1 mM Ca the electrodes could measure the $[Mg^{2+}]$ down to 50 μM and in nominally Ca free solutions they were almost Nernstian down to this concentration.

Résumé

Une méthode a été décrite pour la production de macroélectrodes magnésium-sélectives en utilisant le conducteur neutral ETH 7025 pour le mesurage de la concentration magnésique ionisée ($[Mg^{2+}]$) dans des solutions physiologiques, avec ou sans protéines. Tandis que l'interférence de la part de pH, Na et K est minimale, le Ca réduit la réponse des électrodes. Avec une présence de 1 mM Ca les électrodes pouvaient mesurer le $[Mg^{2+}]$ diminué jusqu'à 50 μM, et dans des solutions nominalement sans Ca elles étaient presque Nernstian basses dans cette concentration.

Introduction

It has been well established that magnesium modulates numerous intracellular processes, but it is the ionized magnesium concentration ($[Mg^{2+}]$) and not the total magnesium concentration ([Mg]) that is the physiological parameter [4]. This being so, it is the $[Mg^{2+}]$ that should be measured intracellularly, extracellularly or in physiological solutions which mimic either the intra- or the extracellular milieu.

Mg selective electrodes provide a direct and convenient way to measure the $[Mg^{2+}]$ and suitable microelectrodes capable of measuring the $[Mg^{2+}]$ intracellulary or in intracellular like physiological solutions have been available for a number of years [8]. However, these electrodes are difficult to manufacture, have an extremely high resistance necessitating specialized recording equipment, characteristics which limit their routine use. In this paper, we describe the manufacture and characteristics of Mg macroelectrodes based on the neutral carrier ETH 7025 [11] suitable for measuring the $[Mg^{2+}]$ in physiological solutions that are similar to either the intracellular or extracellular ionic composition. Such electrodes do not suffer from the disadvantages of the microelectrodes and are capable of measuring the $[Mg^{2+}]$ down to 50 μM or lower. The electrodes are easy and cheap to manufacture and require only simple recording equipment.

This work has been published in abstract form [13, 14].

[1] Karolinska Institute, The Noble Institute for Physiology, Box 60400, S-10401 (Sweden).

[2] Kinderklinik, University Hospital (Inselspital), 3010 Berne (Switzerland).

Materials and Methods

Magnesium Macroelectrodes

Manufacture

The manufacture of these electrodes is based on the method of *Fry* [6] where a detailed description is given. Briefly, these electrodes were made from either PVC tubing or from polyurethane tubing. The polyurethane tubing has the advantage of being more rigid and was obtained as sterile catheters (internal diameter, 1.5 mm) from Vygon, Laboratoire Pharmaceutique, Ecouen, France. A ceramic plug was milled to fit into the end of the tubing and this could be coated with the magnesium selective layer (see Fig. 1).

To prepare the electrodes we used the mixtured proposed by *Spichiger* et al. [11]. Initially the concentration of potassium tetrakis-(4-chlorophenyl)-borate was reduced in this mixture [14] but later work showed that this was not necessary. To manufacture the mixture we carefully cleaned three small glass bottles (about 10 ml in volume) with tetrahydrofuran (THF), allowing the THF to evaporate. Tab. 1 gives the constituents of each bottle.

The PVC is weighed out and the THF is slowly added to it stirring continuously. This is essential, or else it will form

