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# Micro Chemical Analysis System Constructed with Stacked Modules for Multi-Analyte Detection

Hiroaki Suzuki, Takumi Taura and Shimpei Kojima

University of Tsukuba, Institute of Materials Science
1-1-1 Tennodai, Tsukuba, Ibaraki 305-8573, Japan
Phone: +81-298-53-5598   Fax: +81 298 55 7440   E-mail: hsuzuki@ims.tsukuba.ac.jp

## 1. Introduction

Measurement of blood gasses and electrolytes is very important in clinical diagnosis. Over the last decade, intensive efforts have been made to construct various micro total analysis systems (μTAS). Realization of miniaturized blood analysis systems will be one of the targets. Here, micro chemical sensors, micro pumps, micro valves, and integrated circuits for signal processing will be integrated. However, the construction of such a sophisticated multifunctional system is not necessarily easy with the present level of technology. This is because the fabrication processes for the respective components are essentially different. A solution to this problem is to construct the system with several modules with different functions. In this study, a miniaturized system was constructed by stacking sensing and reference electrode modules, and its performance was characterized.

## 2. Experimental

### Structure of the modules

In this study, a reference electrode module and sensing modules for gasses ($pO_2$, $pCO_2$, pH) and electrolytes ($pK^+$, $pNa^+$, $pCl^-$) were fabricated. The modules were fabricated on a glass substrate of 15 mm × 15 mm. Gold backbone patterns were used for all elements. A silver cathode for the oxygen electrode, an iridium oxide indicator electrode for the carbon dioxide electrode and the pH combination electrode, and Ag/AgCl electrodes were formed on the backbone layer. The active areas of these electrodes were delineated with a polyimide layer.

The dimensions and structure of all the Ag/AgCl electrodes were the same. A silver layer was formed on the U-shaped gold backbone pattern and was covered with the polyimide protecting layer. Pinholes of 10 μm × 10 μm were formed in the polyimide layer and AgCl layers were grown from the pinholes [1]. A problem in using the thin-film Ag/AgCl electrode in realistic systems is its durability in concentrated KCl solution and long-term storage on the shelf. The structure with the pinholes is our ultimate solution. Two modes of operation can be used for the element. In the first mode, a small constant current (typically 10 nA) is continuously applied. This mode was used for the reference electrode module. In the second mode, AgCl is grown by applying a relatively high current for a short time prior to measurements. In the carbon dioxide and
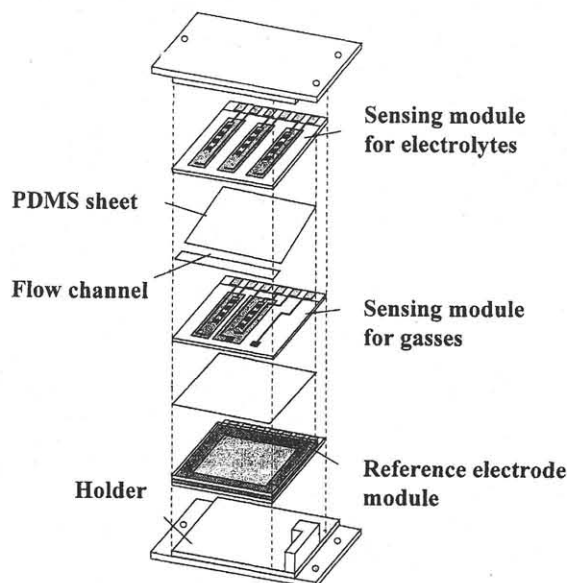


Fig.1 Construction of the system. Decomposed structure showing the respective modules and the holder is shown.

ion-selective electrodes covered with a hydrophobic membrane, it is not possible to reproduce the AgCl layer using an external counter electrode. For these electrodes, a gold counter electrode was additionally formed inside the membranes to use the second mode of operation. This method is advantageous because the small consumption of $Cl^-$ ions will not change the activity of the ions in the internal electrolyte significantly. In KCl solutions of moderate concentration, only a small amount of AgCl will be enough to maintain a stable potential during the time required for the measurement.

Although a liquid-junction reference electrode can be integrated on a sensing module [2], it would be more realistic to separate it as an independent module considering its limited lifetime. Here, six thin-film Ag/AgCl elements were formed along with a gold counter electrode. The container for the electrolyte solution was formed with PDMS and grains of KCl were stuffed there. A liquid junction was formed in one part of the container. Saturated KCl solution was introduced into the container to activate it.

