

Fabrication of a Flow Cell for Electrochemical Impedance Measurements

Kosuke Hayama, Hiroki Tanaka, Myung-Jong Ju, Kenshi Hayashi
and Kiyoshi Toko

Graduate School of Information Science and Electrical Engineering, Kyushu University,
6-10-1 Hakozaki, Higashi-ku Fukuoka 812-8581, Japan

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A flow cell incorporating a liquid junction was fabricated for sequential analysis using the surface-polarity-controlled sensor. In the present flow cell, a porous wall or an S-shaped route acts as a liquid junction to prevent contamination of the reference-electrode solution. The stability of the electric potential of the Ag/AgCl reference electrode in these flow-cell systems was studied, and both of the liquid junctions were highly effective in keeping the electrode potential stable. In the surface-polarity-control method, good agreement of sensor response was confirmed between the batch system and the flow-cell system with the S-shaped junction, although large resistance of the porous wall junction influenced the estimation of the impedance of the working electrode/solution interface. A portable and general-purpose chemical sensor can possibly be realized using the surface-polarity-controlled sensor with a flow-cell system which utilizes flow injection analysis.

1. Introduction

A surface-polarity-controlled sensor measures electrochemical impedance of the electrode surface whose electric potential is dynamically controlled, and the impedance changes by electrode potential are used as chemical sensor output. Chemical substances affect the electrode impedance by the weak and transitory adsorption to the electrode surface. The adsorption condition varied by electrode potential is dependent on the character of chemical substances in the electrode/solution interface. Therefore, analytes can be broadly identified by the sensor output, i.e., the electrode-potential dependency of the impedance is correlated with properties of chemicals adsorbed to or interacted with the

surface. In addition, the sensor can detect both electrolytes and nonelectrolytes (hydrophilic and hydrophobic substances) with high sensitivity.⁽¹⁻³⁾ This sensor has been developed as a multi-purpose chemical sensor with low selectivity, and thus is suitable for application to identify various chemical substances as a screening method before accurate analyses such as GC/MS or LC/MS.

However, the surface-polarity-controlled sensor had a time-consuming sensing procedure for multi-sampling because its sampling was of batch type to observe stable impedance spectroscopy measurements. Furthermore, it is necessary to obtain more information about analytes for multiple purposes and complex sensor application, and hence the method of *in-situ* modification of the electrode surface is required in the surface-polarity-controlled sensor.⁽⁴⁻⁶⁾

Flow-type analyses such as a flow injection analysis (FIA) and liquid chromatography (LC) are indispensable to realize the above requirement. These analyses are useful in sequential sampling and quick detection of the sample. There are various flow-cell systems coupled with electrochemical detection in FIA or LC.

Flow-cell detectors must be miniaturized to combine them with a pumping system. In many cases, however, the miniaturization is limited to the working electrode, and the reference electrode of a normal size is used in the flow-cell detectors. Although a reference electrode without a liquid junction is easy to miniaturize, its use is severely restricted because of susceptible changes in activity of the primary ions (*e.g.*, activity of Cl^- in the Ag/AgCl system). In addition, it is difficult to miniaturize the liquid junction of reference electrodes while still satisfying basic requirements such as stability, reversibility and reproducibility.⁽⁷⁾ Furthermore, the impedance measurements using the flow cell are not easy because of a derivative impedance which comes from a small flow system.

The liquid junction serves as the ionically conducting electrical pathway between the inside of the reference electrode and the bulk of the cell. It has low electrical resistance (under 10 k Ω for the common filling solutions) and a modest leak rate. The electrical resistance of the liquid junction is an important factor in determining the stability and speed of the potentiostat in actual use. High impedances of the liquid junction lead to high iR (electric current multiplied by resistance) drops, which ultimately lead to excess noise, instability of the applied potential and a nonlinear response. On the other hand, the leak rate may be important because of possible contamination of the analyte solution by filling solution of the reference electrode. There is a fundamental trade-off between electrode impedance and leakage rate of reference-electrode solution. The reference electrode, which has a proper electrode impedance and a proper leakage rate, requires routine maintenance. For electrochemical impedance measurements in the surface-polarity-control method, frequency spectra of the working electrode are important and so proper fabrication is required for the cell.