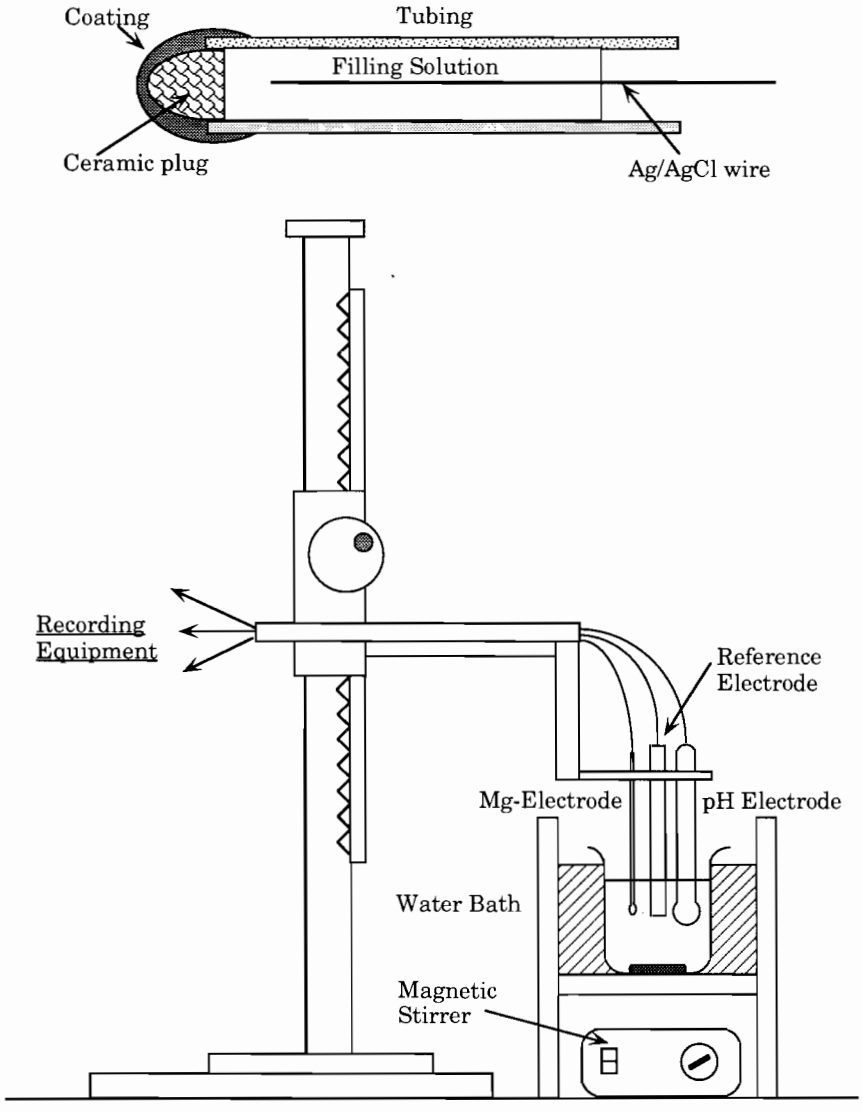

Fig. 1: Diagram of the experimental set-up used to either calibrate the electrodes or to measure apparent binding constants. All measurements were carried out at the same temperature and pH and solutions were pre-warmed to the desired temperature. A diagram of the electrode is shown at the top of the Fig.

Tab. 1: Composition of the magnesium mixture.

Bottle 1	High molecular weight PVC, 150 mg Tetrahydrofuran, 6 ml
Bottle 2	ETH 7025, 60 µl 2-Nitrophenyl octyl ether, 325 mg
Bottle 3	ETH 500, 15 mg K tetrakis-(4-chlorophenol)-borate, 4.4 mg

clumps which then take ages to dissolve. When the PVC is dissolved the contens of bottle 2 are emptied into it, while stirring. Finally, the contents of bottle 3 are also emptied into the PVC. Once all constituents are dissolved, the THF is allowed to evaporate slowly by covering the bottle with several layers of filter paper held in place by a heavy weight. Evaporation takes several days. The mixtures should be stored in the dark and can be kept and reused for months [6].

For the preparation of the electrodes the mixture is re-dissolved in about 1 ml of THF and the mixture stirred until the consistency is like British Tate & Lyle syrup. The electrodes are then prepared by dipping and three coats are recommended. When they are dry, the electrodes can be stored in the dark for several months with no change in their characteristics.

Use

Before use they are backfilled with 150 mM KCl containing 1 mM $MgCl_2$ to which has been added a small lump of AgCl. When backfilling it is essential to get rid of all the bubbles from the tip of the electrode. It pays to refill an unstable electrode for this can often be due to air bubbles at the back of the ceramic plug. A chlorided silver wire was carefully pushed into the electrode to establish electrical contact.

