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# Application of Hydrogen Ion Sensitive Field Effect Transistor to the Kinetic Study of Fast Reaction in Solution

Thoru NAKATSUKA, Michihiro NAKAMURA,† and Takayuki SANO*
Department of Materials Science, Faculty of Science, Hiroshima University, Higashisenda-machi, Hiroshima 730
†Central Research Laboratories, Kuraray Co., Ltd., Kurashiki, Okayama 710
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The ion sensitive field effect transistor(ISFET) developed for pH measurement was incorporated with the stopped flow apparatus for direct detection of the fast change in the hydrogen ion concentration. By the experiments on the dehydration reaction of carbonic acid, it was proved that the pH sensor has a fast response time (<2 ms) beyond the resolution time of stopped flow apparatus, and the constructed apparatus can be satisfactorily applied to the fast reaction kinetics with the sensitivity for the pH change more than 0.01 pH unit.

The stopped flow method is one of most useful methods for study of fast reaction in solution.[1,2] The modes of detection for monitoring the time course of reaction are classified as follows: optical[3,4] (absorption, fluorescence, ORD, CD, etc.), electrochemical[5] (conductivity, electrode detection), thermal,[6] and magnetic resonance[7,8] (ESR, NMR) detections. The choice of the detection mode depends on the information necessary for elucidation of reaction mechanism and on the detectable probe in the reaction system.

Hydrogen ion plays important roles in various kinds of reactions such as not only simple protolysis reactions but also biologically interesting reactions; e.g., proton pump by $H^+$-ATPases in mitochondria, chloroplasts and bacteria in which ATP is synthesized at the expense of a transmembrane proton gradient.[9-11]

On the other hand, the kinetic experiments on the reaction associated with the hydrogen ion have been widely performed using a pH indicator so far.[12] However, an addition of the indicator to reaction system makes its mechanism complicated, and, in some cases, the added indicator inhibits the intended reactions through its direct interaction with reaction species. Hence, the apparatus for direct detection of hydrogen ion with high resolution in both the sensitivity and response time is necessary for the study of the fast reaction in these interesting systems.

A glass electrode can detect directly hydrogen ion concentration. Some applications of the glass electrode to detection of fast reactions have been reported.[13-15] Sirs has applied the glass electrode to the stopped flow apparatus and attained maximum sensitivity of 0.004±0.0005 pH unit using 0.5 ml of solution for each run, but its time resolution is up to half-lives of 0.05 s. Meanwhile, the combination of the glass electrode with the continuous flow method has been tried by Rossi-Bernardi and Berger, and Nakamura, because this method dose not require the fast response of a detector. Rossi-Bernardi and Berger have constructed the apparatus having high resolution in both the reaction rate and sensitivity; the dead time of 3 ms and precision of ±0.005 pH unit, which are superior to those by Nakamura. However, the continuous flow method has disadvantage of requirement of a large quantity of sample solution. Thus, the continuous flow method is not suitable for invaluable samples. Consequently, the pH detector with fast response time was desired to combine with the stopped flow method which requires rather small amount of sample solution.

Recently, a new kind of pH sensor, i.e., ion sensitive field effect transistor (pH-ISFET),[16,17] has been commercially available. The sensor has the characteristics of its small dimensions and faster response time than 0.1 s.[18] In spite of these striking properties of the pH sensor, the application of the sensor to the kinetic study of the fast reaction have not been succeeded in yet. Accordingly, we examined the possibility of a sensor responsive much faster than 0.1 s and constructed a stopped flow apparatus incorporated with that pH sensor.

The present paper describes the applicability of the stopped flow apparatus equipped with a pH-ISFET (ISFET-stopped flow apparatus) to the kinetic study of fast reaction.

## Experimental

The pH sensor (PH-2135, Kuraray Co., Ltd.) used in the present study, which is commercially obtainable as a part of the pH meter (KR-500), is developed for the medical use such as pH monitoring in a blood vessel and stomach. The sensor (diameter, 1.0 mm, 350 mm long) consists of the ISFET (0.15 mm thick, 0.40 mm wide, and 5.5 mm long) integrated with a temperature sensor, reference electrode, and a guard electrode for preventing induced noise, which are packed all together in a catheter. The reference electrode is made of silver-silver chloride. Physiological saline solution retained in the porous hollow fiber is used as a inner solution of the reference electrode.

