# ガラスナノピペット内に作製したセシウムイオン選択膜

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# Cesium ion-selective membrane fabricated in a glass nanopipette

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# Abstract

ガラス製ナノピペット中にセシウムイオン選択膜を作製した. 1,3-alternate thiacalix[4]biscrown-6.6 をイオン選択 リガンドとして用い,その選択性を確認するためにセシウム,カリウム,ナトリウムの各イオンについてそれぞれ 0.01 mM から 100 mM まで濃度を変えていったときの信号強度からイオン選択性を計測した. イオン選択膜の主成分であ るポリ塩化ビニルが,ナノピペット中でゲル化する前段階において,予めナノピペット中に入れている水と混ざってし まうことを防止するために,選択膜ゲル化の前段階においてその選択膜の原料を溶かした溶媒と水との液界面にシクロ ヘキサンの液膜を張っておく手法を開発して,選択膜作製の確度を上げることができた. Keywords: Ion-selective Electrode, Nanopipette, Cesium Ion

#### 1. Introduction

Glass nanopipettes have been used as a bridge between macro world and nano world.<sup>1)</sup> Pipettes have been used as a tool to transfer liquids with various volumes,<sup>2)</sup> and many novel phenomena and applications are involved as going down the size of pipettes to micro or nano-meters.<sup>38)</sup> Nanopipettes could be also used as probes in scanning electrochemical microscopy and scanning ion conductance microscopy for high resolution imaging.<sup>9-11)</sup> Moreover, microinjection is a useful method of transfection to cells,<sup>12)</sup> though the viability of cells after the microinjection is not high enough to obtain statistical and quantitative results.<sup>13)</sup>

An ion-selective electrode with polymer membrane is a universal and known tool for the recognition of ions in a liquid, and various types of ion-selective electrodes have been developed.<sup>14,15)</sup> However, the typical size of a conventional ion-selective electrode is on the order of millimeters, and thus, such as an ionselective electrode cannot be used with a nanopipette. Therefore, we developed a method to prepare an ion-selective membrane in a nanopipette; further we developed a nanopipette-based detection system for subtle signals.<sup>16-20)</sup> The ion detection mechanism of an ion-selective electrode in a nanopipette is discussed in our recent paper.<sup>21)</sup> Further, we applied our method to the determination of local concentrations of target ions in various kinds of living cells. We applied this method to determine the local concentration of potassium or sodium ions in HeLa cells,<sup>22)</sup> rat vascular myocytes, and neuron cells from embryonic stem cells.<sup>23)</sup>

Although the detection of sodium, potassium, and calcium ions could be identified with ion-selective nanopipettes,<sup>16-23)</sup> cesium ion-selective nanopiette, which would have an application of local detection of cesium ions in a living cell, has not been developed yet. Choi *et al.* reported the cesium ionselective glass tube electrode with the ionophore of 1,3alternate thiacalix[4] biscrown-6,6 in poly(vinyl chroride) membrane.<sup>24)</sup> They reported the linear response with a near Nernstian slope of 57.6 mV per decade within the concentration range of 0.001 to 32 mM, and the limit of detection was determined as 0.38  $\mu$ M.<sup>24)</sup>

In this paper, we have succeeded in producing cesium ionselective nanopiette. We have estimated

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the selectivity coefficient and confirmed the negative values of the selectivity coefficients at the concentration of the sample solution of CsCl, KCl, and NaCl from 0.01 mM to 100 mM.

# 2. Experimental methods

The resource of ion-selective membrane was (i) 32 mg of poly(vinyl chloride) (Sigma-Aldrich, high molecular weight, CAS 9002-86-2) for the main resource of the membrane, (ii) 62 mg of 2-Nitrophenyloctylether (NPOE, Dojindo, purity >99.0%, CAS 37682-29-4) for the plasticizer, (iii) 5 mg of 1,3alternate thiacalix[4] biscrown-6,6 for the ion-selective ionophore, and (iv) 1 mg of tetrakis[3,5-bis(trifluoromethyl) phenyl]borate, sodium salt (TFPB, Dojindo, purity >99.0%, CAS 79060-88-1) for the anion repeller. They were dissolved in 1 mL tetrahydrofuran (THF, Sigma-Aldrich, purity >99.9%, with 250ppm dibutylhydroxytoluene (BHT) for the stabilizer, CAS 109-99-9). Here we call the solution "solution A".

