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The preparation of a fine tip calcium ion selective electrode

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ABSTRACT

The purpose of this present study is to make a fine tip Ca^{2+}–selective electrode. Detection limit is found at pCa around 7.5 in Ca^{2+} buffer solution. The changes in electromotive force induced between two solutions for 10–fold change in Ca^{2+} concentration are close to 30 mV. This electrode characteristics were found for single–barrelled microelectrodes of 72.6 nm diameter tip and an internal filling solution buffered for pCa of 6.

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1 INTRODUCTION

Ion–selective electrodes (ISEs) are electrochemical ion sensors that convert the activity of a target ion into an electrical potential as the measurable signal (Hu *et al.*, 2016). There are several advantages such as high selectivity, quick analysis, low cost, wide variety of tested ions, and small sample size in this technology (Vijayalakshmi and Selvi,2013).

In the study of Eriksen *et al.* (2001), the copper in natural water was determined by the use of ISE. Similarly, the determination of lead in drinking water by means of ISE has also been reported by Ceresa *et al.* (2001). In another study, Miller *et al.* (2001) developed an ISE with internal solution for the direct measurement of Na^+ in plant cells. The calcium electrode offers great possibilities for the determination of the total calcium content in blood (Melchior–Rasmussen, 1972). Recently, the determination of calcium ions in sap using carbon nanotube–based ion–selective has been described (Hernandez *et al.*, 2010). In more recent years, a new simple, highly specific and calcium selective electrode has been prepared by Vijayalakshmi and Selvi (2013). This calcium selective electrode was also successfully used in the analysis of concentration of calcium ion in various real samples (Vijayalakshmi and Selvi, 2013).

However, there has been very little research reported on a fine tip ISE. The present work is aimed to manufacture a fine tip Ca^{2+}–selective electrode. This electrode can combine with the scanning ion conductance microscope (SICM–a asembly for scanning living cells without damaging them or a specific scanning protocol that prevents the tip of the micropipette from making direct physical contact with the specimen) (Korchev *et al.*, 1997). For these purposes, a micropipette with a fine tip was prepared in the first step, followed by evaluating characteristics of the Ca^{2+}–selective electrode with phosphate buffered saline as an internal filling solution.

2 MATERIALS AND METHODS

2.1 Materials

N–(Trimethylsilyl)dimethylamine, ethylene glycol–*bis*(2–aminoethylether) –*N,N,N′,N′*–tetraacetic acid (EGTA) were from Sigma. Calcium chloride dehydrate and cocktail A (0.1 mL) were obtained from Fluka. However, it was impossible to obtain information on the chemical composition of cocktail A for commercial confidentiality reasons. Phosphate buffered saline was purchased from Biowhittaker (Table 1).

Table 1: The composition of phosphate buffered saline (PBS)

	Formula		
	KH_2PO_4	**NaCl**	**Na_2HPO_4**
Content, mg/L	144	9,000	795

2.2 Methods

2.2.1 Manufacturing a fine tip microelectrode

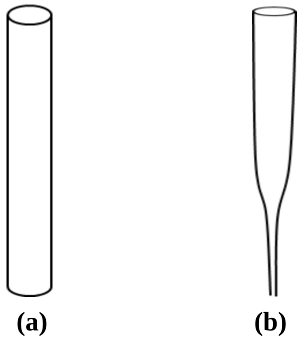

Fig. 1: (a) Scheme of a capillary and (b) a fine tip microelectrode

Fig. 1 displays a capillary was pulled from borosilicate glass with filament (O.D = 1 mm; I.D = 0.5 mm; length = 10 cm) with a P–2000 micropipette puller NANOLAB, KAIST, Korea. Then, the capillaries were placed in a petri dish and backed at least three hours at 150°C to remove traces of water.

2.2.2 Silanization

The structure of silicate glasses (Fig. 2) is dominated by the SiO_4 unit of pure silica (Greaves *et al.*, 1981).

