A Glass Capillary Ultramicroelectrode with an Electrokinetic Sampling Ability

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A glass capillary ultramicroelectrode (tip diameter $\approx 1.2 \mu\text{m}$) having an electrokinetic sampling ability is described. It is composed of a pulled glass capillary filled with an inner solution and three internal electrodes (Pt working and counter electrodes and an Ag/AgCl reference electrode). The voltammetric response of the capillary electrode is based on electrokinetic transport of analyte ions from the sample solution into the inner solution across the conical tip. It was found that the electrophoretic migration of analytes at the conical tip is faster than electroosmotic flow, enabling electrokinetic transport of analyte ions into the inner solution of the electrode. By using $[\text{Fe}(\text{CN})_6]^{4-}$ and (ferrocenylmethyl)trimethylammonium (FcTMA^+) ions as model analytes, differential pulse voltammetric responses of the capillary electrode were investigated in terms of tip diameter of the capillary, sampling voltage, sampling time, detection limit and selectivity. The magnitude of the response depends on the size and charge of analyte ions. With a capillary electrode having a $\approx 1.2\text{-}\mu\text{m}$ tip diameter, which minimizes non-selective diffusional entry of analytes, the response after 1 h sampling at $+1.7 \text{ V}$ is linearly related to $[\text{Fe}(\text{CN})_6]^{4-}$ concentration in the range of $0.50\text{--}5.0 \text{ mM}$ with the detection limit of $30 \mu\text{M}$. Application of a potential of the same sign as that of the analyte ion forces the analyte to move out from the electrode to the solution, enabling reuse of the same capillary electrode. The charge-selective detection of analytes with the capillary electrode is demonstrated for $[\text{Fe}(\text{CN})_6]^{4-}$ in the presence of FcTMA^+ .

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Detection of ions and molecules with ultramicroelectrodes is one of the most useful approaches for knowing local and dynamic concentration changes in biological fluids in tissues, slices and cells.^{1,2} Sophisticated approaches, such as the patch-clamp method,³ *in vivo* voltammetry,^{1,4,5} microdialysis in combination with various analytical methods⁶ and ion-selective microelectrode methods,⁷ have been established and widely used for detecting neurotransmitters, second messengers and other bioactive substances that play a key role in signal cascades of biological cells.

The ultramicroelectrode made of a glass capillary is a unique type, especially used in the so-called patch-clamp method.⁸ The glass capillary electrodes have tip diameters ranging from submicro- to several micrometers, which are much smaller than those of carbon fiber electrodes ($\sim 10 \mu\text{m}$) for *in vivo* voltammetry⁴ and microdialysis tubes (several $100 \mu\text{m}$)⁵ for *in vivo* sampling of analytes, and the glass surface has an affinity to lipids and biocompatibility. They serve as ultramicroelectrodes for detecting ions passing through ion channels in biological membranes. Glass capillary micropipets have also been employed for microiontophoresis to inject exogenous substances into specific regions in biological systems.⁹

Taking an advantage of the very small tip size, pulled glass capillaries have been employed as microholders for constructing electrochemical microsensors, such as carbon fiber microelectrodes,^{10,11} bilayer lipid membrane microsensors^{12–14} and oxygen microsensors,¹⁵ and also in achieving enhanced and variable mass-transfer rates at a hydrodynamic

ultramicroelectrode.¹⁶ The use of glass capillaries with a conical or cylindrical tip ($1.5\text{--}2 \mu\text{m}$) as a diffusional microburet for microtitration^{17–19} and quantitative reagent delivery for fine chemical manipulations^{20,21} has also been reported. Recently, Wei *et al.* have reported that pulled quartz tubes with orifice radii of $\sim 20 \text{ nm}$ form a diffuse electrical double layer within the tip, leading to charge-selective ion movement across the tip.²²