Next, we prepared the sample solutions of CsCl (Sigma-Aldrich, purity >99.5%, CAS 7647-17-3), KCl (Yanagishima, special grade, purity >99.5%, CAS 7447-40-7), and NaCl (Manac, special grade, purity >99.5%, CAS 7447-14-5) at the concentrations of 0.01, 0.1, 1, 10, and 100 mM in distilled water produced with Organo BB-5B/G-35PA (resistivity 5 M $\Omega$ •cm). In this way, we have prepared 15 kinds of sample solutions.



Fig.1 (Color online) The process to prepare ion-selective electrode. The details of the process are described in the text.

Before the preparation of ion-selective nanopipette, we prepared conventional ion-selective electrodes with boron silicate glass tubes (7 mm outer diameter, 0.8 mm thickness, 70 mm length). The cut face of the glass tube was polished with a waterproof abrasive paper. Figure 1 shows the process to fabricate the conventional ion-selective glass tube electrode. The solution A was poured in a glass petri dish and dried for 1 day to prepare PVC membrane film with ionophore. The film was cut and attached to the bottom of the glass tube with an epoxy. After curing the epoxy, 0.01 M CsCl aq was filled in the glass tube. Finally, a 0.20 mm diameter silver wire (Niraco, purity >99.99%) coated with a Ag/AgCl ink (BAS) was inserted into the glass tube to prepare the ion-selective electrode.



Fig.2 (Color online) The process to prepare ion-selective nanopipette. The details of the process are described in the text.



Fig.3 (Color online) Photos of nanopiette tube during the curing process of PVC membrane inside the nanopipette. (A-1): After pouring the PVC solution ("solution A" in the text) on the distilled water covered with cyclohexane. (A-2): Five minutes later after taking the photo (A-1). (B-1): After pouring the PVC solution on the distilled water, without using cyclohexane. (B-2): Five minutes later after taking the photo (B-1).

The Nanopipette was fabricated from boron silicate capillary tubes (Narishige GD-1 outer diameter: 1.0 mm, inner diameter: 0.6 mm) with a puller (Sutter P-2000) at Shizuoka University. The details of the preparation method were written in the previous reports.<sup>25-28)</sup> Figure 2 shows the process to fabricate the ion-selective nanopipette. First, pure water was filled in the nanopipette to the shank end using a micro-

syringe. Then, we poured ca. 0.01 µL cyclohexane (Sigma-Aldrich, purity >99.0%, CAS 110-82-7) on the water. After making the cyclohexane solvent layer on the water, 10  $\mu L$  of "solution A" was poured in the nanopipette, and dried for 1 day to cure the PVC membrane in the nanopipette. Using cyclohexane before pouring the solution A is the improved point in this study. Figure 3 shows how the cyclohexane solvent works between PVC solution and water. The upper photos (A-1) and (A-2) in Fig. 3 indicates that the mixing of water and solution A did not occur even after 5 minutes of pouring (A-2) so that the PVC membrane can be formed firmly in the nanopipette. On the other hand, the lower photos (B-1) and (B-2) shows the mixing of water and solution A because THF can dissolve in water. Moreover, the mixed PVC solution in water got white to start the precipitation after 5



Fig.4 (Color online) Schematic diagram of the experimental set up for conventional glass tube ion-selective electrode (a) and ion-selective nanopipette (b).

minutes (B-2). In this way, we could prevent the mixing of PVC solution with water by using cyclohexane between the PVC solution and water. It is noted that the cyclohexane solvent was finally transpired with THF on the drying process. After the drying process, 0.01 M CsCl aq was filled in the nanopipette, and a 0.20 mm diameter silver wire coated with a Ag/AgCl ink was inserted into the glass tube to prepare the ionselective nanopipette.

Figure 4 shows the schematics of the diagram of the experimental set up for conventional glass tube ionselective electrode (a) and ion-selective nanopipette (b). The signal was amplified with a patch/whole cell clamp amplifier (Nihon Kohden CEZ-2400) at constant current mode, and the output voltage was recoded with a digital multimeter (Fluke 287) and the data were transferred to a personal computer (PC). The red lines indicate the shielded part in the apparatus. We prepared the shield from conventional medium density fiberboard, aluminum mesh, and 0.1 mm thickness aluminum foil, as shown in Fig. 5. Without the shield, 100 Hz noise with 250 mV peak-to-peak from the flickering of fluorescent light hindered the measurement. The prepared shield could reduce the noise less than 5 mV.