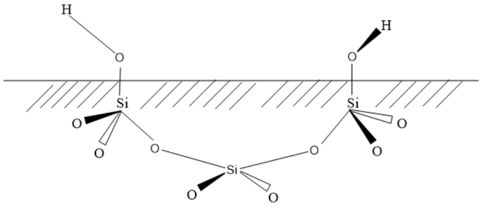

Fig. 2: The structure of the surface of glass

The surface of glass is hydrophilic and a column of liquid sensor (which is hydrophobic) was introduced into the tip of an untreated glass micropipette it would be displaced by any aqueous solution in which the tip was immersed (Munoz *et al.*, 1983).

The process of silanization consists in replacing the hydrogen of the surface hydroxyl group by a covalently bound silicon atom that carries hydrophobic groups (Holland, 1964). As a result, the hydrophilic hydroxyl groups are replaced by hydrophobic groups. Hydroxyl groups on the surface of glass can be made to react with the class of silicon compounds known as silanes (Munoz *et al.*, 1983). In this experiment, the reaction between the surface of glass, R–OH, and silanes $(CH_3)_3Si–N(CH_3)_2$, can be presented by the following equation (Miller JM., 2005):

$$R–OH+(CH_3)_3Si–N(CH_3)_2 \longrightarrow R–OSi(CH_3)_3+ (CH_3)_2NH$$

N– (trimethylsilyl) –dimethylamine was injected into the tip of capillary. The reagent was allowed to react in the vapor phase for 12 hours at 200°C (Ammann *et al.*, 1987).

2.2.3 Filling membrane (coctail A)

By means of a micromanipulator (Scanning Electro Microscope Olympus SZ51 at Biosensor Lab, KRISS, Korea), a short column of the Ca^{2+}–selective sensor was introduced into the tip by capillarity, or suction if necessary.

2.2.4 Preparing for internal solution

In the region of pCa = 2–4 (pCa = $–lg[Ca^{2+}]$), it was found that the solution prepared very carefully by normal dilution techniques was satisfactory (Ammann *et al.*, 1987). At low concentration, Ca^{2+} will be lost by adsorption on glass or reaction with impurities. An alternative is to prepare a metal ion buffer from the metal (Ca^{2+}) and a suitable ligand (EGTA) (Tsien and Rink, 1981). The preparation for the buffer solution has been listed in Table 2 with reference of Ammann *et al.* (1987).

Table 2: The composition of buffer solution

	Mass, g		pH^a	Volume of solution, mL
	$CaCl_2.2H_2O$	EGTA		
$pCa = 6^b$	0.0694	0.1902	7.4	50
pCa = 7	0.0462	0.1902	7.4	50
pCa = 8	0.0107	0.1902	7.4	50

[a] Controlling pH value at 7.4 using KOH solution; [b] pCa = –lg[Ca^{2+}]

2.2.5 Making reference electrode

The most commonly used reference electrode in biological measurements is the AgCl coated Ag wire–the Ag/AgCl electrode. These electrodes are easy to manufacture, and the electrode potential has a relatively small temperature coefficient. If the Ag/ AgCl electrode is placed in saturated KCl, it is written as Ag/AgCl(solid)/KCl(saturated, aqueous) (Fry and Langley, 2002). Reaction at the interface for an Ag/AgCl electrode is:

$$AgCl(s) + \bar{e} \longrightarrow Ag(s) + Cl^-(aq)$$

The following procedure is for the preparation of a reference electrode–the Ag/AgCl electrode: Firstly, a chlorinated silver wire was prepared by soaking silver wire in a solution of HNO_3 about one hour followed by cleaning with distilled water. It was then soaked in a HCl solution about 10 minutes and cleaned by water again. Secondly, reference electrode was prepared from micropipette filled with 3 M KCl solution. Finally, reference electrode was completed by insertion of a chlorinated silver wire which was fixed at the end of the original capillary tubing by a drop of wax.