On the other hand, long, open-tubular capillaries with larger diameters, $50\text{--}100 \mu\text{m}$ inner diameter, have been established as a separating tool in capillary electrophoresis.²³ The electrokinetic movement of analytes from sample solution into one end of a glass capillary has been used for sample injection in capillary electrophoresis.²³ The sample injection is based on the cumulative effects of strong electroosmotic flow of solution and electromigration of ions, which lead to the net movement of both cations and anions in the same direction as the electroosmotic flow. Ultra-low-volume sample introduction into an electrophoresis capillary *via* a glass capillary having an outer tip diameter of $15\text{--}70 \mu\text{m}$ has also been reported for sampling of analytes from biological microenvironment.^{24,25} On the other hand, several studies have been reported on charge-selective sampling of analytes by using a field-amplified technique^{26,27} and electrophoretic preconcentration of analytes in a semipermeable hollow fiber under an injection field.²⁸ In the field-amplified method, the electroosmotic velocity of the bulk solution is much slower than the electrophoretic velocity of sample ions, because an amplified electric field accelerates migration of sample ions.

In the present study, a new glass capillary ultramicroelectrode with a $\approx 1.2 \mu\text{m}$ tip diameter having an electrokinetic sampling ability is described. The sampling principle is based on our finding that electromigration velocity of anions is faster than that

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of electroosmotic flow across the conical tip of a glass capillary, while the electromigration of cations occurs in the same direction as the electroosmotic flow. Analyte ions in solutions are forced to migrate electrophoretically into the inner solution ($\sim 10 \mu\text{l}$) of the capillary under an electric potential gradient and are detected with differential pulse (DP) voltammetry at an underlying Pt electrode. The fundamental response properties of the capillary electrode are investigated with $[\text{Fe}(\text{CN})_6]^{4-}$ and (ferrocenylmethyl)trimethylammonium (FcTMA^+) ions as model analytes in terms of working principle, sensitivity and selectivity.

Experimental

Materials

Potassium ferrocyanide $\text{K}_4[\text{Fe}(\text{CN})_6]$ was obtained from Wako Pure Chemicals Co. (Osaka, Japan). (Ferrocenylmethyl)trimethylammonium (FcTMA^+) bromide was obtained from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Coomassie Brilliant Blue R250 (CBB) was purchased from Fluka (Buchs, Switzerland). 2-[4-(2-Hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid (HEPES) was obtained from Dojindo Laboratories (Kumamoto, Japan). Other chemicals used were all of analytical reagent grade. Milli-Q water (Millipore reagent water system, Bedford, MA) was used throughout the experiments.

Preparation of capillary electrodes

Borosilicate glass capillaries (outer diameter 1.5 mm and inner diameter 0.86 mm, Clark Electromedical Instruments, Pangbourne, England) used for pipet preparation were washed in a mixture of chloroform-ethanol (2:1, v/v) overnight and dried at 65°C for 30 min and stored over silica gel in a desiccator. Capillary pipets having inner tip diameters of $0.9 - 2.3 \mu\text{m}$ were made using a three-pull technique with a Sutter micropipette puller Model P-97 (Sutter Instrument Co., Novato, CA). The tip diameter of the pipet was measured under a microscope (TMS, Nikon, Tokyo, Japan). The glass pipet was filled with a $\sim 10 \mu\text{l}$ aliquot of 0.30 M KCl solution containing 0.10 M HEPES/NaOH (pH 7.4) (abbreviated as KCl-HEPES solution) by backfilling with a disposable microsyringe. The pipets had electric resistance of $7.1 - 12 \text{ M}\Omega$ for a tip diameter of $0.9 \mu\text{m}$, $4.9 - 5.3 \text{ M}\Omega$ for $1.2 \mu\text{m}$, $2.4 - 2.5 \text{ M}\Omega$ for $1.5 \mu\text{m}$ and $0.96 - 1.2 \text{ M}\Omega$ for $2.3 \mu\text{m}$. Working, counter and reference electrodes were set in position in the capillary pipet (Fig. 1). A Teflon-coated Pt wire (ϕ 0.127 mm, Nilaco Co., Tokyo, Japan) was used as an inner working electrode. The Teflon coating on the top ($\sim 2 \text{ mm}$) of the Pt wire was peeled off with a cutter. The electrode area was determined to be 0.53 mm^2 by chronoamperometry with $4.0 \text{ mM } [\text{Fe}(\text{CN})_6]^{4-}$ in 0.10 M KCl.²⁹ The Pt wire electrode was washed with ethanol, acetone and finally Milli-Q water. An Ag/AgCl wire (ϕ 0.20 mm) electrode and a Pt wire (ϕ 0.10 mm) electrode served as the reference and the counter electrode, respectively, in the capillary.