Fig.5 (Color online) Photo of the shield used for the prevention of the noise on the experiments shown in Fig. 4.

Here shows the sequence of the measurement. First, pure water was poured in the glass petri dish and measure the relative voltage between the ion-selective electorode/ nanopipette and reference/counter electorode, Ag/AgCl wire immersed in the dish as shown in Fig. 4. The relative voltage was adjusted to zero after the measuring voltage was settled. Then the dish was changed to 0.01 mM solution and measured the relative voltage. After the measurement of 0.01

mM solution, the dish was changed to 0.1 mM solution. In this way, the solution was changed and measured from 0.01 to 100 mM, and the same sequence was conducted on CsCl, KCl, NaCl solutions.

### 3. Results and discussion

First, the results on the conventional cesium ionselective glass tube electrode are shown. Figure 6 shows the measured relative voltages on Cs, K, and Na ions at the concentrations from 0.01 mM to 100 mM. Table 1 shows the measured relative voltage values on Cs, K, and Na ions at the concentrations of 0.01, 0.1, 1, 10 and 100 mM, respectively. The obtained relative voltage values for cesium ion are larger than those for potassium and sodium ions, which proves the selectivity of the prepared electrode.



Fig.6 (Color online) Measured relative voltages on Cs, K, and Na ions with conventional ion-selective glass tube electrode at the concentrations of 0.01, 0.1, 1, 10, and 100 mM.

Table 1 Measured relative voltage values on Cs, K, and Na ions with conventional ion-selective glass tube electrode and the estimated selectivity coefficients at the concentrations from 0.01 to 100 mM.

C[mM]	0.01	0.1	1	10	100
$V_{ m Cs}$ [V]	-0.763	-0.775	-0.0455	-1.2090	2.280
$V_{ m K}$ [V]	-1.101	-1.162	-0.7403	-0.0293	0.772
$V_{ m Na}$ [V]	-1.063	-1.210	-0.8460	-0.2442	0.518
$V_{\mathrm{Na}}[\mathrm{V}]$	-1.063	-1.210	-0.8460	-0.2442	0.518
$\log K^{Pot}_{Cs,K}$	-5.82	-6.66	-13.5	-21.3	-25.9
$\log K^{Pot}_{Cs,Na}$	-5.16	-7.48	-15.3	-25.0	-30.3

Selectivity coefficient  $\log K_{M,N}^{Pot}$  was estimated from the separate solution method using the following equation,<sup>29)</sup>

where  $E_M$  and  $E_N$  are the response voltages [V] for the M and N ions at the same concentration,  $Z_M$ and  $Z_N$  are the valences of M and N, F is Faraday constant [C mol<sup>-1</sup>], R is gas constant [J K<sup>-1</sup>mol<sup>-1</sup>], T is the temperature [K], and  $C_M$  is the concentration of the solution [M]. Since all the ions detected by the ion-selective electrode in this study are cations, the negative value of the selectivity coefficient  $\log K_{M,N}^{Pot}$ indicates the selectivity. Table 1 shows the estimated selectivity coefficient values. All the estimated values are negative, so that the ion-selectivity of the prepared glass tube electrode has been confirmed at the concentration range from 0.01 to 100 mM. Choi et al. reported that the cesium ion selectivity over potassium  $\log K_{CS,K}^{Pot} = -3.7.^{24}$  Since they measured with fixed interference method, different from the method in this study, direct comparison of the values is not available. In any case, it was confirmed that the selectivity of the ion-selective glass tube electrode prepared in this study is as well as the one in the previous report.<sup>24</sup>



Fig.7 (Color online) Measured relative voltages on Cs, K, and Na ions with ion-selective nanopipette at the concentrations of 0.01, 0.1, 1, 10, and 100 mM.

Table 2 Measured relative voltage values on Cs, K, and Na ions with the ion-selective nanopipette and the estimated selectivity coefficients at the concentrations from 0.01 to 100 mM.