2.2.6 Making a measurement

In order to measure electromotive force (EMF), the following electrochemical cell is constituted:

Ag | AgCl | KCl ‖ bridge electrolyte ‖ sample ‖ membrane ‖ inner filling solution | AgCl | Ag

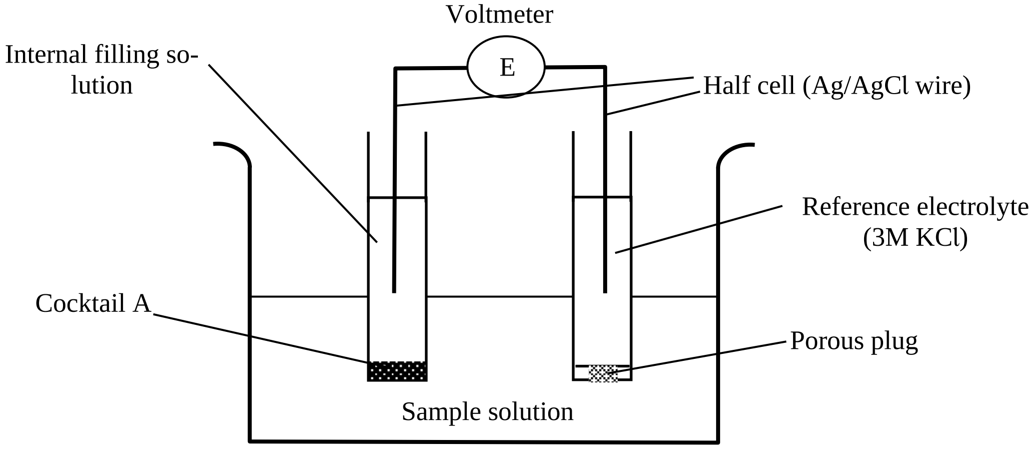

Fig. 3: Schematic diagram of EMF measurement

All potential measurements were made by means of a digital pH meter benchtop–Orion 3 Star at Biosensor Lab, KRISS, Korea.

3 RESULT AND DISCUSSION

3.1 Manufacturing a fine tip Ca^{2+}–selective electrode

A fine tip microelectrode with 72.6 nm diameter tip and very smooth tip surface (Fig. 4) was manufactured in this research. The finding infers that this microelectrode demonstrated the potential as an excellent candidate for scanning ion conductance microscope (SICM) system.

The pipette was mounted on a micromanipulator and immersed into the membrane solution (cocktail A). Cocktail A will easily penetrate into the tip of pipette owing to capillary force (liquid membranes are organic cocktails and hydrophobic). The time to obtain cocktail column length required depends on tip diameter and efficiencies of silanization.

Besides, silanization of charged surfaces produces a monomolecular hyrophobic finish allowing the organic liquid membrane to bed comfortably in the tip of the electrode, no longer being displaced by the electrolyte (Munoz *et al.*, 1983).

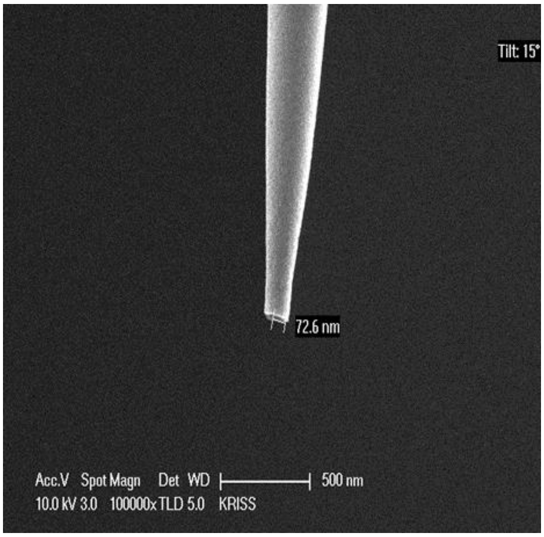

Fig. 4: Scanning electron micrographs of a micropipette tip

(conditioned in: HEAT = 420, FIL = 4, VEL = 30, DEL = 140, PULL = 250)

Membrane column length should be short. If the liquid membrane column within a microelectrode is so long that part of it remains above the surface level of a warmed experimental bath, change in bath temperature or surface level can give rise to changes in electrode output, thereby causing serious noise and errors in quantitative ion measurement.

