The preparation of Ca^{2+}–selective electrodes

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Article info.	ABSTRACT
	Ion–selective electrode (ISE) is a useful tool in the direct determination of ionic species in complex samples. This study relates to the manufacture of Ca^{2+}–selective electrode with single–barrelled microelectrodes and an internal filling solution buffered for pCa (pCa= –lg[Ca^{2+}]) of 6. It is shown that an improved lower detection limit of 10^{-6} M is obtained in Ca^{2+} buffer solution and the changes in electromotive force between two solutions of 10–fold change in Ca^{2+} concentration are close to 30 mV/decade.

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1 INTRODUCTION

Ion–selective electrodes (ISEs) are electrochemical sensors that allow the potentiometric determination of the activity of an ionic species in the presence of other kinds of ions. ISEs are cheap and simple devices that can be miniaturized, allow on–line and in–situ measurements. Ideally, they consume no analyte during the measurement and usually do not need sample preparation (Ceresa, 2001). Hence, ISEs have been extensively studied over the past four decades. They have been successfully applied in many research fields, especially in routine clinical analysis as well as in environmental and industry analyses (Lewenstam *et al.*, 1991; Eriksen *et al.*, 2001).

ISEs have found widespread use for the direct determination of ionic species in whole and diluted blood, serum, urine, tissue, and intracellular samples. Eriksen *et al.* (2001) determined copper in natural water 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. In 2010, Hernandez *et al.* described determination of calcium ions in sap using carbon nanotube–based ion–electrode. Recently, a new simple, highly specific and calcium selective electrode has been prepared by Vijayalakshmi and Selvi (2017). This calcium selective electrode was also successfully used in the analysis of concentration of Calcium ion in various real samples (Vijayalakshmi and Selvi, 2017).

The present work is aimed to report on the manufacture of Ca^{2+}–selective electrode as a useful analytical tool for many applications in practical.

2 MATERIALS AND METHODS

2.1 Materials

N–(Trimethylsilyl)dimethylamine, ethylene glycol–bis(2–aminoethylether)–*N,N,N′,N′*-tetraacetic acid (EGTA), albumin from bovine serum minimum 96% electrophoresis, cellulose acetate, and hydrochloric acid 36.5–38% were from Sigma. Calcium chloride dehydrate, sodium phosphate tribasic dodecahydrate, nitric acid, silve wire,

acetone, and cocktail A (0.1 mL) were obtained from Fluka.

2.2 Methods

2.2.1 Manufacturing Ca²⁺–selective electrode

The capillaries were pulled from borosilicate glass with filament (O.D = 1mm; I.D = 0.5 mm; length = 10 cm; item # BF 100–50–10) with PP–830 single stage glass microelectrode puller at Biochemistry Lab, Chungnam National University, Korea. Then, the capillaries were placed in a petri dish and backed at least 3 hours at 150°C to remove trace of water. Next, *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). After silanization, they were broken to the desired tip size and filled with calcium buffer solution.
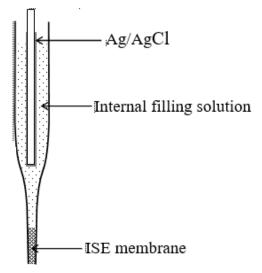

Fig. 1: Construction of Ca²⁺–selective electrode

Using Scanning Electro Microscope Olympus SZ51 at Electrochemistry Lab, Chungnam National University, Korea, a short column of the Ca²⁺–selective sensor was introduced into the tip by capillarity, or suction if necessary. Also, in order to prevent the tip from breaking (the tip of capillary is very fine), the tip of capillary was immersed into the solution consisting of 1 mL of 10% (wt./vol) bovine serum albumin in 50 mmol Na_3PO_4 and 10 µL of 25% glutaraldehyde, and solution of 10% cellulose

acetate in acetone, respectively. Finally, the Ca²⁺–selective electrode was completed by insertion of a chlorinated silver wire which was fixed at the end of the original capillary tubing by using drops of wax.

Ion–selective liquid membranes are organic cocktails and hydrophobic. Freshly, pulled electrodes are, in comparison, hydrophilic. Due to the density of surface hydroxyl groups, (4.6 free OH groups per 10 nm²) charged surfaces produce a monomolecular hydrophobic allowing the organic liquid membrane to bed comfortably in the tip of the electrode, no longer being displaced by the electrolyte. 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). Besides, tip becomes hydrophobic after silanization. The time to obtain required cocktail column length depends on the tip diameter and efficiencies of the silanization.

2.2.2 Making reference electrode

Firstly, a chlorinated silver wire was prepared by soaking silver wire in a solution of HNO_3 *ca.* one hour followed by cleaning with distilled water. It was then soaked in a HCl solution about ten 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.

2.2.3 Preparing for internal solution

In the region of pCa = 2–4, it was found that the solution prepared very carefully by normal dilution techniques was satisfactory (Ammann *et al*., 1987). At low concentration, Ca²⁺ will be lost by adsorption on glass or reaction with impurities. An alternative is to prepare a metal ion buffer from the metal (Ca²⁺) and a suitable ligand (EGTA) (Tsien and Rink, 1981). Also, a buffer solutions with a constant pH of 7.40 used in this work (Ammann *et al*., 1987). The preparation for the buffer solution has been describe in the literature (Ruzica *et al*., 1973).