We fabricated two flow cells with liquid junctions, *i.e.*, a porous-wall separated junction and an S-shaped junction which has sufficiently small size for practical application. A gypsum porous pellet or an S-shaped flow path acts as a liquid junction. These flow cells have two channels, one for the analyte solution and the other for the reference-electrode solution. This structure is also useful for the purpose of restoration of the reference-electrode solution. Because the exchange of solutions takes only a few minutes,

it is easy to keep the concentration of the solution uniform and to control the electric potential of the reference electrode. Moreover, these electrodes, which are fixed onto the flow cells, can be easily removed and hence the surface of the electrode can be cleaned thoroughly before and after use.

The purpose of the present study is the development of the sensing system using surface-polarity-controlled sensor to realize a functional and multi-purpose chemical sensor. Firstly, the reliability of the liquid junction was investigated by measuring the electric potential between Ag/AgCl electrodes. Next, the flow-cell system was evaluated by measuring some chemicals using the surface-polarity-control method.

2. Materials and Methods

2.1 Fabrication of flow cells for impedance measurement

We fabricated two flow cells, which have a liquid junction as shown in Fig. 1. Holes and ditches were dug with 2 mm diameter as a channel, in which liquid streams, in an

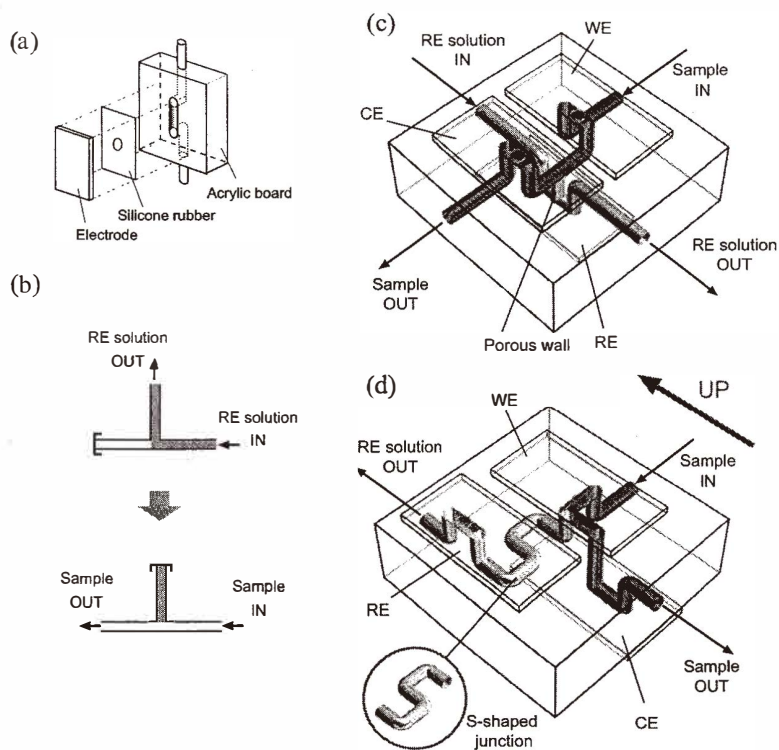


Fig. 1. (a) Electrode on flow route, (b) solution flow sequence, (c) porous wall cell and (d) S-shaped junction cell.

acrylic block. Three electrodes were attached on the acrylic board using a 0.2-mm-thick silicone rubber with a 2-mm-diameter hole as shown in Fig. 1(a). The electrodes were fixed with Teflon tape. Since the electrodes can be removed easily, we can pretreat them by annealing and alumina rubbing before all the experiments. The pretreatments of the electrode are important to obtain high reproducibility of the experiments. The size of the flow cell ($3 \times 3 \times 1$ cm) is sufficiently suitable for the present purpose, which is the fabrication of flow cells for electrochemical impedance measurement.

Platinum was used as both the working electrode (WE) and the counter electrode (CE), and Ag/AgCl was used as the reference electrode (RE). The size of all the electrodes was $10 \text{ mm} \times 20 \text{ mm}$ and 0.5 mm in thickness. These flow cells have two channels, one of which is for the sample solution and the other is for the internal filling solution of the reference electrode. As shown in Fig. 1(b), the valve of the sample outlet side is closed first, and the RE solution is filled in the channel for the RE. Next the valve of the RE side is closed, and carrier solution streams through the WE path. The porous wall in Fig. 1(c) or the S-shaped flow path in Fig. 1(d) separates the electrolyte for RE from the sample solution.