3.2 Electromotive force measurements

EMF is defined as the difference between the potentials of two electrodes immersed into a solution. A pair of electrodes immersed into a solution makes a galvanic cell. One of the electrodes is called indicator electrode in the galvanic cell and obeys the Nernst equation. The other electrode is the reference one, and its potential is constant.

A calibration curve is a plot of the potential differences of an ion–selective electrode/reference electrode pair against the logarithm of the ionic activity or concentration of a particular species (called an ion–selective electrode assembly). The potential differences should be plotted on the ordinate (vertical axis) with the more positive potentials at the top of the axis and the logarithm of the ion activity (or concentration) plotted on the abscissa with increasing activity (or concentration) to the right (i.e. decreasing pIon values to the right) (Fry and Langley, 2002).

Calibration curve representing EMF *versus* the logarithm of the ion concentration revealed a typical ion–selective electrode response shown in Fig. 5. The linear working range was determined to be $-7 < \lg[Ca^{2+}] < -3$ (i.e. $3 < pCa = -\lg[Ca^{2+}] < 7$).

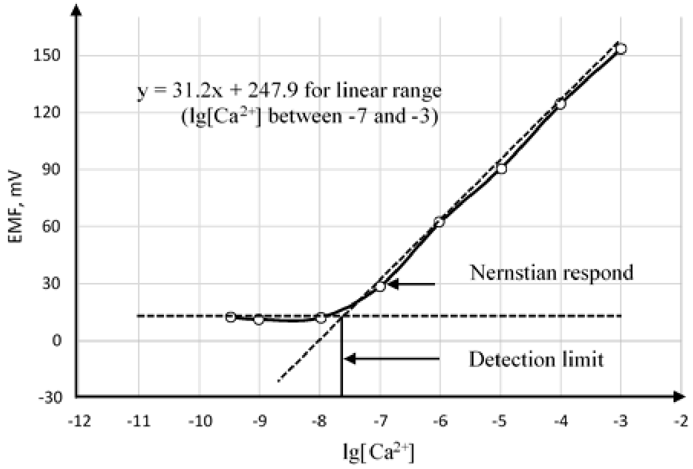

Fig. 5: A calibration curve showing detection limit and the region of Nernstian response of Ca^{2+}–selective electrode

Detection limit is estimated from the calibration curve as the concentration defined by the intersection of the extrapolated two linear regions of the curve. Fig. 5 shows that the detection limit was estimated to be $\lg[Ca^{2+}] \approx -7.5$ (i.e. $[Ca^{2+}] = 10^{-7.5}$ M).

An ion–selective electrode assembly has a Nernstian response over a given range of ion concentration, C_i (or activity, a_i) if the calibration curve is linear with a slope of $2.303103\times RT/z_iF$ mV per 10–fold change of C_i (per unit change of pC_i). At 25°C, this is $59.16/z_i$ mV per unit change of pC_i.

The slope of the curve in the linear range (i.e. the sensitivity of the Ca^{2+}–selective electrode) was determined to be 31.2 mV/decade, which is in complete accordance with the Nernst equation for positively–charged divalent ions.

The time between when the ion–selective electrode assembly is brought into contact with a new solution and when the measured potential differences is within 1 mV of the final steady–state value. The response time is an important factor for ion selective electrodes. Response time was recorded over a Ca^{2+} concentration range of 10^{-7}–10^{-3} M. The electrode reached equilibrium within a very short period of 10 seconds, so Ca^{2+}–selective electrode can be used for SICM system.

4 CONCLUSIONS

The electrode exhibited good properties with regard to the tip diameter (72.6 nm), the slope (≈30mV/decade), lower detection limit (≈7.5), and response time (<10 s). It is concluded that the Ca^{2+}–selective electrode demonstrated the potential as a promising candidate for as a useful analytical tool for many practical applications. Especially, it possible to combine with SICM for imaging a living cell, however, this application requires further studies.

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