Table 2: The composition of buffer solution

	Mass, g		pHᵃ	Volume of solution, mL
	CaCl₂.2H₂O	EGTA		
pCa = 6	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

2.2.4   Making a measurement

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

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

All potential measurements were made using a digital pH meter benchtop–Orion 3 Star at

Electrochemistry Lab, Chungnam National University, Korea. In order to evaluate influences of internal filling solution on Ca^{2+}–selective electrode, a set of five different ISEs were prepared. They consisted of 10^{-2} M Ca^{2+} (ISE–2), 10^{-3} M Ca^{2+} (ISE–3), 10^{-4} M Ca^{2+} (ISE–4), 10^{-5} M Ca^{2+} (ISE–5), and 10^{-6} M Ca^{2+} (ISE–6). All calibration curves were measured from the higher (10^{-1} M Ca^{2+}) to the lower concentrated (10^{-10} M Ca^{2+}).
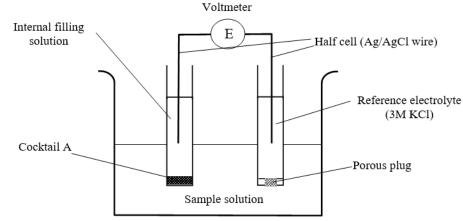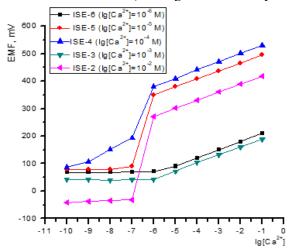

Fig. 2: Scheme of EMF measurement

3   RESULTS AND DISCUSSION

3.1   Influences of internal filling solution

The EMF is the difference between the potentials of two electrodes immersed into a solution. A pair of electrodes immersed into a solution makes the so–called galvanic cell. One of the electrodes (the so–called indicator electrode) in the galvanic cell obeys

the Nernst equation, while the potential of the other electrode (reference electrode) is constant. Figure 3 demonstrates that the EMF of ISE–2, ISE–4 and ISE–5 are strongly affected by the change in Ca^{2+} concentration in the range of 10^{-7}–10^{-6} M. In the range of 10^{-10}–10^{-7} M, it can be observed that the EMF is independent of Ca^{2+} concentration.
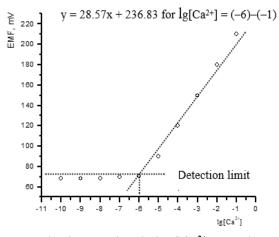

Fig. 3: Response of five ISEs with identical but different in internal filling solutions: ISE–2 (lg[Ca²⁺] = 10⁻² M), ISE–3 (lg[Ca²⁺] = 10⁻³ M), ISE–4 (lg[Ca²⁺] = 10⁻⁴ M), ISE–5 (lg[Ca²⁺] = 10⁻⁵ M), ISE–6 (lg[Ca²⁺] = 10⁻⁶ M)

Fig. 4: Detection limit of Ca²⁺– selective electrode, conditioned in: the Ca²⁺ concentration in internal filling solution of 10⁻⁶ M Ca²⁺ (ISE–6)

For any ISEs in present study, a rather good lower detection limit of around 10^{-6} M is obtained and the slope of the plot was found to be 30 mV/decade. However, the concentration of free calcium is very low to eliminate small flux of primary ion (Ca^{2+}) from internal filling solution into calibration solution (or sample solution). If small flux of primary ion enters the sample solution, EMF value is not stable this means that the voltage of the cell will drift with time. Therefore, ISE–6 is the best choice since Ca^{2+} concentration is the lowest among ISEs made.

3.2 Ca^{2+}–electrode characterization

Plotting the steady–state potentials obtained from measurements *versus* the logarithm of the ion concentration revealed a typical ion–selective electrode response (Figure 3). The linear working range was determined to be $-6 < \lg[Ca^{2+}] < -1$, which approximately corresponds to a range of Ca^{2+} concentration from 10^{-6} M to 10^{-1} M. While the detection limit was estimated to be $\lg[Ca^{2+}] = -6$ (i.e. $[Ca^{2+}] = 10^{-6}$ M), the slope of the curve in the linear range (i.e. the sensitivity of the Ca^{2+}–selective electrode) was determined to be 30 mV/decade, which is in complete accordance with the Nernst equation for positively–charged divalent ions.

4 CONCLUSIONS

These findings make it clear that the developed electrodes showed response time of 10 seconds. A dynamic linear range of 1×10^{-6}–1×10^{-1} mol L^{-1}, Nernstian slope of 28.57 mV $decade^{-1}$ and detection limit of 10^{-6} mol L^{-1} were obtained for the electrode. It is too early to claim that Ca^{2+}–selective electrode made demonstrated the potential as a promising candidate because its applications requires further study, however, a detailed procedure for making a Ca^{2+}–selective electrode was presented in this work.

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