Determination of acid dissociation constants by feedback-based flow ratiometry constituted of ramp and hold modes

Hideji Tanaka[1,*] and Arata Shogenji[2]

[1] Institute of Health Biosciences and [2] Faculty of Pharmaceutical Sciences,
Tokushima University, Shomachi, Tokushima 770-8505, Japan

Abstract

A feedback-based flow ratiometry constituted of two operation modes, ramp and hold modes, is proposed for the determination of acid dissociation constants K_a. The former mode is used for the determination of the equivalence point EP, while the latter is used for the measurement of pH at the half equivalence point (the midpoint of the titration) $EP_{1/2}$. The two modes, which are put into operation alternately, are controlled through an advanced algorithm that saves time for the measurement. A titrant (e.g., HCl) is delivered at the flow rate of F_R, which is varied in response to a control voltage V_{c1} generated from a computer. A titrand (e.g., NH_3) is aspirated and merged with the titrant at the flow rate of $F_T - F_R$, where F_T is the total flow rate. Downstream, pH of the mixed solution is monitored with a flow through glass electrode. In a ramp mode, V_{c1}, and thus F_R are increased or decreased linearly. At the instant EP is sensed, V_{c1} that gives the equivalence composition (V_E) is computed by taking into account the lag time between the merging of solutions and their being sensed with the sensor. Then, in a hold mode, the flow rate ratio of titrant/titrand is held at the $EP_{1/2}$ level, which is estimated from the value of V_E. The pH corresponding to $EP_{1/2}$ is used for the determination of pK_a after the activity correction. Next ramp mode starts from the terminal V_{c1} value in the previous ramp mode to a reverse direction (e.g., downward direction if the previous ramp is to upward direction) without waiting for the completion of acquiring invariable pH data. At the instant EP is sensed, the hold mode starts again. By repeating these processes automatically, pK_a can be determined efficiently (19 ~ 38 s per determination) with reasonable precision (R.S.D. = ca. 0.3 %).

Keywords Flow ratiometry; Flow titration; Feedback-based control; Dissociation constant; Acidity constant

1. Introduction

Acid dissociation constant K_a is one of the most important constants for studying the physicochemical and/or biochemical functions of molecules. Especially, in biological system, the conditions of matrix around a molecule may readily varied and easily affect the behavior of the molecule. It is, therefore, important to develop a rapid method for estimating K_a in varying conditions. Several methods based on flow injection analysis (FIA) [1-5] and related techniques [6-8] have been studied for this purpose.

In the previous papers [9,10], we proposed a new method for the determination of K_a based on a feedback-based flow ratiometry [11,12]. The half equivalence point $EP_{1/2}$ was directly determined by rapid upward and downward scans of titrant/titrand flow rate ratio. The method was applied to low molecular weight acids whose pK_a values are ranging from 2.1 to 10.3. In the earlier work of them [9], the system was very susceptible to detector noise (R.S.D. of pK_a was ca. 3%), although high throughput was attainable (26 s per determination). Improvement in the configuration of flow system and the noise filtration technique increased the reliability of the system (R.S.D. = ca. 1%), but was incompatible with high throughput (56 s per determination) [10].

In the more recent paper [13], we proposed an alternative approach for K_a determination. The method was based on the detection of the equivalence point EP by a feedback-based flow ratiometry and the succeeding measurement of pH at $EP_{1/2}$. The determination of EP is less affected by the noise than that of $EP_{1/2}$, because the pH-jump around EP is large compared to the noise. The precision was, therefore, improved by an order of magnitude (R.S.D. < 0.3 %). The method, however, still needed long time for the determination (53 s per determination).

In the present study, therefore, this method based on the EP detection is further investigated in order to improve the efficiency of the measurement by improving both the algorithm for operating the system and the manifold.