The cell with the S-shaped path is turned in the direction of the arrow in Fig. 1(d), and is set on a supporting board. The S-shaped path set in this way can prevent the filling electrolyte from mixing, caused by the gravity-forced translocation, with the sample stream.

We evaluated the stability of electric potential of these flow cells using KCl responses of WE made by replacing platinum with Ag/AgCl. When two Ag/AgCl electrodes are immersed in KCl solutions of different concentrations, an electric potential difference between the two electrodes obeys the Nernst equation given by

$$E(\text{Ag}/\text{AgCl}) = E_0 - 2.303(RT/F)\log a(\text{Cl}^-), \quad (1)$$

where $a(\text{Cl}^-)$ is activity of Cl^- . At room temperature (25°C), $2.303(RT/F)$ is evaluated at 59.16 mV.

2.2 Surface-polarity-control method

2.2.1 Experimental setup

Figure 2 shows the experimental setup used to measure electrochemical impedance using the flow cell. The electrodes were connected to a potentiostat. The electric potential between the working electrode and the reference electrode was adjusted and controlled using the potentiostat. The alternating input voltage was generated by a function generator (YOKOGAWA, FG110). The amplitudes and phases of both input and output signals were measured with a lock-in amplifier (NF Electronic Instruments, LI 5640). The impedance of the electrode was calculated by the ratio of amplitude and the difference of phase between the input voltage and the output current. The measurement process was controlled by a computer connected to an apparatus with GPIB.

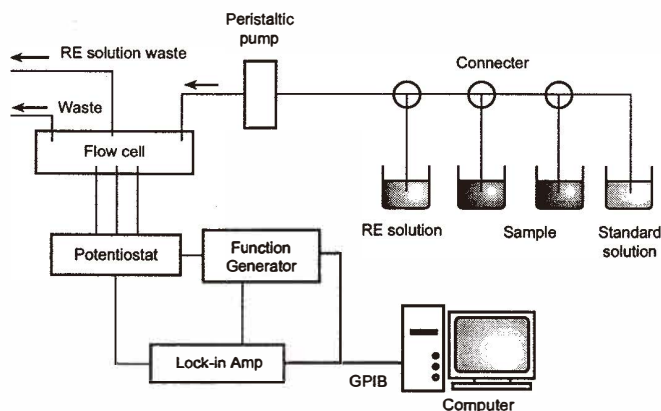


Fig. 2. Experimental setup.

2.2.2 Calculation of CPE impedance

Simple equivalent circuits were estimated from experimental data under the condition where redox reactions such as that of hydrogen or oxygen production did not occur. Figures 3(a) and (b) represent the equivalent circuits for modeling the electrode/electrolyte solution interface in the batch method and the flow method, respectively. R_s and Z_{CPE} denote solution resistance and impedance of the constant phase element (CPE), respectively. The parallel circuit of R_d and C_d in Fig. 3(b) is the derivative component coming out from between the cell-potentiostat circuit and the route in flow cells, thus it does not contain information on the interface. The semicircular also appears in the CPE with parallel resistance, where the semicircular is depressed.⁽⁸⁾ In our results, the semicircular was not depressed and did not depend on electrode potential, therefore we concluded that the semicircular was caused by derivative components from the flow cell. We pay much attention to the CPE part because sensor information can be obtained by CPE changes. Therefore, the part of the impedance-semicircular locus that occurred due to the flow cell should be neglected in the measurement of chemical substances because Z_{CPE} representing the condition of the interface is the only notable element of the impedance on the surface-polarity-control method. As shown in Fig. 3(c), consequently, $R_s' = R_s + R_d$ was regarded as a quasi solution resistance in the flow measurement.

Mathematically, CPE impedance is given by^(8,9)

$$1 / Z_{CPE} = Y_{CPE} = Q (j\omega)^n, \quad (2)$$

where Q becomes the admittance ($1/|Z|$) at $\omega=1$ rad/s.