The stopped flow apparatus (RA-401, Union Giken Co., Ltd.) used is the gas pressure-driven type. The dead time of this apparatus is determined to be 3 ms by test measurement

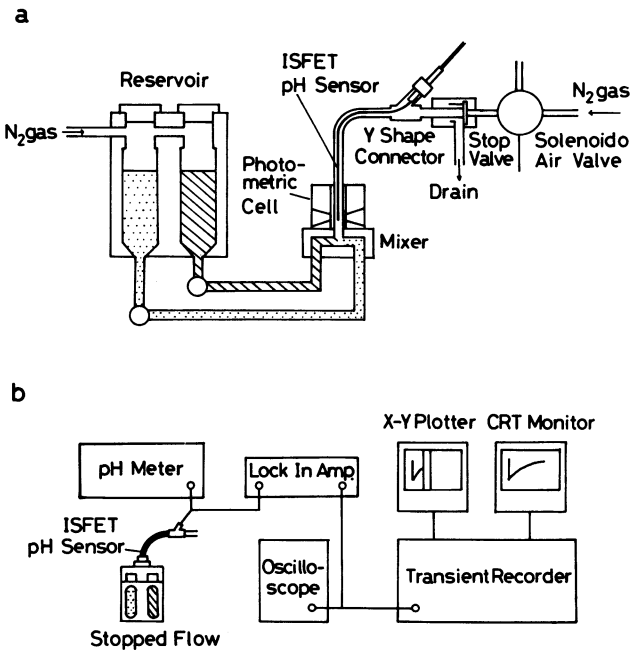


Fig. 1. (a) Combination of the ISFET pH sensor with the stopped flow apparatus. (b) Block diagram of the whole apparatus.

using optical detection. The solution of 0.2 ml is necessary for each run.

The sensor is inserted into the observation cell through the drain tube using the Y shape connector, as shown in Fig. 1(a). Figure 1(b) represents a block diagram of whole apparatus. For the rapid measurement of pH, the signal from the sensor is directly led to a lock-in amplifier and recorded with a transient recorder. A remarkably improved signal to noise ratio was obtained by shielding the equipment and by using a voltage regulator. The maximum sensitivity of the pH sensor was 0.0018 pH unit. For the optical detection experiments, Bromophenol Blue was used as a pH indicator. All experiments were carried out at 25±1 °C.

## Results and Discussion

The response time of the pH sensor combined with stopped flow was examined by detecting pH change after mixing of $NaHCO_3$ and HCl solutions in which the pH change comes from the replacement of the mixed solution in equilibrium with new one prior to the slow reaction. The same measurement was performed with a pH indicator Bromophenol Blue. The rapid pH change detected in these ways is shown in Fig. 2 where the time constants were estimated to be 2 ms for both experiments. As a result, the response time of the sensor was proved to be so faster than 2 ms beyond the dead time of stopped flow apparatus.

Next, the experiments on the dehydration reaction of carbonic acid were carried out to check the validity of the ISFET-stopped flow apparatus. Figure 3 shows the reaction curves observed by two detecting ways:

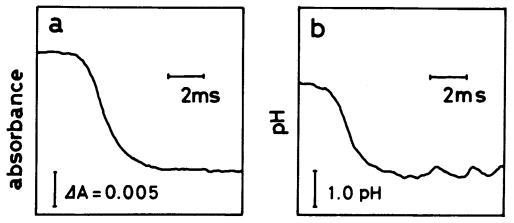


Fig. 2. Measurement of the response time of the pH sensor. A rapid pH change was caused by mixing $2.5\times10^{-3}$ M HCl (1 M=1 mol $dm^{-3}$) and $5.0\times10^{-3}$ M $NaHCO_3$ solution containing Bromophenol Blue (the concentrations are those after mixing) at 25 °C. (a) The rapid pH change detected spectrophotometrically at 435 nm and (b) that by using the pH sensor. The magnitude of absorbance change $\Delta A$ and that of pH change are indicated by the bar in the lower left-hand corner.