Voltammetric measurements

DP voltammetric measurements were performed with a Cypress Model CS-1090 electroanalytical system (Cypress Systems, KA) with a capillary electrode in a three-electrode configuration. All measurements were carried out at room temperature. The experimental setup used in the present study is schematically shown in Fig. 1. The capillary electrode was vertically immersed in a KCl-HEPES solution (*vide supra*) containing various concentrations ($0.10 \mu\text{M} - 5.0 \text{ mM}$) of $[\text{Fe}(\text{CN})_6]^{4-}$ or FcTMA^+ . An additional Pt electrode (outer Pt

Fig. 1 (A) Schematic representation of the experimental setup and capillary electrode used in this work. (B) Geometry of a capillary. (C) A photograph of the tip of a capillary electrode.

electrode, ϕ 0.50 mm) was immersed in the solution for electrokinetic sampling of ions. The distance between the tip of the capillary and outer Pt electrodes was *ca.* 1 cm. A dc voltage (hereafter called sampling voltage) from an outer source (Good Will Instrument, laboratory dc power supply Model GPS-3030) was applied between the capillary electrode (Pt counter electrode) and the outer Pt electrode for a given time, usually 10 – 40 min. The electrokinetic sampling circuit was switched off and DP voltammetric measurements were performed. The capillary and outer Pt electrodes were then transferred into a KCl-HEPES solution without analytes and kept at -1.5 V or $+1.5 \text{ V}$ to drive out the sampled ions until DP peaks in the KCl-HEPES solution disappeared.

Determination of concentration of analyte ions in capillary electrodes

Ten microliter aliquots of inner solutions containing various concentrations ($0.10 \mu\text{M} - 5.0 \text{ mM}$) of $[\text{Fe}(\text{CN})_6]^{4-}$ were taken in capillary pipets. The electrodes were set as above in each capillary pipet and DP voltammograms were recorded. The DP peak currents were plotted against the concentration of $[\text{Fe}(\text{CN})_6]^{4-}$ ions. A calibration curve for FcTMA^+ was prepared in the same manner as for $[\text{Fe}(\text{CN})_6]^{4-}$. The concentration of $[\text{Fe}(\text{CN})_6]^{4-}$ or FcTMA^+ ions transported into the inner solution of a capillary electrode was determined from each calibration curve.

Fig. 2 (A) Photographs of a capillary filled with a NaCl-HEPES solution without CBB. The tip of the capillary was dipped in a NaCl-HEPES solution containing 0.48 mM CBB. The photographs were taken (a) 1 h after applying a potential of +1.7 V and (b) after transferring the capillary into a NaCl-HEPES solution containing no CBB and waiting for 105 min under an applied potential of -1.5 V. (B) Photographs of a capillary filled with a NaCl-HEPES solution containing 0.48 mM CBB. The tip of the capillary was dipped in a NaCl-HEPES solution without CBB. The photographs were taken (c) before applying potential and after applying a potential of -2.0 V for (d) 40 min, (e) 60 min and (f) 70 min. The diameter of the capillary was $1.2\ \mu\text{m}$.

It should be noted that the electrolysis proceeded in a very narrow space confined to the tip of the capillary, and hence bulk electrolysis resulted: the magnitudes of the DP peak current in the capillary were not identical to those obtained in a bulk solution. Consequently, preparation of a calibration curve was necessary for determining concentration of an analyte transported into the electrode inner solution.