2. Experimental

2.1 Flow system

Figure 1 shows the flow system. Two peristaltic pumps (P_1 and P_2; Rainin Dynamax RP-1) were used for delivering solutions. Titrant (R; 0.1 mol dm^{-3} HCl or NaOH) and titrand (S; typically 0.2 mol dm^{-3} base or acid solution) were continuously helium-degassed in order to prevent the influence of CO_2 from ambient air. No supporting electrolyte was added to the solutions. The experimental environment was air-conditioned at 25°C, but beyond this the experimental temperature was not controlled. The pump tubes used were 0.51 mm i.d. Pharmed tubing. Control voltages of V_{c1} and V_{c2} (0 ~ 5 V) were used to control the rotation speed of P_1 and P_2 pumps, respectively. Both the voltages were generated from a computer (PC; a Toshiba Dynabook Satellite 1800 SA95C/4) through an A/D-D/A converter (Measurement Computing PC-CARD-DAS16/12-AO).
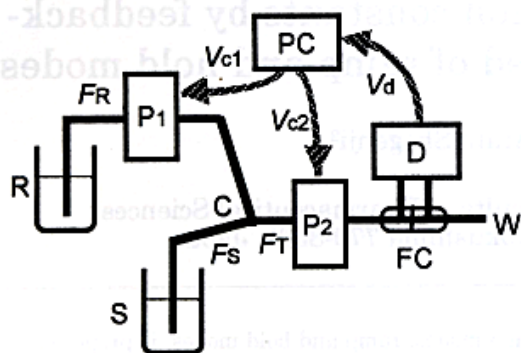
Fig. 1 Schematic diagram of flow system. S: titrand (typically 0.2 mol dm^{-3}); R: titrant (0.1 mol dm^{-3} HCl or NaOH); P$_1$ and P$_2$: peristaltic pump; C: polypropylene T-union; FC: flow-through glass electrode equipped with a reference electrode; D: pH meter; PC: computer; W: waste; F_S, F_R and F_T: flow rate of titrand, titrant, and mixed solution, respectively; V_{c1} and V_{c2}: output voltages for controlling P$_1$ and P$_2$ pumps, respectively; V_d: detector output voltage.

A titrant, delivered by using P$_1$ pump at the flow rate of F_R, was merged with a titrand that was aspirated to the confluence point (C: polypropylene T-union) at the flow rate of $F_T - F_R$, where F_T means the total flow rate. The mixed solution was led to a Horiba 6941-15C flow-through glass electrode (FC) equipped with a Horiba 2461A-15T reference electrode. The electrodes were calibrated daily before the measurements with commercial standard pH buffers in flow mode. Output signal (V_d) from a Horiba F-13 pH meter (D; -0.7 ~ +0.7 V for pH 0 ~ 14) was acquired in the computer through the converter as Microsoft Excel format at the frequency of 20 Hz. A program written in Visual BASIC in-house was used to generate V_{c1} and V_{c2}, acquire V_d, analyze them and graphically display the results automatically.

2.2 Reagents

Reagents used in the present study were analytical reagent grade purchased from Kanto Chemicals (Tokyo, Japan), Nacalai Tesque (Kyoto, Japan) or Wako Pure Industries (Osaka, Japan) and used without further purification. Milli-Q SP grade deionized water was used throughout. Titrant (0.1 mol dm^{-3} HCl and NaOH) was each prepared by diluting commercial 2 mol dm^{-3} HCl or NaOH standard solution (Kanto Chemicals) with deionized water.

3. Principle

3.1 Algorithm for operating the system

The newly devised algorithm is consistited of two operation modes: ramp and hold modes. The former is used for determining the V_{c1} (V_E) that gives the solution composition corresponding to the equivalence

point (EP) at the confluence point. The latter is used for measuring the pH at the half equivalence point (EP$_{1/2}$), which is estimated from the value of V_E. Two kinds of approaches (Approachs 1 and 2) were examined for the hold mode. The principle of the two approaches is shown in Figs. 2 and 3, respectively, by using real data, where 0.1 mol dm^{-3} NH$_3$ was titrated with 0.1 mol dm^{-3} of HCl.

Initially, the controller output voltage V_{c1}, and thus the HCl flow rate F_R increase linearly, while V_{c2} that controls the total flow rate F_T is held constant. The value of V_{c1} changes the composition of the merged solutions at the confluence point. The pH of the mixed solution being detected decreases after a delay. This time lag t_{lag} consists primarily of the physical transit time of the merged solutions to reach the flow cell and minutely of the response time of the electrode. In the present configuration, the physical transit time is constant as long as V_{c2} is held constant. At the instant the detector senses the equivalence point EP, where the slope of the pH curve ($|dpH/dt|$) becomes maximum (a in Figs. 2 and 3), the ramp direction of V_{c1} is reversed to downward. The V_{c1} at this inflection point constitutes V_H. The pH of the mixed solution continues to decrease because of t_{lag}, and then increases after reaching a minimum. The EP is sensed again in the course of this downward scan (b in Figs. 2 and 3). The V_{c1} at this point constitutes V_L. In the previous system [13], F_T and thus t_{lag} were varied with titant flow rate. On the other hand, in the present system, the influence of t_{lag} can be offset simply by averaging the most recent V_H and V_L [11,12], assuming that the electrode response time is constant. The V_E can, therefore, be expressed as follows.