A consequence of the above simple equation is that the phase angle of the CPE impedance is independent of the frequency and has a value of $-(90 \times n)$ degrees. When n is equal to 1, the equation becomes the same as that for the impedance of a capacitor, where $Q = C$. When n is close to 1.0, the CPE resembles a capacitor, but the phase angle is not

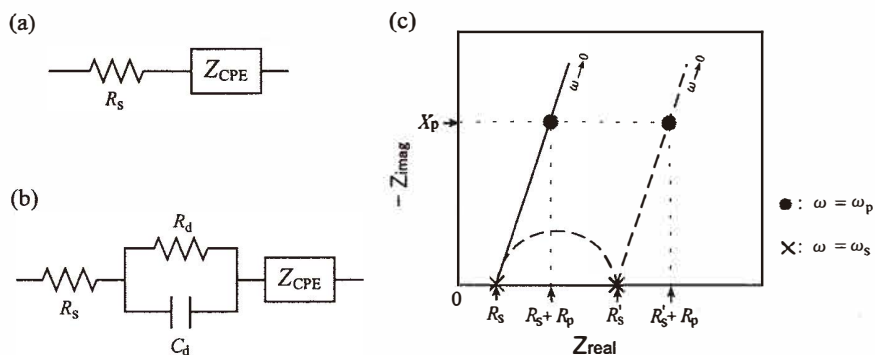


Fig. 3. Equivalent circuits (a) in the batch cell and (b) in the flow cell. (c) Complex impedance plane plot of CPE impedance; solid line represents the circuit (a); dashed line represents the circuit (b). For an ideal CPE, the plot is a straight line which makes an angle of $n \times 90^\circ$ with the x-axis.

90° . It is somewhat less than 90° in the real electrode/solution interface in the wide frequency range.

One physical explanation for the CPE behavior is the roughness of the electrode surface.⁽⁹⁾ Such fractal characteristics of the electrode surface would change the interactions between chemical substances and a solid electrode. The surface-polarity-control method evaluates the CPE impedance and detects the changes on the electrode surface. We measured the impedance at the angular frequencies of ω_p and ω_s , and then estimated the real part (R_p) and the imaginary part (X_p) of the CPE impedance as shown in Fig. 3(b), where $Z_{CPE} = R_p - jX_p$.

Impedance spectra from 10 Hz to 20 kHz were measured and estimated the characteristic values of CPE impedance. The measurement results were compared with those of the conventional batch method.^(2,3)

2.2.3 Dependence of impedance on electrode potential

Using the surface-polarity-control method and the flow-cell detector with the S-shaped junction, we measured some chemical substances. While the electrode potential was swept from 0.55 V to -0.45 V in 0.05 V steps, electrochemical impedance was measured at $f_p = 35$ Hz and $f_s = 260$ Hz and R_p and X_p were estimated, where $f = \omega/2\pi$. A profile of electrochemical impedance as a function of the electrode potential in a 10 mM KCl solution indicates the dependences of the electric potentials of R_p and X_p . ΔR_p and ΔX_p denote the changes of R_p and X_p , respectively, by the addition of a chemical substance to 10 mM KCl solution. The sensor response of this method is called the profile change hereafter. The profile change representing the electrode-potential dependences of the impedance is diversified by chemical features of analytes, such as surface-active functional groups, flexible side chains, aromaticity and pH. Accordingly, pattern analyses of profile changes realized the unspecific broad sensing of chemical substances based on their physical and chemical nature.

We measured continuously five chemical substances: NaCl, HCl, sucrose, quinine-HCl and monosodium glutamate (MSG). The profile changes obtained by this method were compared with those of the batch method. All the response measurements were performed three times.

3. Results and Discussion

3.1 Stability of electrode potential

The stability of electric potential of fabricated flow cells was evaluated by measuring the electric potential of Ag/AgCl electrode (WE) at four different KCl concentrations using Ag/AgCl reference (RE) in 100 mM KCl. Figure 4(a) shows the potential difference that was measured 10 minutes after filling the cell with solution. In both the flow cells, the potential difference has the value close to that of the relation of the Nernst equation. This result shows that the porous wall and the S-shaped route separated different electrolytes well and served as good ionically conducting electrical pathways between the reference-electrode side and the sample side.