Photographic observation of dye movement in a capillary

A NaCl-HEPES solution ($0.12\ \text{M}$ NaCl and $5.2\ \text{mM}$ HEPES/NaOH, pH 7.2) containing $0.48\ \text{mM}$ CBB as a color probe was taken into an open capillary pipet by backfilling with a disposable microsyringe. A Pt wire as an internal electrode was inserted into the CBB solution, avoiding the situation where the dye solution creeps up between the Pt wire and the wall of the capillary. The pipet was immersed in a NaCl-HEPES solution in a chamber placed on the stage of a microscope (BX-60M, Olympus, Tokyo). An outer Pt electrode was set in the chamber solution and a dc voltage was applied between Pt electrodes in the pipet and chamber solutions. Electrophoretic migration of CBB anions in the capillary under the influence of an electrical potential was imaged and their images were collected as photographs taken with $10\times$ objective and $3.3\times$ photographing lenses.

Results and Discussion

Observation of dye movement in a capillary electrode

Application of a dc voltage (sampling voltage) to the Pt counter electrode in the capillary against the outer Pt electrode in solution creates an electrical potential gradient that induces electromigration of ions and an electroosmotic flow of the inner solution in the electrode. We have observed the movement of CBB anions as a dye tracer under the influence of an electrical potential. A glass capillary was dipped in a NaCl-HEPES solution containing CBB and a positive sampling voltage of $+1.7\ \text{V}$ was applied. Figure 2 shows photographs of the capillary tip taken under the dc voltage. No color changes at the tip of the capillary were observed after waiting for 1 h (graph A-a), because concentration of CBB transported in the inner solution of the capillary was low. But when the capillary was transferred into a NaCl-HEPES solution (containing no CBB) and a negative potential was applied, enrichment of the dye at the tip of the capillary was seen (graph A-b). The enrichment of the dye is due to the electromigration of the dye from the bulk of the inner solution to the tip region against the electroosmotic flow (*vide infra*). The results provide evidence that CBB anions in the sample solution were actually sampled electrokinetically into the inner solution of the electrode by applying a positive sampling voltage. With a capillary filled with a CBB dye ($0.48\ \text{mM}$) solution, the electromigration behavior of the dye at a negative potential was observed. The tip region gradually turned to dark blue, showing the enrichment of CBB. After waiting for 40 min, formation of a band of CBB is seen (graph B-d). This suggests that electromigration and electroosmotic flow compete with each other for transporting CBB anions. Further waiting led to distribution of the band to the whole tip region (graph B-e) and a gradual decrease in the band width (graph B-f), showing that CBB anions moved out from the capillary tip. These observations suggest that analyte ions sampled in the inner solution of the electrode can be electrokinetically driven out from the electrode by reversing the sign of the applied potential.

The results obtained above demonstrate that at a positive potential the electromigration velocity of CBB anions, which migrate against the electroosmotic flow, is faster than that of the electroosmotic flow, leading to electrokinetic sampling of the anion into the tip interior. This phenomenon is explained by the shape of the capillary tip, *i.e.* the conical tip. The conical tip with a position-dependent cross sectional area (Fig. 1B) generates a sharp increase in the electric resistance at the tip of the capillary, leading to the enhanced electric field at the tip region. The enhanced electric field strength increases both the electromigration velocity of ions and the electroosmotic velocity of the solution at the tip region. However, the electroosmotic velocity is a bulk property and has to be averaged over the whole capillary according to the continuity principle in a non-compressible fluid:

$$Q(\text{m}^3/\text{s}) = A v_{\text{eo}} = \text{const} \quad (1)$$

where Q is the volume flow rate, A is a cross sectional area (m^2) of the capillary tube and v_{eo} is the electroosmotic velocity (m/s) of the solution. As a result, the solution velocity does not increase even if the electric field is enhanced locally at the tip. Therefore, the electromigration of analyte anions across the conical tip becomes faster than the electroosmotic flow.

