Introduction

Cationic surfactants (CSs) are widely used for rinsing, disinfectant and so on, but they have become one of the environmental pollution sources, since its consumption increases because of toxicity of CSs to humans. Solvent-extraction/spectrophotometry and two-phase titration, which are conventional analysis methods of CSs, have drawbacks, such as complicated operation, being time-consuming and using a large amount of harmful organic solvents. Therefore, the development of simpler analytical methods for CS with less load on the environment is required.

A microfluidic chip with microfluidic channels formed on the substrate, has excellent characteristics, such as reduction of the amount of the sample, waste and reagent solution. Among electrochemical detection methods, an ion-selective electrode (ISE) and an ion-sensitive field effect transistor (ISFET) are effective as detection methods of a microfluidic chip. This is because ISE and ISFET are easy to miniaturize and can easily be fabricated at low cost, and the sensitivity of ISE and ISFET does not decrease, even if their size is reduced. We have already developed a new microfluidic polymer chip with an embedded ISE, where a channel on the chip was prepared by using a small-diameter wire as a template of the channel. On the other hand, ion-selective optical sensor (Optode) membranes are suited for a detector of microfluidic chips, compared with the ISE and the ISFET. From the viewpoints of applications to microfluidic technology, optodes are superior to ISEs because they are not subject to electrical noise, and no contacts to microfluidic chips are required for measurements. Furthermore, no reference electrode is needed for measurements using optodes. However, only a few studies regarding microfluidic chips with embedded miniaturized optodes have been reported.

Flow-injection analysis (FIA) is one of the analytical methods utilizing a flow system originated by Ruzicka et al. In the FIA, after a sample solution is injected into a carrier solution, continuously flowed by a pump and mixed with a reagent solution, a target component in the sample is quantified by a peak signal obtained by a downstream detector. The FIA has attracted much attention as a rapid analytical method capable of precisely controlling the mixing and reaction between a reagent solution and a sample solution in a flow system. As results, the characteristics of the FIA method result in high reproducibility, high sample throughput, low consumption of reagents, and simpler procedures over the batchwise methods. Based on the advantages of the FIA system and microfluidic chips with embedded miniaturized optodes, the combination of the FIA system with a microfluidic chip with an embedded CS-optode as a detector, is expected to be an ideal analytical method for the determination of CSs.

In this paper, we propose a new FIA system for the determination of CSs using our microfluidic chip with an embedded CS-optode used as a detector.

Experimental

Reagents and chemicals

Tetradecyldimethylbenzylammonium chloride (zephiramine) used as a cationic surfactant, was obtained from Dojindo Laboratories. Cetyltrimethyammonium bromide (CTAB) and
cetylpyridinium chloride (CPC) used as cationic surfactants, were of reagent grade and were obtained from Tokyo Kasei Ind. Co. Poly(vinyl chloride) (PVC) (degree of polymerization: 1100) powder and tetrabromophenolphthalein ethyl ester potassium salt (TBPE-K) were obtained from Wako Pure Chemicals Co. 2-Nitrophenyl octyl ether (NPOE) was also obtained from Dojindo Laboratories. All other chemicals of reagent grade were used as received.

Preparation of CS optode membrane based on TBPE

The CS optode membrane based on TBPE was prepared as follows, according to a method used in a previous paper.19 TBPE-K (0.0140 g), NPOE (1.0 g) and 3 M HCl (4.0 mL) were stirred for 4 h. As a result, TBPE-H was extracted into the NPOE phase. After the NPOE phase and the water phase were separated by centrifugation (5 min), the NPOE phase and PVC powder (0.40 g) were dissolved in 10 mL of tetrahydrofuran (THF). The resulting THF solution was poured into a φ32 × 15 mm glass petri dish and left for 24 h for optode master membrane preparation.