$$V_E = \frac{V_H + V_L}{2} \qquad (1)$$

This ramp mode is succeeded by the hold mode. The titrant/titrand flow ratio is fixed at the EP$_{1/2}$ level (described in detail in the section 3.2) for bare minimum period for obtaining an invariable pH corresponding to EP$_{1/2}$. In one approach (Approach 1), V_{c1} is changed to the level V_{c1}* corresponding to EP$_{1/2}$ and held there under invariant V_{c2}, as shown in Fig. 2. In the other approch (Approach 2), V_{c2} and thus the total flow rate are changed to the voltage V_{c2}* that corresponds to EP$_{1/2}$, while V_{c1} is fixed at V_E, as shown in Fig. 3. The first 13 ~ 15 s (depending on the chemical species and titration step (1st. or 2nd.)) is the waiting period to ensure that pH has been reached the stable pH$_{1/2}$ corresponding to EP$_{1/2}$ and the next 1 s is then devoted to signal integration and data acquisition. The pH$_{1/2}$ thus obtained is used for calculating the pK_a of analyte.

The next ramp mode is started from V_L (c in Figs. 2 and 3) to upward direction, without waiting for the completion of the data processing for pH$_{1/2}$. This scheme contributes to the improvement in the throughput rate of the measurement. When the second V_H is located, the hold mode is applied again to obtain the value of pH$_{1/2}$ in the same way. The third ramp mode is started not from V_L but from V_H (d in Figs. 2 and 3), in this case, to downward direction.
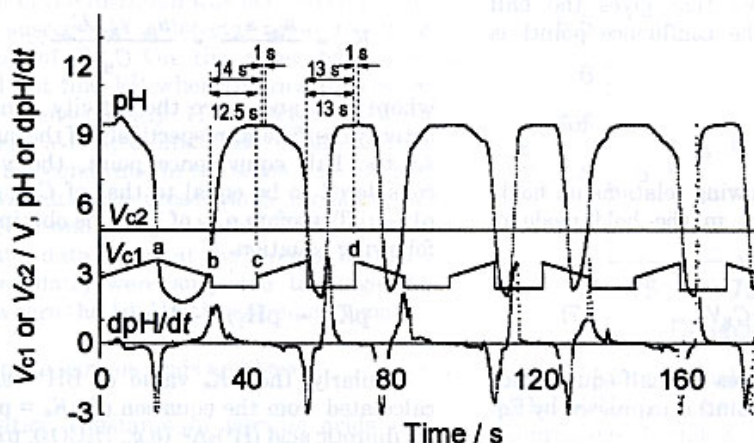
Fig. 2 The principle of Approach 1 for the pK_a determination by a flow ratiometry constituted of ramp and hold modes. The real experimental results for the titration of 0.1 mol dm^{-3} NH$_3$ with 0.1 mol dm^{-3} HCl are shown here. Scan rate of V_{c1} is 50 mV s^{-1}. V_{c2} was set at 4.8 V corresponding to F_T of ca. 2.2 cm^3 min^{-1}.
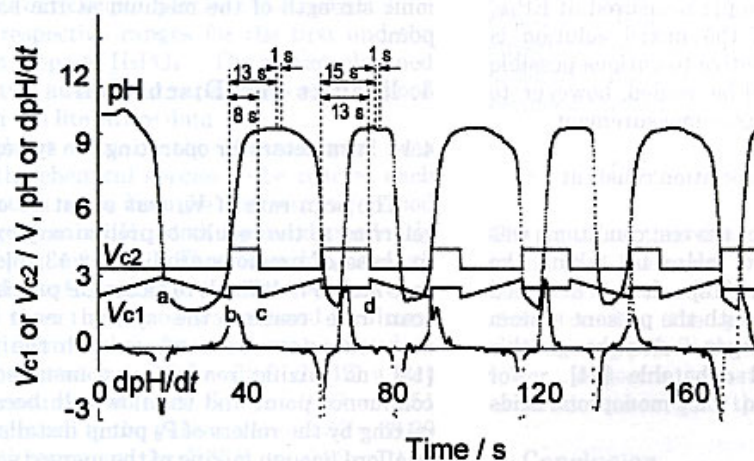

Fig. 3 The principle of Approach 2 for the pK_a determination by a flow ratiometry constituted of ramp and hold modes. The real experimental results for the titration of 0.1 mol dm^{-3} NH$_3$ with 0.1 mol dm^{-3} HCl are shown here. Scan rate of V_{c1} is 45 mV s^{-1}. Initial V_{c2} was set at 3.36 V corresponding to F_T of ca. 1.54 cm^3 min^{-1}.