After backfilling the electrodes have to be placed in a background solution containing 1 mM calcium for 48 hours. This step is essential to improve electrode function, but the reason for the improvement is unknown.

Recording

The indifferent electrode was an Orion reference electrode (number 900200). Recording was via a WPI 4-channel high input impedance interface and the potential could then be read from a digital voltmeter or recorded on a pen recorder. In the majority of experiments an AD converter (Mac Lab) was used. The sweeps were then transferred to a Macintosh computer. Each sweep was for a measurement period of 15 seconds and was averaged on-line. The time interval between each measurement was 1 minute. (The simplest way to record would be to use a pH meter as a voltmeter, for in this case an impedance buffer amplifier is incorporated into the meter.)

Calibration

Calibration was carried out in background solutions similar to the solutions in which the measurements were going to be carried out in, and at the same temperature and pH (Fig. 1). Beakers of 100 ml were used and the solution was continuously stirred. Calibration was carried out in solutions resembling either the extra or intracellular milieu. The "extracellular" calibration solutions contained in mM:

NaCl, 150; KOH, 2,5; KCl, 2.5; $CaCl_2$, 1. The composition of the "intracellular" calibration solutions was as follows: KCl, 138; KOH, 2,5; NaCl, 14.6. The $MgCl_2$ concentrations were 10, 6, 4, 1.5, 0.8, 0.5, 0.25, 0.15 and 0.05 mM. A 1 M $MgCl_2$ was bought from Sigma Chemicals, Switzerland, because $MgCl_2$ is very deliquescent and preparation of an accurate solution is difficult. The solutions were buffered to either pH 6.4 or pH 7.0 and 7.4 with 5 mM Pipes or Hepes respectively. To maintain constant Na and K concentrations in these solutions they were back titrated to the desired pH with 1M HCl [see 5]. When studying the effect of changes in Na and K on the dissociation constant, the calibration solutions were modified accordingly. In the calibration procedure, the potential in 10 mM Mg was taken as zero and all other potentials referred to this arbitrary defined zero potential (Appendix in [2]).

Results

Macroelectrodes

General characteristics

Drift:

Despite screening the electrodes, there was over the time course of the experiments either a small upward or downward drift of the zero reference potential. A mean drift ± SD of 0.072 ± 0.046 mV/minute (n = 31) at 25 °C and 0.069 ± 0.061 mV/minute (n = 69) at 37 °C was found for PVC electrodes calibrated in the intracellular solutions. The drift of the polyurethane electrodes calibrated in extracellular solutions was less, being 0.014 ± 0.013 mV/minute at 25 °C (n = 21) and because of this we now routinely use polyurethane instead of PVC tubing for the manufacture of the electrodes. Over a 60 minute period the drift could amount to some 3 to 4 mV. It was thus necessary to take this drift into account when calibrating the electrodes or when carrying out an actual measurement. Calibration was first carried in the magnesium calibration solutions, the 10 mM calibration solution being measured at the beginning and end of the calibration run. As stated in the Methods section the potential in 10 mM is defined as "zero" potential. For each measurement not only was the potential measured, the actual time of the measurement was noted as well. On the assumption that the drift was linear it could be calculated in mV/minute and it was then possible to calculate the "zero" potential at the time of the measurement. The difference between this zero and the potential in the calibrating solution was then the desired potential.

Typical response and life span of the electrodes:

Typical calibration curves obtained at 37 °C in the intracellular solutions are shown in Fig. 2. The lower points (circles) are the measured calibration after manufacture and the upper points (squares) are the calibration eight days and 17 calibrations later. This drift down with time and use was a consistent finding and meant that at 37 °C after about one to two weeks the electrode had to be replaced by a new one.