Procedures for the fabrication of a microfluidic polymer chip

Figure 1 shows procedures for the fabrication of a microfluidic polymer chip. An axis of a M6 × 10L bolt was cut to 3 mm in length. A hole (φ5 mm) was dug into the top of a M6 × 10L bolt for inserting optical fiber. An optode membrane (φ4 mm) was pasted onto the tip of the axis of the M6 × 10L bolt (part H). Seven pieces of boards (2 cm wide, 5 cm long and 1.7 mm thick) were cut from a B4-size PLA-PLATE made of polystyrene (ITEM 70128 500, Tamiya Co. Ltd., Shizuoka, Japan). One of the cut boards is referred to as part A. A piece of board (part C), which was 2 cm wide, 5 cm long and 0.4 mm thick, was cut from a B4-size PLA-PLATE. Three pieces of part A were sandwiched between two glass plates and pressed by a double clip and put into an oven at 110°C for 30 min for thermal fusion (part A3). A hole, at the distances of 1 cm from the lower side of the part A3, and 2 cm from the right side of it, was dug for the insertion of an optical fiber by using a 3.3-mm drill. The fabricated part is referred to as part D. A hole, at a distance of 2 cm from the right side of another part, A3, and 1 cm from its lower side, was drilled by using a 3.5-mm drill, and a screw was cut in the hole by using a M6 × 10L tap for inserting a bolt with an optode membrane (part F). A hole, at distance of 2 cm from the right side of part C, and 1 cm from its lower side, was drilled by using a 3.3-mm drill.

Then, channels of the polymer-based microfluidic chip in part C were fabricated using stainless-steel wires (diameter of approximately 0.5 mm, SUS304-W1, Waki Industrial Co. Ltd., Osaka, Japan) as a template of the channel, according to our method (part B).11-12,14-17 Next, part B, part C and part D (C is the middle) were sandwiched between two glass plates and pressed by a double clip, and put into an oven at 110°C for 30 min for thermal fusion of parts B, C and D (part E0). A hole was drilled using a 1.05 mm drill at 0.5 cm length and 1 cm width of both sides of part E0 for fabricating a channel of a sample solution and waste (part E). Parts F and E were sandwiched between two glass plates and pressed by a double clip. It was then put in an oven at 110°C for 30 min for thermal fusion of parts F and E (part G). Finally, the optode membrane fixed at the tip of the M6 × 10L bolt (part H) was attached in part G. Part H involved a removable type, and can be easily replaced with a new one.

FIA system for the determination of CSs

A schematic diagram of the μ-FIA system used in this work is shown in Fig. 2. The μ-FIA system was a two-channel system composed of two syringe pumps, a sample injector, a polymer-based microfluidic polymer chip detector, a tungsten/halogen lamp as a light source, a fiber-optic spectrometer (USB 2000, Ocean Optics, USA) and a personal computer. The flow rate of distilled water (carrier solution) and the regeneration solution of an optode membrane (0.18 mol dm–3 HCl) was 50 μL min–1, respectively. A 10-μL aliquot of the sample solution, which was prepared by mixing the CS solution with 10 (v/v) % of 0.1 mol dm–3 CH3COOH/CH3COONa buffer solution (pH 4.0), was injected to the carrier solution. A P400-1-UV/VIS 400 μm fiber (Ocean Optics, USA) was used to transport light from the tungsten/halogen lamp to the sensing membrane fixed at the tip of the M6 × 10L bolt in the microfluidic polymer chip and the other P400-1-UV/VIS 400 μm fiber to collect and transmit light via the sensing membrane to the spectrometer. When the sample solution was introduced into the TBPE-K-based optode detector in the microfluidic polymer chip, the absorbance was monitored with the fiber-optic spectrometer at 622 nm, and recorded as a peak signal on a personal computer using “SpectraSuite” software (Ocean Optics, USA).

Results and Discussion

Effect of concentration of a HCl solution as a regeneration solution on the reversible response of the optode membrane to CSs

The response mechanism of the optode membrane to CSs was as follows: At first, CS is extracted into the optode membrane and a CS-TBPE ion associate is formed in the optode membrane. As a result, the color of the membrane changes from yellowish green to blue (absorption maximum: 622 nm). The ion-association reaction (1) is as follows, and must be reversible in order to use the optode membrane as a microfluidic detector for the FIA of CSs:

\[(CS)_{aq}^+ + (PBE–H^+)_{mem} \leftrightarrow (TBPE–CS)_{mem} + (H^+)_{aq} \tag{1}\]

where, CS⁺ is a cationic surfactant, memb is an optode membrane, and aq is an aqueous solution.