Fig. 3 Differential pulse voltammograms at a capillary electrode with an inner tip diameter of 1.5 μm in 0.30 M KCl-0.10 M HEPES/NaOH solutions (pH 7.4) containing (A) 5.0 mM $[\text{Fe}(\text{CN})_6]^{4-}$ and (B) 5.0 mM FcTMA^+ . The voltammograms for $[\text{Fe}(\text{CN})_6]^{4-}$ were recorded in series after sampling at (a) an open circuit, (b) -1.5 V and (c) $+1.5$ V. Each voltammogram was recorded at the given time. The voltammograms for FcTMA^+ were recorded in the same manner after sampling at (a) an open circuit, (b) $+1.5$ V and (c) -1.5 V. Voltammetric conditions: pulse height, 50 mV, step height, 1 mV, cyclic period, 130 ms, pulse width, 40 ms, sample time, 35 ms.

Fig. 4 Differential pulse voltammograms at capillary electrodes with inner tip diameters of (a) 0.9 μm , (b) 1.2 μm , (c) 1.5 μm and (d) 2.3 μm in a 0.30 M KCl-0.10 M HEPES/NaOH solution (pH 7.4) containing 5.0 mM $[\text{Fe}(\text{CN})_6]^{4-}$. The voltammograms were recorded immediately after immersion in the solution (curve 1) and after keeping at an open circuit for 30 min (curve 2). The voltammetric conditions were the same as in Fig. 3.

Voltammetric response behavior

The DP voltammetric behavior of a capillary electrode was examined in a KCl-HEPES solution containing 5.0 mM $[\text{Fe}(\text{CN})_6]^{4-}$ ions as a model analyte. The DP voltammograms obtained with and without applying sampling voltages of ± 1.5 V are shown in Fig. 3A. The electrode at an open circuit gave a small peak (graph A-a), owing to diffusional entry of $[\text{Fe}(\text{CN})_6]^{4-}$ ions from the outer solution (initially 5.0 mM) to the inner solution (initially zero) of the electrode. The DP peak, however, diminished and finally disappeared when a sampling voltage of -1.5 V was applied for 40 min (graph A-b). This is because $[\text{Fe}(\text{CN})_6]^{4-}$ ions were moved out of the electrode against the electroosmotic flow by the applied negative potential. Thus, the electrode is reusable after applying the same sign of sampling voltage as that of the target ion. When a positive potential of $+1.5$ V was applied, a DP peak due to oxidation of $[\text{Fe}(\text{CN})_6]^{4-}$ ions emerged after 10 min and its height increased with time (graph A-c). These results demonstrate that the capillary electrode accumulated $[\text{Fe}(\text{CN})_6]^{4-}$ anions in its inner solution.

Similarly, when FcTMA^+ cations (5.0 mM) were used as a model analyte, a DP peak due to FcTMA^+ was observed by applying a sampling voltage of -1.5 V (Fig. 3B). The sampled FcTMA^+ ions could be driven out of the electrode by applying a voltage of $+1.5$ V.

Effect of tip sizes

The capillary electrode is expected to have the ability of discriminating ions (analyte) from other ions of opposite charge based on electrophoretic ion transport selectivity. However, if transport of ions by diffusion predominates, the electrophoretic selectivity of ion transport is lost, because diffusion is a charge-independent process that occurs spontaneously. To