The electric potential of Ag/AgCl electrode (WE) in 100 mM KCl was measured using Ag/AgCl reference (RE) in 10 mM KCl. Figure 4(b) shows the variation of electric potential which was measured every 5 minutes over 10 hours. When the working electrode was used in 10 mM KCl, the electric potential was zero within the variation of 1 mV (data not shown). In the case of 100 mM for the sample solution, the electric potential drifted to zero at the rate of 2 mV/h, as shown in Fig. 4(b). The rate was lower in the beginning, and it was less than 30 $\mu\text{V}/\text{min}$ for the first 2 hours. In the present flow system, the internal solution of the flow cell can be exchanged every few minutes, and consequently, the influence of this drift on the measurement data can be neglected. The reference electrode can hence maintain its potential value.

These evaluation results confirm that the system can actually be used for any low-frequency electrochemical measurement.

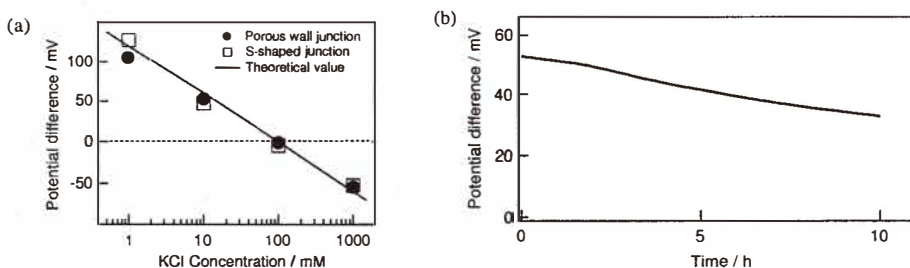


Fig. 4. (a) Changes of electric potential of Ag/AgCl working electrodes with KCl concentration. The KCl concentration for RE is 100 mM. (b) Temporal variation of electric potential of Ag/AgCl working electrode in 100 mM KCl in the S-shaped junction cell at 10 mM KCl concentration for RE.

3.2 Electrochemical impedance response

The complex impedance plane plots of electrochemical impedance in 10 mM KCl solution are shown in Fig. 5. Electrochemical impedance was measured with three cells, i.e., the batch cell, the porous-wall junction cell and the S-shaped junction cell.

In all the cells, the locus of Z showed the straight line of CPE behavior at low frequencies. In the high-frequency range, however, the Z showed a capacitive semicircular locus in the flow cells. A parallel circuit of a resistor and a capacitor represents a semicircle on the complex impedance plane plots. The present experiment was performed under the condition of ideal polarization, and hence, the semicircle was not based on a redox reaction because the capacitive semicircle usually originates from the charge transfer resistance and the interfacial capacitance. In addition, over the hydrogen (or oxygen)-generated region on Pt, this semicircular behavior was observed in parallel with the semicircle that stemmed from a redox reaction, besides, R_d is the diameter of the semicircle at higher frequencies was independent of the electrode potential and was constant. Accordingly, the semicircle plotted in the high-frequency range is probably a derivative component coming out from the capacitance of the cell-potentiostat circuit and the route-structured resistance in flow cells. Parallel capacitance of the cell-potentiostat circuit, which is smaller than the interfacial capacitance, indicates the semicircular behavior at higher frequencies on the complex impedance plane since smaller capacitance responds faster.

The diameter of a semicircle is the value of resistance connected in parallel with capacitance. Since the liquid-junction resistance in the porous wall cell is larger than that in the S-shaped junction cell, this semicircle would be related to the relatively large resistance component and a stray capacitance of the liquid junction. The locus of Z was a semicircle above 300 Hz in the S-shaped junction cell and above 50 Hz in the porous wall

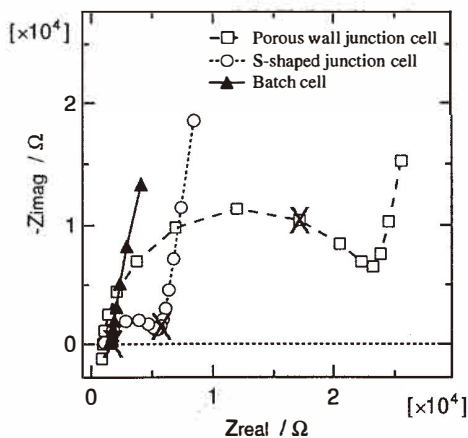


Fig. 5. Changes in the impedance complex plane due to the structure of electrode cells and frequency. X marks impedance at 260 Hz.

cell. Since the existence of a semicircle at lower frequencies influences the estimation of CPE impedance, the flow cell including the porous wall is neither suitable for impedance spectroscopy nor for the surface-polarity-control method.