Such calibrations can be fitted by the *Nicolsky-Eisenman* equation:

$$\text{Potential} = U_o + s \log (\gamma_{\pm}^2[Mg^{2+}] + \Sigma\, K_i a_i^{z_{Mg}/z_i})$$

Where U_o is a constant of the recording system, s the slope and $\gamma_{\pm}$ the mean activity coefficient of $MgCl_2$ · $\gamma_{\pm}^2$ represents the magnesium single ion activity coefficient according to the *Debye-Hückel* convention. The contribution of the interfering ions is given by $\Sigma\, K_i a_i^{z_{Mg}/z_i}$ (review [10]).

A convenient modification for curve fitting routines is:

$$\text{Potential(mV)} = U_o^I + s \log (10^{-pMg} + K)$$

where, $U_o^I = (1000{*}U_o + s \log \gamma_{\pm}^2)$, $K = \frac{\Sigma\, K_i a_i^{z_{Mg}/z_i}}{\gamma_{\pm}^2}$, the slope $s = \frac{1000{*}RT}{zF}$ and R, T, F and z have their usual meaning.

The measured points in Fig. 2 have been fitted by this modification and in both case an excellent fit was obtained. Both responses are almost Nernstian down to a concentration of 0.25 mM (pMg = 3.6) and measurement is possible down to 0.05 mM (pMg = 4.3).

The characteristics of the electrodes at 25 °C were very similar and again measurement was possible down to 0.05 mM. At 25 °C the drift down was slower, electrodes used at this temperature having a life span of about a month.

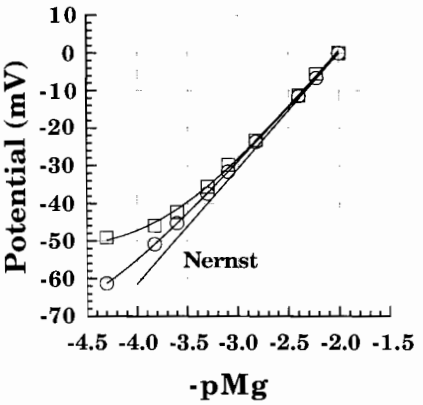

Fig. 2: Typical electrode calibration at 37 °C, circles immediately upon use and eight days later, squares. Both curves have been fitted by the modified *Nicolsky-Eisenman* equation. Coefficients of correlation, r were 0.9999 and 0.9994 respectively.

In the course of these experiments we obtained 52 calibrations before and after an experiment at 37 °C. Not all these experiments were successful, but they did allow us to compare the difference in the $[Mg^{2+}]$ when calculated from the calibration curves before and after the experiment. Such a comparison showed that as the $[Mg^{2+}]$ increased, the same alteration in potential, gave rise to larger deviations in the estimated $[Mg^{2+}]$.

Interference by other cations:

Changing pH from 6.4 to 7.4 had no influence on the calibration curves (four experiments) as had adding EGTA to the calibration solution to reduce the calcium concentration from the contamination level of between 10 to 20 μM [9] to less than 10 nM in nominally calcium free solutions.

The main interference was from Ca and Fig. 3A shows the mean ± SD of seven measurements on three electrodes to increasing calcium concentrations in the extracellular calibration solutions. In nominally zero calcium (10 to 20 μM [9]) the response of the electrode is virtually Nernstian down to a $[Mg^{2+}]$ 0.05 mM (pMg = 4.3). As the calcium is increased first to 1 mM and then to 2.5 mM the response of the