C[mM]	0.01	0.1	1	10	100
	-0.0351	0.0621	0.1250	0.1321	0.158
	-0.0185	0.0152	0.0019	0.0584	0.107
	-0.0190	0.0559	0.0746	0.0891	0.118
	-1.063	-1.210	-0.8460	-0.2442	0.518
	-0.921	-0.807	-2.122	-1.270	-0.867
	-0.277	-0.108	-0.872	-0.741	-0.687

Next, the results on the cesium ion-selective nanopipette are shown. Figure 7 shows the measured relative voltages on Cs, K, and Na ions at the concentrations from 0.01 mM to 100 mM. Table 2 shows the measured relative voltage values on Cs, K, and Na ions at the concentrations of 0.01, 0.1, 1, 10 and 100 mM, respectively. The obtained relative voltage values for cesium ion are larger than those for potassium and sodium ions, which proves the selectivity of the prepared ion-selective nanopipette. Also, the selective coefficients are estimated, as shown in Table 2. The negative values indicates that the ionselectivity of the prepared ion-selective nanopipette has been confirmed at the concentration range from 0.01 to 100 mM.

In principle, the ion-selective electrode has the selectivity that the increase of the concentration of targeted ion increases the signal significantly while the increase of the concentration of non-targeted ions increases the signal little.<sup>29)</sup> Table 3 shows the estimated slopes and the Pearson relative coefficient  $R^2$ . In case of conventional glass tube electrode, the slope value for Cs is 1.65 times for K and 1.95 times for Na. On the other hand, the slope value for Cs is 1.07 times for K and 1.36 times for Na, which means that the selectivity with the ion-selective nanopipette is not as well as that with conventional glass tube electrode.

Table 3 Slopes and the Pearson relative coefficient (R<sup>2</sup>) estimated from the data shown in Figs. 6 and 7.

Electrode	Ion species	Slope	$\mathbb{R}^2$
Conventional glass tube	$egin{array}{c} { m Cs}^{+} \ { m K}^{+} \ { m Na}^{+} \end{array}$	0.807 0.488 0.413	0.924 0.886 0.844
Nanopipette	$egin{array}{c} \mathrm{Cs}^{+} \ \mathrm{K}^{+} \ \mathrm{Na}^{+} \end{array}$	0.0315 0.0295 0.0231	0.936 0.860 0.975

The reason of the insufficient selectivity in case of nanopipette might attribute to the existence of the ionic leaking part in the PVC membrane, which is the assignment of the next study. The leaking problem due to the mixing of the PVC membrane with water on the curing process has been mostly fixed in this study, as shown in Fig. 3. However, we recognized the precipitation of PVC in water even using cyclohexane during the curing process, confirmed with the optical microscope, owing to the difference in solubility of PVC between THF and cyclohexane. Also, we sometimes observed the slight detachment of the PVC membrane from the nanopipette inner wall, which causes the ionic leakage. The control of the curing process, including the humidity and temperature is also the assignment of the further study.

#### 4. Conclusion and Remarks

We have prepared the cesium ion-selective nanopipette with the ionophore 1,3-alternate thiacalix[4]biscrown-6,6. Compared the relative voltage of the CsCl, KCl, and NaCl solutions at the various concentrations from 0.01 to 100 mM, the ion selectivity was confirmed. For the application of the cesium ionselective nanopipette to the practical use, the curing process of the PVC membrane in the nanopipette should be reconsidered in order to obtain higher selectivity coefficient.