Fig. 5 Differential pulse voltammograms at a capillary electrode with a 1.2- μm tip diameter in 0.30 M KCl-0.10 M HEPES/NaOH solutions (pH 7.4) containing (A) 5.0 mM $[\text{Fe}(\text{CN})_6]^{4-}$ and (B) 5.0 mM FcTMA^+ . The voltammograms were recorded immediately after immersion (curve 1) and after sampling of 15 min (curve 2). Sampling voltages were +1.5 V for $[\text{Fe}(\text{CN})_6]^{4-}$ and -1.5 V for FcTMA^+ . Between the two measurements, the sampled $[\text{Fe}(\text{CN})_6]^{4-}$ ions were driven out of the capillary electrode by applying a voltage of -1.5 V. Differential pulse voltammograms at a bare Pt electrode in 0.30 M KCl-0.10 M HEPES/NaOH solutions (pH 7.4) containing (C) 50 μM $[\text{Fe}(\text{CN})_6]^{4-}$ and (D) 50 μM FcTMA^+ are also shown. The voltammetric conditions were the same as in Fig. 3.

investigate the effect of the tip diameter of the electrode on the diffusional transport of an analyte, differential pulse voltammetric responses at capillary electrodes with various tip diameters (0.9, 1.2, 1.5 and 2.3 μm) were examined after standing for 30 min in KCl-HEPES solutions containing 5.0 mM $[\text{Fe}(\text{CN})_6]^{4-}$ (without applying sampling voltages). The results obtained are shown in Fig. 4. The electrodes with >1.2 μm tip diameters gave a DP peak due to $[\text{Fe}(\text{CN})_6]^{4-}$ ions, owing to the diffusional entry of the analyte into the inner solution. However, the smaller the tip diameter was, the smaller the DP peak became. With tip diameters ≤ 1.2 μm , no detectable peaks were observed, showing that the diffusional transport of the analyte within 30 min is negligible. Considering these results, we purposely used the electrode having a tip diameter of 1.2 μm (resistance 4.9 – 5.3 M Ω).

Effect of charge of analyte ions

The DP voltammograms at a capillary electrode were recorded in sequence in KCl-HEPES solutions containing 5.0 mM $[\text{Fe}(\text{CN})_6]^{4-}$ and 5.0 mM FcTMA^+ , otherwise under the identical condition. Between the measurements, the sampled $[\text{Fe}(\text{CN})_6]^{4-}$ was driven out of the electrode, so that the same electrode was used throughout. The DP voltammograms obtained are shown in Fig. 5. It is seen that the peak height due to $[\text{Fe}(\text{CN})_6]^{4-}$ is almost identical to that due to FcTMA^+ (note that at a bare Pt electrode the DP peak of $[\text{Fe}(\text{CN})_6]^{4-}$ is smaller than that of FcTMA^+). The concentration of $[\text{Fe}(\text{CN})_6]^{4-}$ in the inner solution of the electrode was determined to be 20 μM , which is larger than that (12 μM) of FcTMA^+ . This demonstrates that the transport of $[\text{Fe}(\text{CN})_6]^{4-}$ ions occurred more effectively than that of FcTMA^+ ions.

Table 1 Effects of sampling voltage and sampling time on the DP voltammetric response of the capillary electrode with an inner tip diameter of 1.2 μm

Sampling condition		DP peak	$[\text{Fe}(\text{CN})_6]^{4-}$
Voltage (V)	Time (min)	current (μA) ^a	(μM) ^b
+0.80	5	— ^c	— ^c
	10	0.011	6.8
	15	0.030	17
+1.5	5	— ^c	— ^c
	10	0.017	10
	15	0.057	34
+1.7	5	0.0057	3.4
	10	0.043	25
	15	0.085	51

a. Concentration of $[\text{Fe}(\text{CN})_6]^{4-}$ ions in the sample solution was 5.0 mM.

b. Concentration of $[\text{Fe}(\text{CN})_6]^{4-}$ ions transported into the inner solution.

c. No peaks appeared.

Fig. 6 $[\text{Fe}(\text{CN})_6]^{4-}$ concentration dependence of the DP peak height at a capillary electrode with a 1.2- μm tip diameter. Differential pulse voltammograms were recorded after sampling of 15 min at +1.7 V. The sampled $[\text{Fe}(\text{CN})_6]^{4-}$ ions were driven out of the electrode after each DP voltammetric measurement. (○) First and (●) second runs. The inset figure shows the concentration dependence in a lower concentration range obtained with 1 h sampling. The solution and voltammetric conditions were the same as in Fig. 3, except for the concentration of $[\text{Fe}(\text{CN})_6]^{4-}$.