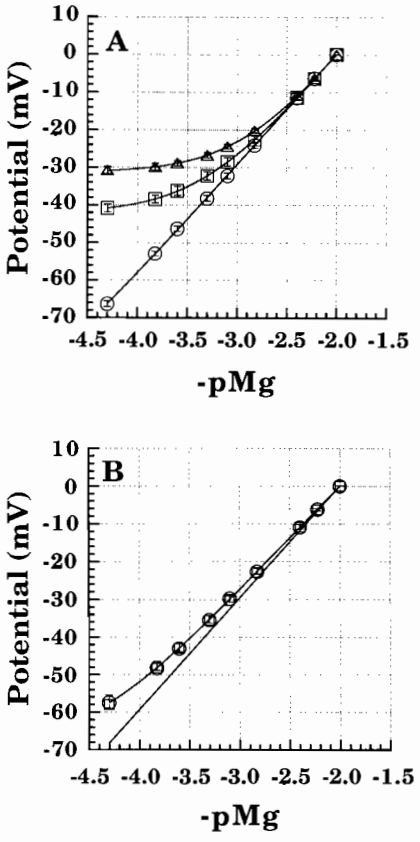

Fig. 3: Electrode interference at 25 °C. A.: Mean ± SD for seven measurements on three electrodes to demonstrate the effect of increasing the calcium concentration from zero (○), 1 mM (□) and 2,5 mM (△); r was in all three cases 0.9999. B.: To show the lack of interference from changes in the K and Na concentrations. Mean ± SD of three electrodes. Circles normal intracellular background solutions, squares, background solutions in which the Na concentration was 53.3 mM and the K 101.8 mM; nominally calcium free, r, 0.9999.

electrode decreases. However, in the presence of physiological [Ca^{2+}], namely 1 mM measurement is still possible down to 50 μM.

Mixing the intracellular and extracellular solutions (calcium omitted) in the proportions of 5 to 2 give a sodium concentration 53.3 mM and a potassium concentration of 101.8 mM had no effect on the characteristics of the electrodes when compared to calibration in the intracellular solution. This is illustrated in Figure 3B, where the mean ± SD of seven measurements in the intracellular solution and five measurements from the mixture with three different electrodes is shown. The curves are identical. These experiments were carried out in nominally calcium free solution, but similar experiments in solutions containing 1 mM calcium with five different electrodes also gave the same response. There was however, some interference from potassium since calibrating the same electrode in the intracellular and extracellular solutions (calcium omitted) showed a very slight decrease in the response of the electrode in the high potassium solution. However, such large changes in potassium and sodium would not be encountered in an experimental situation.

It was previously reported [14] that is was not possible to use the electrodes in protein containing solutions. The electrodes had been tested by the addition of 5% bovine albumin to the calibration solutions and it had been found that this markedly decreased the response of the electrodes. This response now turns out to be due to impurities in the bovine albumin and successful measurements have now been performed in cell homeogenates and human serum using these electrodes.

Discussion

Magnesium Macroelectrodes

Characteristics

There has been one other, but rather brief report on the manufacture of magnesium selective macroelectrodes [9], using not only ETH 7025, but also its predecessor ETH 5282. The composition of these electrodes was different from that described in this paper and in physiological solutions the performance was poorer. In nominally calcium free solution, the measured potential difference between 10 and 1 mM Mg was some 40 mV, less than the 58 mV shown in Fig. 3A, for the same concentration change in very similar calibration solutions. Moreover, the limit of detection using these electrodes was 100 μM in contrast to 50 μM or lower in our electrodes. *Brookes* and *Fry* [3] successfully measured ionized magnesium in plasma with their electrodes and we have also been able to make measurements of ionized magnesium in serum with these electrodes.

The Mg macroelectrodes manufactured from ETH 7025 were sufficiently selective to measure the [Mg^{2+}] down to 50 μM in both nominally calcium free and in the presence of 1 mM calcium. In calcium free solutions, lower concentrations of [Mg^{2+}] could no doubt be measured (see Fig. 2 and 3) but this would have entailed the manufacture of accurate magnesium buffer solutions and because of this, concentrations lower than 50 μM were not tested. However, as demonstrated in this paper they have their disadvantages. When used, their selectivity declines slowly with time, the rate of loss of selectivity being a function of both use and temperature. In general, the life span is around two weeks at 37 °C and a month at 25 °C after they have been back-filled. Moreover, despite selection of the electrodes, drift of around 4 mV/hour could be present. This was allowed for during both calibration and experiment, by noting the time of the measurement and assuming that the drift was linear.