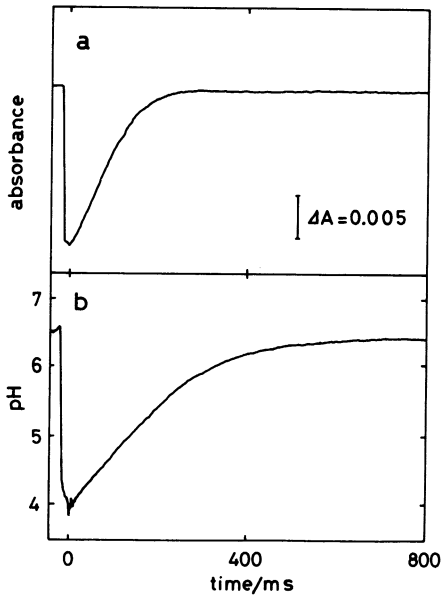


Fig. 3. Reaction curve of dehydration of carbonic acid; (a) by pH indicator and (b) by the pH sensor. The experimental conditions are the same as in Fig. 2.

one is to use the sensor and the other to use Bromophenol Blue as a pH indicator. As can be seen in Fig. 3, their time courses are apparently different. This is because the ordinates of the two curves are different, i.e., the scale for the sensor is pH, while that for the indicator is absorbance. In case of the optical detection, the relaxation time was obtained from the slope of the semilogarithmic plot of the absorbance change against time, as shown in Fig. 4(a). On the other hand, for the case of the pH sensor, the output signal should be converted to hydrogen ion concentration which was plotted in Fig. 4(b). The relaxation times estimated are 17.5±0.5 $s^{-1}$ and 17.0±1.3 $s^{-1}$ for the sensor and indicator, respectively. This good

agreement suggests the pH sensor incorporated with the ISFET-stopped flow apparatus is working correctly. In order to confirm this utility, kinetic experiments were continued under various concentrations.

The dehydration reaction of carbonic acid is represented by next reaction scheme

$$HCO_3^- + H^+ \underset{}{\overset{K_1}{\rightleftharpoons}} H_2CO_3 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} CO_2 + H_2O, \quad (1)$$

where $K_1$ and $K_2$ denote the equilibrium constants of the first and the second steps, and $k_2$ and $k_{-2}$ forward and backward rate constants of the second step, respectively. Since the first step in the above reaction is faster than the second one, the relaxation time $\tau^{-1}$ for the second process can be represented by

$$\tau^{-1} = k_2 \left( \frac{[H^+] + [HCO_3^-]}{K_1 + [H^+] + [HCO_3^-]} + \frac{1}{K_2} \right). \quad (2)$$

According to this equation, the relaxation time obtained was plotted against concentration term which can be calculated by using $K_1$ and $K_2$. By using the values of $1.72\times10^{-4}$ M$^{-1}$ reported for $K_1$[19] and $4.7\times10^2$ assumed for $K_2$, a best straight line passing the origin was obtained as can be seen in Fig. 5. The slope of the straight line gives the value of (18±1) s$^{-1}$ for $k_2$ and the value of $k_{-2}$ was calculated to be $(4.0\pm0.2)\times10^{-2}$ M$^{-1}$ s$^{-1}$ from $K_2$ and $k_2$. As for the dehydration reaction, the values of (13.7—27.9) s$^{-1}$ for $k_2$ and $(3.6—4.3)\times10^{-2}$ M$^{-1}$ s$^{-1}$ for $k_{-2}$ have been reported.[14,20-23] The values estimated in the present work are in good accordance with these reported so far. Consequently, the ISFET-stopped flow apparatus constructed was proved to be nicely working.

As for the comparison of the two data obtained by pH sensor and pH indicator, it should be emphasized that pH indicator has some disadvantages; the change in absorbance of pH indicator can correctly reflect that in the hydrogen ion only for the small change in the concentration. In practice, obvious deviation from the straight line in Fig. 4(a) was recognized. Further, a single pH indicator can be used only at limited pH