For the electrodes other than the reference electrode module, containers for the internal electrolyte were formed with a thick-film photoresist, SU-8. Internal electrolyte solutions were impregnated in a hydrogel filled in the containers. For the oxygen and carbon dioxide electrodes, a PDMS gas-permeable membrane was applied to the entire area other than the pads. For the ion-selective electrodes, ion-selective membranes were formed only on the sensitive areas, and other areas were partially covered with PDMS. The electrodes were activated by introducing water vapor into the internal electrolyte layer by osmosis.

*Construction of the system*

A miniaturized system to measure the six analytes was constructed with the three modules. PDMS sheets were intercalated between the sensing modules (Fig.1). The space formed with the two sheets and the two modules became a flow channel. The sensitive areas of the respective electrodes were placed there. The reference electrode module and either of the sensing modules were placed back to back. The liquid-junction of the reference electrode module was placed at the downstream of the flow channel. The constructed structure was fixed with the help of PMMA substrates as shown in Fig.1.

## 3. Results and discussion

*Performance of the thin-film Ag/AgCl element and the reference electrode module*

By using the novel Ag/AgCl element with the first mode, its lifetime in saturated KCl solution was expanded by 2000 times compared with the conventional thin-film Ag/AgCl element. Variation of the potential of the reference
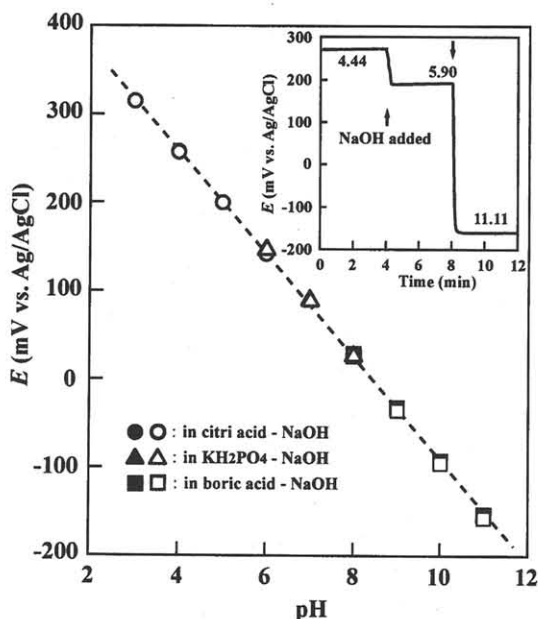


Fig.2 Calibration curves of the pH combination electrode at 37°C. The potentials were measured with respect to the reference electrode module (open symbols) and a macroscopic commercial reference electrode (closed symbols). The inset shows a response curve taken with respect to the reference electrode module.

electrode module accompanied a liquid-junction potential of 2-3 mV. However, the electrode could maintain a potential expected as a Ag/AgCl reference electrode for nearly ten days with the first mode of operation. Concerning the Ag/AgCl element used as the internal reference electrode, the lifetime became longer as the amount of AgCl increased with the second mode of operation. When the layers were grown only for 5 min at 1 μA, the element could maintain an expected stable potential for longer than one day. In both cases, variation of potential at each moment was within ±0.1 mV, which was below the detection limit of our instrument.

*Performance of the sensing modules*

In Fig.2, the calibration curves and the response curve of the pH combination electrode is shown as a representative case. The indicator electrode potential was measured with respect to the reference electrode module and a macroscopic commercial reference electrode. Linear range could be observed in a wide range in both cases. Coincidence of the data obtained with the two types of reference electrodes was excellent. Similar results were obtained with the oxygen and carbon dioxide electrodes and the other ion-selective electrodes.

*Measurement of the analytes in the completed system*

The six analytes in a standard solution could be measured in the flow channel as in a batch-style measurement. In using the constructed module, a possibility will be to use it as an on-line system. A tube with a needle can be attached to one end of the flow channel, and blood samples can be introduced into the system either manually using a syringe or mechanically using an appropriate micro pump. We have already developed such micro pumping mechanisms. The incorporation of the micropumps will be reported in the near future.

## 4. Conclusions

A micro analysis system for multi-analyte detection can easily be constructed with stacked modules. A liquid-junction reference electrode prepared as a discrete module can be used successfully in combination with potentiometric electrodes. This will be a good approach to construct a micro system with many integrated chemical sensors.

**References**
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[2] H. Suzuki, T. Hirakawa, S. Sasaki, and I. Karube, *Anal.Chim.Acta* **405**, 57 (2000)