The expected difference can be estimated theoretically by differentiation of the *Nicolsky-Eisenman* equation. With the concentration in mM, differentiation gives:

$$\frac{d\,[Mg^{2+}]}{d\ potential} = \frac{10^3}{s}\ln(10)^*10\,\frac{(Potential - U_o^I)}{s}$$

Calculation using this equation shows that at a concentration of 0.05 mM a difference of 1 mV would give a difference of 0.016 mM and at 10 mM a difference 0.8 mM would be expected.

The drift, although small was the major disadvantage of these electrodes and a reduction in the drift would improve the accuracy of measurements with the electrodes. To reduce the drift to a minimum, electrodes are now routinely manufactured from polyurethane tubing and a method recommended by *Fry* [6] for chloriding silver wire is now used. Drift is less of a problem when making measurements of the [Mg^{2+}] in solutions, for drift can be held to a minimum by intermittently, during a series of measurements recalibrating in 10 mM Mg solution, i.e. the solution which is defined as zero.

Interference, junction potentials, changes in ionic strength and temperature

In the calibration procedure and when making measurements with the Mg macroelectrodes it is implicitly assumed that interference from other ions is constant, that the changes in the potential of the reference are either minimal or can be neglected, and that changes in ionic strength and temperature do not interfere with the measurements. To overcome these problems the composition and pH of the calibrating solutions were made identical to that of the solutions in which the measurements were carried out in, and calibration and measurement were carried out at identical temperatures. This meant that interference from Na, K, Ca and pH and the effect of temperature on the Mg and reference electrodes would be the same in each case.

In the calibrating solutions both the ionic strength and the chloride concentration increased due to the addition of $MgCl_2$. On going from 50 μM to 10 mM, the ionic strength increased by 0.03 M from 0.158 M ("extracellular" solution) and 0.155 M ("intracellular" solution). At the same time the chloride concentration icnreased by 19.9 mM. The effect of the increase in ionic strength would be to slightly decrease the Mg mean activity coefficient ($\gamma\pm$) from 0.579 to 0.565 ("extracellular" solution) and from 0.580 to 0.567 ("intracellular" solution) at 37 °C (for the calculation of $\gamma\pm$, see [10]). This change was not allowed for when plotting the calibration curves but a measured potential in an experimental solution is always compared to the corresponding potential, at the same ionic strength in a calibrating solution. A similar argument also holds for any change in the potential of the reference electrode that could be introduced by the change in ionic strength and/or Cl concentration.

Commercial Mg analysers

There are three analysers designed to measure the $[Mg^{2+}]$ in blood or plasma available namely, Nova [1], Kone [7] and AVL [12]. However, these instruments are designed to measure the $[Mg^{2+}]$ in blood and are not suitable for measurement of the $[Mg^{2+}]$ in physiological solutions. This is due either to the introduction of spurious junction potentials due to the normally high chloride concentration in physiological solutions and/or to the lack of calcium in the solution [16]. For measurements of the $[Mg^{2+}]$ in physiological solutions, self manufactured Mg macroelectrodes are still the method of choice.

Conclusions

Magnesium macroelectrodes manufactured with the magnesium sensor ETH 7025 provide a straightforward method to directly measure the $[Mg^{2+}]$ in physiological solutions. At present these electrodes are more suitable for such measurements than commercially available Mg analysers and their performance is better than those reported in [3]. Since it is also possible to make calcium macroelectrodes [6] both the $[Mg^{2+}]$ and $[Ca^{2+}]$ can now be directly measured and it is no longer necessary to calculate these concentrations with all the associated inaccuracies [9].

Finally, the electrodes could be used to estimate binding of magnesium to ligands and could well be suitable for measuring magnesium fluxes from cells or vesicles.

Acknowledgements

This work was supported by the Swiss National Science Foundation (No 31-30189.90) and the Stanley Thomas Johnson Foundation, Berne. *Anita C. Truttmann* was supported by the Roche Research Foundation. We wish to thank Professor *Silvio Weidmann* for commenting on the manuscript and *Rosemay McGuigan* for mathematical advice.