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#### 参考文献

- 1) T. Takami, B. H. Park, and T. Kawai, Nano Convergence 1, 17 (2014).
- 2) C. A. Morris, A. K. Friedman, and L. A. Baker, Analyst 135, 2190 (2010).
- 3) Y. Zhao, S. Inayat, D. A. Dikin, J. H. Singer, R. S. Ruoff, and J. B. Troy, Proc. IMechE, Part N: J. Nanoengineering and Nanosystems 222, 1 (2008).
- 4) M. Malboubi, Y. Gu, and K. Jiang, Microelectron. Eng. 87, 778 (2010).
- 5) S. An, C. Stambaugh, G. Kim, M. Lee, Y. Kim, K. Lee, and W. Jhe, Nanoscale 4, 6493 (2012).
- 6) A. P. Suryavanshi and M. F. Yu, Appl. Phys. Lett. 88, 083103 (2006).
- 7) A. P. Suryavanshi and M. F. Yu, Nanotechnology 18, 105305 (2007).
- 8) F. O. Laforge, J. Carpino, S. A. Rotenberg, and M. V. Mirkin, Proc. Nat. Acad. Sci. U.S.A., 104, 11895 (2007).
- 9) C. B. Prater, P. K. Hansma, M. Tortonese, and C. F. Quate, Rev. Sci. Instrum. 62, 2634 (1991).
- 10) Y. Takahashi, A. I. Shevchuk, P. Novak, Y. Murakami, H. Shiku, Y. E. Korchev, and T. Matsue, J. Am. Chem. Soc. 132, 10118 (2010).
- 11) P. Elsamadisi, Y. X. Wang, J. Velmurugan, and M. V. Mirkin, Anal. Chem. 83, 671 (2011).
- 12) R. E. Hammer, V. G. Pursel, C. E. Rexroad Jr., R. J. Wall, D. J. Bolt, K. M. Ebert, R. D. Palmiter, and R. L. Brinster, Nature 315, 680 (1985).
- 13) H. Matsuoka, M. Saito, and H. Funabashi, in "Embryonic Stem Cells - Basic Biology to Bioengineering" (M. S. Kallos, Ed.), InTech; 2011, p. 149.
- 14) E. Bakker, P. Bühlmann, and E. Pretsch, Chem. Rev. 97, 3083 (1997).
- 15) E. Bakker, P. Bühlmann, and E. Pretsch, Talanta 63, 3 (2004).
- 16) J. W. Son, T. Takami, J. K. Lee, B. H. Park, and T. Kawai, Appl. Phys. Lett. 99, 033701 (2011).

- 17) T. Takami, J. W. Son, J. K. Lee, B. H. Park, and T. Kawai, Jpn. J. Appl. Phys. 50, 08LB13 (2011).
- 18) T. Takami, J. W. Son, J. K. Lee, B. H. Park, and T. Kawai, in "Physics, Chemistry and Applications of Nanostructures", ed. by V. E. Borisenko, S. V. Gaponenko, V. S. Gurin, and C. H. Kam (World Scientific, Singapore, 2011), pp. 535-538.
- 19) X. L. Deng, T. Takami, J. W. Son, T. Kawai, and B. H. Park, Appl. Phys. Express 5, 027001 (2012).
- 20) E. J. Kang, T. Takami, X. L. Deng, J. W. Son, T. Kawai, and B. H. Park, J. Chem. Phys. B 118, 5130 (2014).
- 21) X. L. Deng, T. Takami, J. W. Son, E. J. Kang, T. Kawai, and B. H. Park, J. Nanosci. Nanotech. 13, 5413 (2013).
- 22) T. Takami, F. Iwata , K. Yamazaki , J. W. Son , J. K. Lee , B. H. Park , and T. Kawai, J. Appl. Phys. **111**, 044702 (2012).
- 23) T. Takami, J. W. Son, E. J. Kang, X. L. Deng, T. Kawai, S. W. Lee, and B. H. Park, in *"Physics, Chemistry and Applications of Nanostructures"*, ed. by V. E. Borisenko, S. V. Gaponenko, V. S. Gurin, and C. H. Kam (World Scientific, Singapore, 2013), pp. 305-308.
- 24) Y. S. Choi, H. S. Kim, J. K. Lee, S. H. Lee, H. B. Lim, and J. S. Kim, Talanta 64, 975 (2004).
- 25) F. Iwata, S. Nagami, Y. Sumiya and A. Sasaki, Nanotechnology 18, 10531 (2007).
- 26) S. Ito and F. Iwata, Jpn. J. Appl. Phys. 50, 08LB15 (2011).
- 27) T. Takami, F. Iwata , K. Yamazaki , J. W. Son , J. -K. Lee , B. H. Park , and T. Kawai, J. Appl. Phys., 111, 044702 (2012).
- 28) F. Iwata, K. Yamazaki, K. Ishizaki, and T. Ushiki, Jpn. J. Appl. Phys. 53, 036701 (2014).
- 29) Japanese Industrial Standard "General rules for ion selective electrode method", JIS K 0122:1997 (1997).