3.3 Dependence of response profile on chemical substances

Using the surface-polarity-control method using the S-shaped flow cell and the batch cell, we measured the responses for five chemical substances. Since the concentration of the internal solution of the reference electrode varies, the potential ranges of the flow-cell system were adjusted in preliminary experiments. The reference-electrode potential (Ag/AgCl/10 mM KCl) in the flow cell is 0.15 V vs. Ag/AgCl/saturated KCl. In the case of the explanation about profile changes, the electrode potential of the flow cell is described mainly here and that of the batch cell is shown in parentheses hereafter. Figure 6 shows profile changes (ΔR_p) due to five test substances: NaCl, HCl, sucrose, quinine-HCl and MSG. Profile changes (a) were measured using the S-shaped junction flow-cell system. Profile changes (b) were obtained in the batch cell using the same working electrode and the ordinary-sized Ag/AgCl reference electrode in a saturated KCl solution and platinum wire counter electrode.

Profile changes were attributed to a mechanism on the electrode/electrolyte interface, although it is difficult to clarify the relationship between the impedance changed by the addition of chemical substances and the electrode potential. Many chemical-adsorption mechanisms onto the electrode surface are not clear, despite many research studies on the electrochemical reaction on the electrode surface. Our broad chemical detecting sensor does not utilize a specific chemical reaction or high-affinity adsorption which occurs in highly selective systems such as molecular recognition in the biological system. Therefore, our discussion is based on speculation.

The feature of the profile change, ΔR_p , of MSG which has an amino group was a peak that appeared at around -0.35 V for the flow cell (-0.2 V for the batch cell) and identified

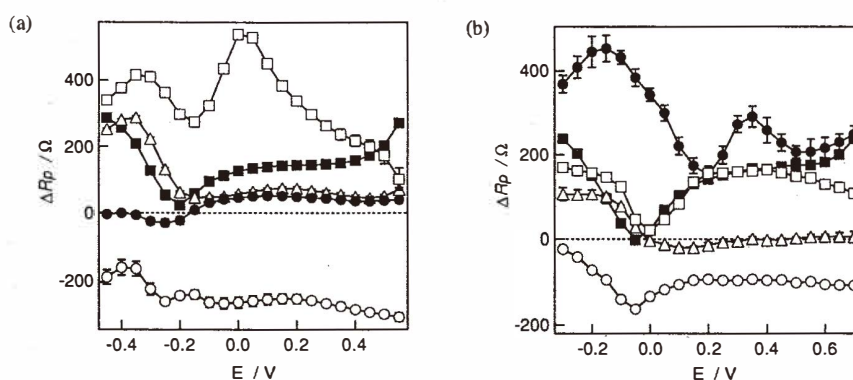


Fig. 6. Profile changes due to chemical substances in flow measurement using the S-shaped junction flow cell (a) and batch measurement (b). \circ : 100 mM NaCl, \bullet : 0.3 mM HCl, \square : 300 mM sucrose, \blacksquare : 0.3 mM quinine-HCl and \triangle : 3 mM MSG.

at concentrations above 0.1 mM in both the cells. Here, sensitivity was described by the concentration where the chemical could be identified by the profile change. The lowest concentration where the impedance began to change was smaller than that concentration. Compounds including an N atom such as amino (-NH₂) groups mainly indicate this feature. The results of thin-layer electrochemistry⁽¹⁰⁾ suggest that Nitrogen heteroaromatic rings (such as pyridine) are N-bonded perpendicularly to the surface, unless the N atom has been protonated or modified. Accordingly, the peak near -0.35 V (-0.2 V) in the surface-polarity-control measurement of MSG indicates the -NH₂ group.