References

[1] *Altura, B. T.; Shirey, T. L.; Young, C. C.; Hiti, J.; Dell'Orfano, K.; Handwerker, S. M.; Altura, B. M.:* A new method for the rapid determination of iuonized Mg^{2+} in whole blood, serum and plasma. Meth. Find. Exp. Clin. Pharmacol. **14** (1992) 297-304.

[2] *Blatter, L. A.; McGuigan, J. A. S.:* Estimation of the upper limit of the free magnesium concentration measured with Mg-sensitive microelectrodes in ferret ventricular muscle; (1) use of the Nicolsky-Eisenman equation and (2) in calibrating solutions of the appropriate concentration. Magnesium 7 (1988) 154-168.

[3] *Brookes, C. I. O.; Fry, C. H.:* Ionized magnesium and calcium in plasma from healthy volunteers and patients undergoing cardiopulmonary bypass. Brit. Heart J. **69** (1993) 404-408.

[4] *Buri, A.; McGuigan, J. A. S.:* Intracellular free magnesium concentration in ferret heart muscle: its measurement and regulation. In: *Lasserre, B.; Durlach, J.* (ed.): Magnesium a Relevant Ion. John Libbey, London 1991, pp. 201-214.

[5] *Chapman, R. A.; Coray, A.; McGuigan, J. A. S.:* Sodium/calcium exchange in mammalian ventricular muscle: a study with sodium-sensitive micro-electrodes. J. Physiol. **343** (1983) 253-276.

[6] *Fry, C. H.:* Ion-selective electrodes. Manufacture and use in biological systems. March, Biomesstechnik-Verlag, Germany 1990.

[7] *van Ingen, H. E.; Huijgen, H. J.; Kok, W. T.; Sanders, G. T. B.:* Analytical evaluation of Kone Microlyte determination of ionized magnesium. Clin. Chem. **40** (1994) 52-55.

[8] *McGuigan, J. A. S.; Blatter, L. A.; Buri, A.:* Use of ion selective microelectrodes to measure intracellular free magnesium. In: *Strata, P.; Carbone, E.* (ed.): Mg^{2+} and Excitable Membranes. Springer-Verlag, Berlin 1991, pp. 1-19.

[9] *McGuigan, J. A. S.; Lüthi, D.; Buri, A.:* Calcium buffer solutions and how to make them: A do it yourself guide. Can. J. Physiol. Pharmacol. **69** (1991) 1733-1749.

[10] *McGuigan, J. A. S.; Buri, A.; Chen, S.; Illner, H.; Lüthi, D.:* Some theoretical and practical aspects of the measurement of the intracellular free magnesium concentration in heart muscle: consideration of its regulation and modulation. In: *Birch, N. J.* (ed.): Magnesium and the Cell. Academic Press, London 1993, pp. 91-120.

[11] *Spichiger, U. E.; Eugster, R.; Schaller, U.; Haase, E.; Simon, W.:* Magnesium-selective electrodes: state of the art. Mag.-Bull. **13** (1991) 140-144.

[12] *Truttmann, A. C.; Gerber, D. M.; Lüthi, D.; McGuigan, J. A. S.:* Measurement of the ionized magnesium concentration ($[Mg^{2+}]$) in physiological solutions, human blood, plasma and serum. J. Physiol. **477** (1994) 3P.

[13] *Truttmann, A.; Zhang, W.; Lüthi, D.; McGuigan, J. A. S.:* The use of self manu-

factured Mg^{2+} macroelectrodes to measure the Mg-ATP dissociation constant. Experientia **50** (1994) A38, Abstract number S12-05.

[14] *Zhang, W.; Truttmann, A.; Lüthi, D.; Mc Guigan, J. A. S.:* The use of Mg^{2+} macroelectrodes to measure the Mg-ATP dissociation constant. J. Physiol. **473** (1993) 6P.

Correspondence to:
Prof. *John A. S. McGuigan,* Physiologisches Institut, Bühlplatz 5, Ch-3012 Berne, Switzerland.