The reversible response of the optode membrane to CSs depends on the concentration of the H⁺ ion in the regeneration solution of the optode membrane, as presumed from Eq. (1). Therefore, at first, we examined the effect of the concentration of the H⁺ ion in a regeneration solution (HCl solution) on the reversible response of the optode membrane to CSs. When the concentration of HCl solution was 0.030, 0.060, 0.090, 0.12 and 0.15 mol dm–3, the response signals obtained by an embedded CS-optode returned to the initial baseline absorbance. This means that a reversible response of the optode membrane to CSs is obtained by using a 0.18 mol dm–3 HCl solution as a regeneration solution.

From this result, concentration of the HCl solution as a regeneration solution was determined to be 0.18 mol dm–3.

Calibration curve for CS obtained by using a microfluidic polymer chip with an embedded CS optode in the FIA system

At first, we examined the effect of the flow rate of distilled water (carrier solution) and the regeneration solution of the optode membrane, on the sensitivity and sample throughput of
zephiramine. The flow rate at each channel was the same, and was changed to 50 μL, 75 μL, and 100 μL min⁻¹. An increase of the flow rate leads to a decrease in the peak height (sensitivity) and an increase in the amount of waste. On the other hand, as the flow rate was decreased, the peak height (sensitivity) was increased, and the sample analysis time became very long. From these results, we determined that the optimal flow rates of the two channels are 50 μL min⁻¹, respectively. Next, we examined the effect of the sample volume on the sensitivity and sample throughput of zephiramine. The sample volume was changed to 5, 10, and 20 μL. In the case that the sample volume was 5 μL, even 400 μmol dm⁻³ zephiramine was not detected. In the case that the sample volume was 10 μL and 20 μL, the sensitivity of zephiramine was very similar. However, the sample analysis time at 20 μL of the sample volume was approximately twice that at 10 μL of the sample volume. From the result, the optimal sample volume was determined to be 10 μL. Figure 3 shows the typical calibration peaks for zephiramine obtained under the optimal conditions described above. A linear relationship ($r = 0.993$) was found to exist between the peak height in the absorbance unit, and the concentration of zephiramine in a concentration range from 50 to 400 μmol dm⁻³ under the optimal conditions described above. The graph equation is $Y = 2.00 \times 10^{-4}X + 4.76 \times 10^{-2}$.
for zephiramine. Here, $Y$ is the peak height in the absorbance unit and $X$ is the micromolar concentration of zephiramine in the injected sample solution. The lower limit of detection, defined as $S/N = 3$, was approximately 30 μmol dm$^{-3}$. The relative standard deviations of the peak heights examined by 3 injections of the 50 and 200 μmol dm$^{-3}$ zephiramine were 2.6 and 3.2%, respectively. The sample throughput for the determination of 300 μmol dm$^{-3}$ zephiramine was around 11 samples h$^{-1}$. The calibration curves for CSs other than zephiramine, i.e., CTAB and CPC, were also obtained for the same concentration range as those for zephiramine. The graph equation is $Y = 1.00 \times 10^{-4} X + 2.93 \times 10^{-2}$ for CTAB, and $Y = 7.50 \times 10^{-5} X + 3.50 \times 10^{-2}$ for CPC, respectively. This means that the total CSs in commercial products, such as disinfectants, are determined by the optode membrane. The order of the sensitivity (slope of the calibration curve) of the CS-optode for CSs is zephiramine > CTAB > CPC, and may be identical with the order of the hydrophobicity of CSs. The lower limit of detection, defined as $S/N = 3$, was approximately 50 μmol dm$^{-3}$ for CTAB and 50 μmol dm$^{-3}$ for CPC, respectively.