Quinine-HCl is a hydrophobic and aromatic compound. Aromatic compounds adsorb irreversibly⁽¹¹⁾ onto the platinum surface by π orbital adsorption.⁽¹²⁾ The adsorption of aromatic compounds is less dependent on the electrode potential, therefore the impedance change of quinine-HCl increased except around -0.2 V (-0.05 V) at PZC (point of zero charge) of Pt. In the electrode potential around PZC, the concentration of ions near the electrode surface was extremely low, therefore the impedance change of adsorbed compounds to the surface would be insignificant. Aromatic and other compounds adsorbable on the Pt surface are detected using the highly sensitive surface-polarity-controlled sensor; quinine-HCl was identified at concentrations above 0.01 mM in both the cells.

NaCl, a strong electrolyte, significantly decreased the solution resistance and increased the double-layer capacitance at high concentration, and consequently, R_p decreased. NaCl was identified at concentrations above 3 mM in both the cells.

Hydroxyl groups (-OH) included in the structure of sucrose probably adsorbed on the surface at around -0.1 V (0.05 V) by hydrogen bonding.⁽¹³⁾ The adsorption of hydroxyl groups affected the interface impedance in the broad range of the electrode potential near -0.1 V (0.05 V). However, the profile change of sucrose occurred (blank squares with solid curve) because of the influence of PZC upon the impedance. The values of ΔR_p were larger in the flow cell than in the batch cell although the sucrose profile change was identified at concentrations above 10 mM in both the cells. On the other hand, the sensitivity of HCl was lower in the flow cell. ΔR_p of HCl increased abruptly above 0.3 mM in the batch cell and similarly the increase of ΔR_p was observed above 1 mM in the flow cell. The profile change of HCl may be relative to the adsorption of hydrogen and the underpotential deposition process but this detailed reason is obscure at present.

The measurements of MSG, quinine-HCl and NaCl in the flow cell were equal to those in the batch cell, however the results of sucrose and HCl were different with respect to the impedance change. It is highly likely that sucrose and HCl influence each other on the Pt surface. In fact, as a result of measurements of sucrose responses obtained in coexisting HCl solution, ΔR_p of sucrose responses was inhibited by HCl. Accordingly, it is suggested that the pre-adsorbed proton can hinder hydroxyl groups (-OH) from binding to the Pt surface. The adjustment of pH of the test solution would solve the disagreements of profile changes of sucrose and HCl between measurements in the flow cell and those in the batch cell in the future.

The surface-polarity-control method using a flow-cell detector can perform sequential analysis in a short time and reduce waste fluids. Moreover, it has a wide range of applications and can be used for easy and quick measurements of the properties of various chemical substances, which require high sensitivity, such as endocrine disruptors. The present flow system would be useful as an easy screening method for accurate analyses.

References

- 1 K. Hayashi and K. Moriyama: *Trans. IEEJ* **117**-E (1997) 95.
- 2 K. Hayama, M. Habara, K. Hayashi and K. Toko: *Trans. IEEJ* **121**-E (2001) 215.
- 3 M. J. Ju, K. Hayashi and K. Toko: *Sensors and Materials* **13** (2000) 169.
- 4 K. Hayama, K. Hayashi and K. Toko: *Proc. Biosensors 2002* (Kyoto, Japan, 2002) P3-6.21.
- 5 M. J. Ju, K. Hayama, T. Onodera, K. Hayashi and K. Toko: *Proc. IWIE* (Suwon, Korea, 2002) p. 87.
- 6 M. J. Ju, K. Hayama, K. Hayashi and K. Toko: *Sensors and Actuators B* (in press).
- 7 H. Suzuki, T. Hirakawa, S. Sasaki and I. Karube: *Sensors and Actuators B* **46** (1998) 146.
- 8 M. J. Rodriguez Presa, R. I. Tucceri, M. I. Florit and D. Posadas: *J. Electroanal. Chem.* **502** (2001) 82.
- 9 U. Rammel and G. Reinhard: *Electrochim. Acta.* **35** (1990) 1045.
- 10 M. P. Soriaga and A. T. Hubbard: *J. Am. Chem. Soc.* **104** (1982) 2735.
- 11 I. Bakos, S. Szabo, M. Bartok and E. Kalman: *J. Electroanal. Chem.* **532** (2002) 113.
- 12 M. Heckl, F. M. Marassi, K. M. R. Kallury, D. C. Stone and M. Thompson: *Anal. Chem.* **62** (1990) 32.
- 13 N. Israelachvili: *Intermolecular and Surface Forces*, Second Edition (Academic Press, 1991).