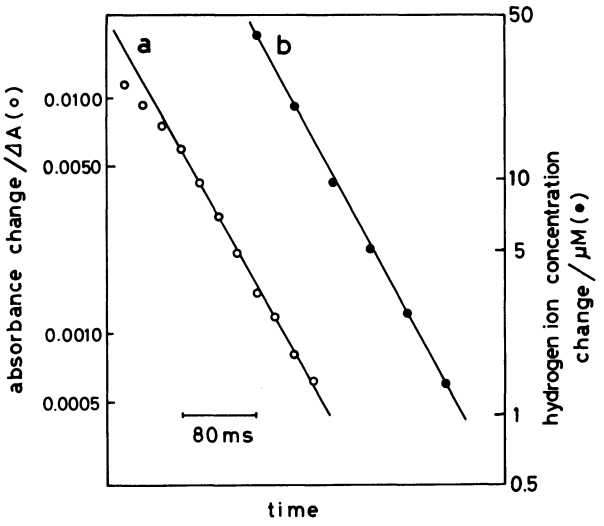


Fig. 4. Semilogarithmic plots of the observed reaction curve against time; (a) absorbance change detected by pH indicator and (b) hydrogen ion concentration change by the pH sensor.

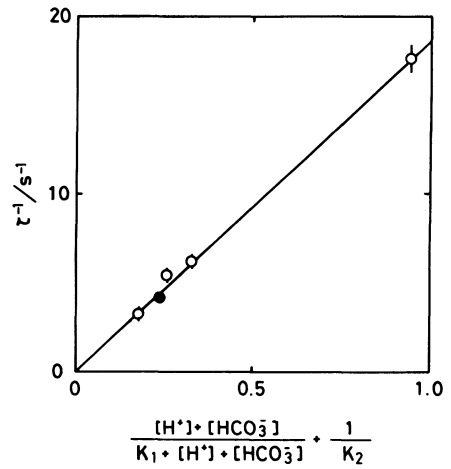


Fig. 5. A plot of $\tau^{-1}$ vs. the concentration term in Eq. 2. (●) denotes the data at ionic strength of 0.1 M.

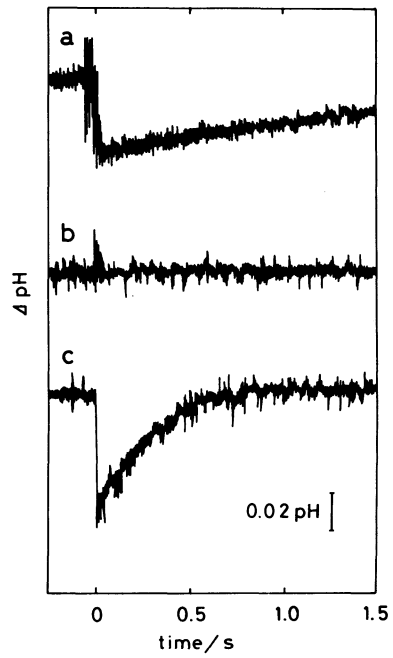


Fig. 6. Measurement of small pH change. (a) The artifact arisen from the flow of the $10^{-4}$ M NaCl solution. (b) Elimination of the artifact by the flow of 0.1 M NaCl solution. (c) Reaction curve of dehydration of carbonic acid at ionic strength of 0.1 NaCl; $1\times10^{-4}$ M HCl and $5\times10^{-5}$ M $NaHCO_3$ solution were mixed.

range. Meanwhile, the single pH sensor can be satisfactorily used for the kinetic study in solution over wide range of pH value 1—13.

In general, the pH measurement using an electrode is affected by the ionic strength of solution. This effect comes from the distortion and restoration of the ionic atmosphere around the tip of the reference electrode, and brings about the slowing down of its response time and the instability of the pH value. In the case of the pH sensor, such effect was observed as an artifact in the stopped flow experiments as shown in Fig. 6(a). However, as shown in Fig. 6(b), the artifact can be eliminated by holding the ionic strength at 0.1 M, and then the reaction curve could be nicely detected even for small change of 0.04 in pH unit as in Fig. 6(c); the relaxation time obtained from this data is also reasonably on the line in Fig. 5. In addition, most kinetic studies, especially for biological system, have been carried out at relatively high ionic strength around 0.1 M, therefore, the artifact must be not so serious problem.

In the present work, the pH-ISFET was, for the first time, combined with the stopped flow apparatus, and proved to be useful for the kinetic studies of the fast reaction up to 3 ms associated with the large change as well as small change in pH. Further, the present results may suggest the similar applicability of the ISFET developed for other ion to the kinetic investigation of fast reactions associated with biologically interesting ions such as $Ca^{2+}$, $Mg^{2+}$, $K^{+}$, and $Na^{+}$.

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