Fig. 7 Differential pulse voltammograms at a capillary electrode with a 1.2- μm tip diameter in 0.30 M KCl-0.10 M HEPES/NaOH solutions (pH 7.4) containing (A) 5.0 mM $[\text{Fe}(\text{CN})_6]^{4-}$ and (B) 5.0 mM $[\text{Fe}(\text{CN})_6]^{4-}$ and 5.0 mM FcTMA^+ . The voltammograms were recorded immediately after immersion (curve 1) and after sampling of 15 min at +1.7 V (curve 2). The sampled $[\text{Fe}(\text{CN})_6]^{4-}$ ions were driven out of the electrode between the measurements. (C) A differential pulse voltammogram at a bare Pt electrode for a mixture of 5.0 mM $[\text{Fe}(\text{CN})_6]^{4-}$ and 5.0 mM FcTMA^+ in a 0.30 M KCl-0.10 M HEPES/NaOH solution (pH 7.4). The voltammetric conditions were the same as in Fig. 3.

The electrophoretic velocity v_{eo} of an ion under the influence of an electric field is generally given by²⁷

$$v_{\text{ep}} = \mu_{\text{ep}} E \quad (2)$$

$$\mu_{\text{ep}} = \frac{q}{6\pi\eta r} \quad (3)$$

where μ_{ep} is the electrophoretic mobility of the ion, E is the local electric field strength, q is the charge on the ion, η is the buffer viscosity and r is the radius of the ion. Therefore, the electrophoretic velocity is faster for the ions having a smaller radius and a larger charge when the strength of the electric field is constant. The ionic size of $[\text{Fe}(\text{CN})_6]^{4-}$ (minimum diameter about 9 Å)³⁰ is not significantly different from that of FcTMA^+ ; the ferrocene group of FcTMA^+ fits well into the cavity of β -cyclodextrin³¹ having an inner diameter of about 8 Å. On the other hand, the charge of a $[\text{Fe}(\text{CN})_6]^{4-}$ ion is larger than that of a FcTMA^+ ion. Consequently, the electrophoretic velocity of $[\text{Fe}(\text{CN})_6]^{4-}$ is faster than that of FcTMA^+ . Taking this into consideration, the present electrode could accumulate $[\text{Fe}(\text{CN})_6]^{4-}$ ions more efficiently than FcTMA^+ ions in its inner solution.

Enhancement of sensitivity

The response of the capillary electrode necessitates electrokinetic transport of analyte ions into the inner solution. The sensitivity of detecting an analyte, therefore, depends on the amount of the analyte transported into the inner solution. DP voltammetric measurements for $[\text{Fe}(\text{CN})_6]^{4-}$ were performed at different sampling voltages. As given in Table 1, the amplitude of the DP current, as well as the concentration of the ions transported into the inner solution, increased with the sampling voltage. Also, the longer the sampling time was, the larger the DP peak height was. Thus, the sensitivity of the capillary electrode is enhanced by applying a larger sampling voltage or prolonging the sampling time.

The DP peaks after sampling of 5 min were very small or were not detected with all the sampling voltages. The inner Pt working electrode was able to be positioned 1.5 mm apart from the top of

the capillary (Fig. 1C), due to the diameter (0.127 mm) of the Pt working electrode. The $[\text{Fe}(\text{CN})_6]^{4-}$ ions to be detected at the underlying Pt electrode migrated this distance, resulting in a delay of the voltammetric response.