These processes are repeated over automatically.

3.2 Estimation of the half equivalence point

Generally, when a titrand (concentration: C_S) reacts with a titrant (concentration: C_R) in a flow system, the following relationship holds for the n-th equivalence point.

$$nC_S F_S = C_R F_R \qquad (2)$$

In the present configuration, F_S corresponds to the difference of F_T and F_R, as expressed by Eq. 3.

$$F_S = F_T - F_R \qquad (3)$$

Assuming that F_R and F_T are proportional to the respective contol voltage completely in the same manner (i.e., $F_R = k_1 V_{c1}$, $F_T = k_2 V_{c2}$, and $k_1 = k_2 = \text{const}$) because the same model of pump and pump tubing were used for P$_1$ and P$_2$, Eq. 2 is rewritten for the n-th equivalence point.

$$nC_S(V_{c2} - V_E) = C_R V_E \qquad (4)$$

In the hold mode of Approach 1, the following relationship holds for the half equivalence point EP$_{1/2}$.

$$nC_S(V_{c2} - V_{c1}^{\bullet}) = \frac{2n}{2n-1} C_R V_{c1}^{\bullet} \qquad (5)$$

From Eqs. 4 and 5, $V_{c1}{}^*$ (V_{c1} that gives the half equivalence composition at the confluence point) is expressed by Eq. 6.

$$V_{c1}{}^* = \frac{(2n-1)V_{c2}V_E}{2nV_{c2} - V_E} \qquad (6)$$

On the other hand, the following relationship holds for the half equivalence point in the hold mode of Approach 2.

$$nC_S(V_{c2}{}^* - V_E) = \frac{2n}{2n-1}C_R V_E \qquad (7)$$

Therefore, $V_{c2}{}^*$ (the V_{c2} that gives the half equivalence composition at the confluence point) is expressed by Eq. 8.

$$V_{c2}{}^* = \frac{2nV_{c2} - V_E}{2n-1} \qquad (8)$$

Thus computed $V_{c1}{}^*$ and $V_{c2}{}^*$ were used to keep the solution at the hald equivalence point in the hold mode of the Approaches 1 and 2, respectively.

The influence of the error arising from the flow rate seemed to be marginal because pH measured at $EP_{1/2}$, where the buffer capacity of the mixed solution is maximal, was relatively insensitive to various possible sources of error [13]. It would be needed, however, to calibrate the pumps for more strict measurement.

3.3 Determination of acid dissociation constant

The pK_a of the analyte under the test conditions was determined from the mean of $pH_{1/2}$ by taking the activity correction into account [13]. It was assumed that the pH values measured with the present system reflected the activity of hydrogen ion, although this ordinary assumption is still debatable [14]. For example, K_a is expressed by Eq. 9 for monoprotic acids of H^+A^- (e.g., CH_3COOH).

$$K_a = \frac{a_{H^+}\,a_{A^-}}{a_{HA}} = \frac{a_{H^+}\,\gamma_{A^-}\,C_{A^-}}{C_{HA}}, \qquad (9)$$

where a, C and γ are the activity, concentration and activity coefficient, respectively, of the subscript species. At the half equivalence point, the value of C_{A^-} is considered to be equal to that of C_{HA}, and $-\log a_{H^+} = pH_{1/2}$. Therefore pK_a of H^+A^- is obtained through the following equation.

$$pK_a = pH_{1/2} - \log \gamma_{A^-} \qquad (10)$$

Similarly, the pK_a value of BH^+ (e.g., NH^{4+}) was calculated from the equation of $pK_a = pH_{1/2} + \log \gamma_{BH^+}$; for diprotic acid $(H^+)_2A^{2-}$ (e.g., H_2CO_3), $pK_{a1} = pH_{1/2} - \log \gamma_{HA^-}$ and $pK_{a2} = pH_{1/2} - \log \gamma_{A^{2-}} + \log \gamma_{HA^-}$. Activity coefficient of the ion is calculated according to the Davies equation [15]:

$$\log \gamma_i = -0.51 z_i{}^2 \left(\frac{\sqrt{I}}{1 + \sqrt{I}} - 0.3 I \right) \qquad (11)$$

where z_i is the charge magnitude of the ion and I is the ionic strength of the medium at the half equavalence point.