The optode membrane showed an operational lifetime of 1 week, at least, by wrapping the membrane with an aluminum foil and storing it at room temperature. One CS-optode membrane can be used for about 20 times injections, at least, without showing any deterioration in the performance due to long-term use. Furthermore, if the microfluidic polymer chip with the embedded CS-optode shows deterioration in the performance during long-term use, the CS-optode can be easily replaced with a new one.

**Effect of coexisting inorganic electrolytes in the sample solution on the determination of CS**

Inorganic electrolytes are often contained in real samples, such as environmental water, commercial disinfectants, antifriction, and emulsifying agents. Therefore, the effect of these coexisting inorganic electrolytes on the determination of CSs should be examined in order to apply the present method to the determination of CSs in real samples. The effects of coexisting inorganic electrolytes on the determination of 200 μmol dm$^{-3}$ zephiramine are given in Table 1.

Table 1 indicates that the coexistences of inorganic electrolytes, such as NaCl, KCl, CaCl$\text{$_2$}$, MgCl$\text{$_2$}$, NH$\text{$_4$}$Cl, Na$\text{$_2$}$SO$\text{$_4$}$ at 100 times, NaNO$\text{$_3$}$ at 20 times excess to zephiramine did not interfere with the determination of zephiramine. The reason why interference of NaNO$\text{$_3$}$ for the determination of zephiramine is relatively large, may be due to that the NO$\text{$_3$}$– ion forms an ion associate with zephiramine; as a result, the concentration of dissociated zephiramine decreases.
Table 2  Recovery tests of CPC added to dental rinse samples

| Dental rinse samplea | Added/μmol dm–3 | Found/μmol dm–3 | Recovery, % |
|---------------------|----------------|----------------|------------|
| Sample 1            | 0              | 360 ± 1        |            |
|                     | 150            | 505 ± 5        | 97 ± 3     |
| Sample 2            | 0              | 153 ± 1        |            |
|                     | 134            | 283 ± 5        | 98 ± 4     |

a. Dental rinse samples were diluted 5.0 times with distilled and deionized water.

Table 3 Comparisons between the concentrations of CPC in dental rinse samples obtained by the proposed FIA method and those by the conventional two-phase titration method

| Dental rinse samplea | Concentration/μmol dm–3 | Error, % |
|---------------------|-------------------------|----------|
|                     | Proposed FIA method     | Two-phase titration method |
| Sample 1            | 360 ± 1                 | 358 ± 0.4 | + 0.6    |
| Sample 2            | 153 ± 1                 | 167 ± 0.4 | – 9.0    |

a. Dental rinse samples were diluted 5.0 times with distilled and deionized water.

Application of the FIA system for the determination of CS in dental rinse samples

The results of a recovery test for CPC added to dental rinse samples, obtained by using the CS-optode detector in the present FIA system, are given in Table 2. The dental-rinse samples were 5.0-fold diluted with distilled and deionized water. For both of the two dental rinse samples examined in this work, the recovery of CPC was roughly 97 – 98%. This result indicates that there is little matrix effect on the determination of CPC in dental-rinse samples. Table 3 shows comparisons between the concentration of CPC in dental-rinse samples obtained by the proposed FIA method, and that by the conventional two-phase titration method.6 The analytical results of CPC in dental rinse samples were in good agreement with those obtained by the two-phase titration method. These results mean that the present FIA system is applicable for the determination of CS in dental-rinse samples.

Conclusions

A new plastic microfluidic polymer chip with an embedded CS-optode was developed as a detector for the determination of CSs in the FIA system. The present FIA system has the advantages of a decreased use of reagent and lower sample volumes, and no toxic organic solvent over other analytical methods. Anionic surfactants will interfere with the determination of CS in the present FIA system because the hydrophobicity of anionic surfactants is much higher than that of NO3-. Therefore, the present FIA system is not applied to the determination of CS in environmental samples at this stage. If an anion-exchange column is introduced in the present FIA system, CS in an environmental sample will be determined in the present FIA system without any interference of anionic surfactants, as the authors reported previously.24 The present FIA system is useful for the determination of CS in dental-rinse samples. The CS-optode can be easily replaced with a new one, if the microfluidic polymer chip with the embedded CS-optode shows a deterioration in performance during long-term use.

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