Concentration dependence

The relationship between the concentration of $[\text{Fe}(\text{CN})_6]^{4-}$ ions in a KCl-HEPES solution and the DP peak height was investigated at a sampling voltage of +1.7 V. The sampling voltage was applied for 15 min. The results obtained are shown in Fig. 6. The DP peak current is proportional to the concentration of $[\text{Fe}(\text{CN})_6]^{4-}$ ions in the range of 0.50 – 5.0 mM. After the sampled $[\text{Fe}(\text{CN})_6]^{4-}$ ions were driven out of the electrode, the measurement was repeated. The result of the second measurement agreed with the first one within an experimental error. The relative standard deviation of the three measurements at 1.0 mM $[\text{Fe}(\text{CN})_6]^{4-}$ was 13%. The detection limit was lowered (improved) to 30 μM $[\text{Fe}(\text{CN})_6]^{4-}$ by using 1 h electrokinetic sampling at +1.7 V (inset in Fig. 6). The concentration of $[\text{Fe}(\text{CN})_6]^{4-}$ ions transported from the sample solution (1.0 mM $[\text{Fe}(\text{CN})_6]^{4-}$) into the capillary electrode after electrokinetic sampling for 15 min at +1.7 V was found to be 9.8 μM . Assuming that the concentration of $[\text{Fe}(\text{CN})_6]^{4-}$ in the inner solution ($\sim 10 \mu\text{l}$) is homogeneous throughout the solution, we estimate the number of $[\text{Fe}(\text{CN})_6]^{4-}$ ions transported to be $\sim 10^{-10}$ moles. This amount is negligibly small as compared with that (3×10^{-6} moles) in the sample solution (3 ml), showing that the present electrode does not perturb the sample solution even if the analyte was sampled.

The slope of the linear plot varied among the capillary electrodes, owing to the variation of the size and shape of the pulled capillary tips. Therefore, preparation of the calibration curve is necessary for each capillary electrode, as is usual for voltammetric analysis with conventional electrodes.

Selectivity

The capillary electrode has been applied to selective detection of $[\text{Fe}(\text{CN})_6]^{4-}$ anions in the presence of FcTMA^+ cations. Figure 7 shows DP voltammograms recorded in series for 5.0 mM $[\text{Fe}(\text{CN})_6]^{4-}$ in the absence and presence of 5.0 mM FcTMA^+ . For

comparison purposes, a DP voltammogram at a bare Pt electrode in a KCl-HEPES solution containing $[\text{Fe}(\text{CN})_6]^{4-}$ and FcTMA^+ is also shown. At the bare Pt electrode, $[\text{Fe}(\text{CN})_6]^{4-}$ (0.20 V) and FcTMA^+ (0.36 V) currents were clearly seen (Fig. 7C). On the other hand, with the capillary electrode, only a $[\text{Fe}(\text{CN})_6]^{4-}$ peak was seen at 0.16 V and no FcTMA^+ peak was observed (curve 2 in Fig. 7B). The amplitude of the $[\text{Fe}(\text{CN})_6]^{4-}$ peak current in the presence of FcTMA^+ was almost identical to that in the absence of FcTMA^+ (curve 2 in Fig. 7A). These results demonstrate that the capillary electrode selectively detected $[\text{Fe}(\text{CN})_6]^{4-}$ anions in the presence of FcTMA^+ cations based on the charge-selective sampling ability.

Conclusion

The glass capillary electrode described here has an inner tip diameter of $\approx 1.2 \mu\text{m}$, across which analyte ions are sampled electrokinetically into its inner solution. The electrokinetic sampling is based on the fact that the electromigration velocity of anions is faster than the velocity of electroosmotic flow in the capillary electrode having a conical tip. The present capillary electrode is useful as a new ultramicroelectrode for detecting ionic species and has the potential for sensitive and selective detection of bioactive substances in biological tissues. However, the response time of the present ultramicroelectrode is slow (5 – 10 min) for monitoring the molecular events proceeding in biological signal transduction. To overcome this feature, we are now trying to introduce a very small volume of the inner solution by chemically modifying the inner surface of the capillary pipet near the tip with octadecyltrichlorosilane.

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