4. Results and Discussion

4.1 Parameters for operating the system

The scan rate of V_{c1} was set at 45 or 50 mV s^{-1} by referring to the results of preliminary experiments and to those of previous studies [12,13,16]: too high scan rate makes it difficult to locate EP precisely and too low scan rate renders the system more susceptible to detector noise. In contrast with the previous study [13], no mixing reactor was installed between the confluence point and the flow cell, because the active mixing by the rollers of P2 pump installed there seemed to afford enough mixing of the merged solutions [16,17]. A moving average filter was used for the noise filtration.

Table 1 Dissociation constant of acids determined by the proposed method

Analyte	Titrant	Approach 1		Approach 2		Literature values
		pK_a	S.D. (n)	pK_a	S.D. (n)	
Formic Acid (HCOOH)	NaOH	3.75$_4$	0.01$_7$ (16)	3.75$_4$	0.00$_6$ (14)	3.75 [18,19]
Acetic Acid (CH$_3$COOH)	NaOH	4.76$_3$	0.00$_5$ (7)	4.75$_3$	0.01$_1$ (12)	4.756 [18], 4.76 [19]
Propanoic acid (C$_2$H$_5$COOH)	NaOH	4.89$_9$	0.01$_1$ (9)	4.86$_8$	0.01$_4$ (6)	4.87 [18,20], 4.88 [19]
n-Butanoic acid (C$_3$H$_7$COOH)	NaOH	4.83$_5$	0.01$_2$ (15)	4.83$_0$	0.01$_9$ (13)	4.82 [19], 4.83 [18]
n-Pentanoic acid (C$_4$H$_9$COOH)	NaOH	4.89$_8$	0.01$_3$ (16)	4.86$_4$	0.01$_8$ (18)	4.83 [18]
Phenol (C$_6$H$_5$OH)	NaOH	9.93$_2$	0.00$_9$ (18)	10.10$_0$	0.03$_0$ (8)	9.99 [18], 10.00 [20]
Phosphoric acid (H$_3$PO$_4$), pK_{a1}	NaOH	2.12$_8$	0.11$_8$ (17)	2.13$_7$	0.00$_2$ (9)	2.16 [18]
pK_{a2}	NaOH	7.20$_7$	0.15$_5$ (8)	7.21$_4$	0.07$_8$ (8)	7.21 [18]
Oxalic acid ((COOH)$_2$), pK_{a2}	NaOH	3.92$_9$	0.07$_9$ (11)	3.91$_1$	0.05$_9$ (11)	3.81 [18], 4.28 [20]
Ammonium ion (NH$_4^+$)	HCl	9.19$_9$	0.02$_4$ (27)	9.18$_7$	0.01$_8$ (21)	9.25 [18]
Carbonic acid (H$_2$CO$_3$) pK_{a1}	HCl	6.28$_9$	0.03$_2$ (14)	6.31$_7$	0.05$_0$ (11)	6.35 [18]
pK_{a2}	HCl	10.24$_7$	0.03$_7$ (16)	10.24$_1$	0.01$_3$ (6)	10.33 [18]

Concentration of titrant: 0.1 mol dm^{-3}.

When the extent of the filtration was not sufficient, the system became susceptible to detector noise, resulting in false location of EP. On the other hand, the algorithm could not find EP when the raw data were over-smoothed. Consequently, 11 consecutive raw V_d (pH) values were averaged and thus obtained data were processed a second time in the same way. When thus obtained V_d came in a preset range including EP (pH 3 ~ 7 for NH_3 and pH 6 ~ 10 for CH_3COOH, for example), recent 4 data points at the interval of 0.15 s (i.e., every third data) were subjected to judge the location of EP, where the $|dpH/dt|$ became maximal.

4.2 Applications to various acids and bases

The dissociation constants of various acids and bases determined by the proposed method are listed in Table 1 along with the literature values [18-20]. In the case of H_2CO_3 and NH_4^+, their conjugate bases, CO_3^{2-} (taken as Na_2CO_3) and NH_3, were titrated with 0.1 mol dm^{-3} HCl. For H_3PO_4 and H_2CO_3, the first or second dissociation constants were independently determined by setting the pH range for the EP judgment in order to confine the system to the desired titration step. For example, pH 3.0 ~ 6.5 and pH 7.8 ~ 11.8 were the respective ranges for the first and the second titration steps of H_3PO_4. The values obtained by the present two automated approaches were in good agreement with the literature data.

The throughput rate of the measurement was dependent on the chemical species to be reacted each other and titration step, because the waiting period before the integration of pH$_{1/2}$ data were independently optimized for each reaction. That is, a long enough waiting period was initially set as a software parameter and then the period was refined to almost the bare minimum value by analyzing the data obtained. For Approach 1, 19.5 ~ 32.0 (25.42) s was needed per measurement at R.S.D. of 0.09 ~ 1.51 (0.29) %; for Approach 2, the values were 22.33 ~ 38.00 (28.89) s per titration at the R.S.D. of 0.09 ~ 2.01 (0.26) %, where the values in the parentheses mean the median of the each data.

4.3 Effect of dielectric constant

The applicability of the present system to follow the pK_a along with various dielectric constant (ε) was tested by adding an organic solvent to both the titrant and titrant. Acetonitrile (MeCN) was selected for this purpose because the solvent freely mixed with water and the ε and pH of the mixed solvent system have been studied extensively [21,22]. The electrode was pre-calibrated with aqueous standard pH buffers. The pH in the mixed solvent system (^s_wpH) measured by using the electrode was corrected by taking into account the mole fraction of MeCN, X_{MeCN}, according to Eq. 12 obtained experimentally by Espinosa et al. [21].

$$^s_spH = {}^s_wpH + 3.81 X_{MeOH}^2 \qquad (12)$$

where ^s_spH means the pH in an MeCN-H_2O medium, referred to the standard pH buffers containing MeCN

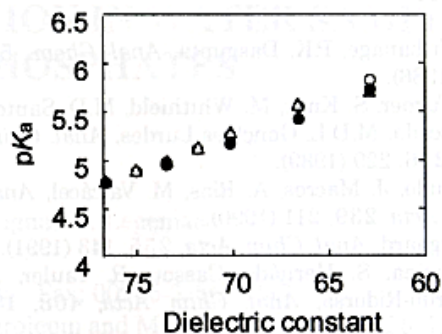Fig. 4 Effect of dielectric constant on the pK_a of CH_3COOH. ○ and △: pK_a obtained through Approaches 1 and 2, respectively. ●: literature data.

at the same X_{MeCN} as in the sample. The ^s_wpH at EP$_{1/2}$ was corrected in this way. The pK_a was estimated by using pH$_{1/2}$ thus corrected. The obtained values are plotted as a function of ε in Fig. 5 by the open circles (Approach 1) and open triangles (Approach 2). The closed circles are the literature values [21,22]. The dielectric constant ε of the MeCN−H_2O media were estimated from the Eq. 13 [21].

$$\varepsilon^{-1} = 0.01547 X_{MeCN} + 0.01305 \qquad (13)$$

The values obtained in the present study agreed well with the literature values; the pK_a values for CH_3COOH increased with the decrease of ε. From these results, the present system is capable of determining pK_a in the mixed solvent with matrices of lower dielectric constant, at least within the experimental range of the present study.

5. Conclusion

The proposed feedback-based flow ratiometry constituted of two operation modes, ramp and hold modes, greatly improved the throughput rate of the measurement by a factor of about 2 compared with the previous method [13]. The system made it possible to measure pK_a at the efficiency around 25 s per determination with reasonable precision (R.S.D. = ca. 0.3%). As for the hold mode, two kinds of approaches were examined for measuring the pH at EP$_{1/2}$. There was little difference in the quality of data between the two approaches. However, Approach 1 (the total flow rate F_T is invariable irrespective of the mode) seems superior to the Approach 2 (F_T in the hold mode is higher than that in the ramp mode) with regard to the efficiency. That is, the latter approach needs to set V_{c2} at relatively lower value in the ramp mode by taking the margin for its raise in the subsequent hold mode into account. This makes the lag time t_{lag} longer, resulting in the decrease of the efficiency of the measurement. In contrast, such the consideration is not necessary in Approach 1, and higher value of V_{c2} (~5 V) can be used throughout the measurement.

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