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

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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 $E_P$, while the latter is used for the measurement of $pH$ at the half equivalence point. 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 $F_h$, which is varied in response to a control voltage $V_h$ generated from a computer. A titrant (e.g., NaOH) is aspirated and merged with the titrant at the flow rate of $F_l - F_h$, where $F_l$ is the total flow rate. Downstream, $pH$ of the mixed solution is monitored with a flow through glass electrode. In a ramp mode, $V_h$, and thus $F_h$ are increased or decreased linearly. At the instant $E_P$ is sensed, $V_h$ that gives the equivalence composition ($V_{eq}$) 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/titrant is held at the $E_{P_{eq}}$ level, which is estimated from the value of $V_{eq}$. The $pH$ corresponding to $E_{P_{eq}}$ is used for the determination of $pK_a$ after the activity correction. Next ramp mode starts from the terminal $V_{eq}$ 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 $E_P$ 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 $E_{P_{eq}}$ was directly determined by rapid upward and downward scans of titrant/titrant 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 (66 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 $E_P$ by a feedback-based flow ratiometry and the succeeding measurement of $pH$ at $E_{P_{eq}}$. The determination of $E_P$ is less affected by the noise than that of $E_{P_{eq}}$ because the pH-jump around $E_P$ 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 $E_P$ 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. Beyond this the experimental temperature was not controlled. The pump tubes used were 0.51 mm i.d. Pharmohex tubing. Control voltages of $V_h$ and $V_{eq}$ (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 SA86C/4) through an A/D-D/A converter (Measurement Computing PC-CARD-DAS16/12-AC).
A titrant, delivered by using $P_1$ pump at the flow rate of $Fr$, was merged with a titrant that was aspirated to the confluence point (C: polypropylene T-junction) at the flow rate of $Ft - Fr$, where $Ft$ means the total flow rate. The mixed solution was led to a Horiba 5941-16C flow-through glass electrode (PC) equipped with a Horiba 2401A-16T reference electrode. The electrodes were calibrated daily before the measurements with commercial standard pH buffers in flow mode. Output signal ($V_o$) from a Horiba B-13 pH meter (D): 0.7 - 10.7 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 $V_o$ and 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 consisted of two operation modes: ramp and hold modes. The former is used for determining the $V_o$ that gives the solution composition corresponding to the equivalence point (CP) at the confluence point. The latter is used for measuring the pH at the half equivalence point (EPₕₜ), which is estimated from the value of $V_o$. Two kinds of approaches (Approach 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 NH₃ was titrated with 0.1 mol dm⁻³ of HCl.

Initially, the controller output voltage $V_o$ and thus the HCl flow rate $Fr$ increase linearly, while $V_o$ that controls the total flow rate $Ft$ is held constant. The value of $V_o$ 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 Allows 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 $V_o$ is held constant. At the instant the detector senses the equivalence point EPₕₜ where the slope of the pH curve (1dpH/dt) becomes maximum (a in Figs. 2 and 3), the ramp direction of $V_o$ is reversed to downward. The $V_o$ at this instant point constitutes $V_{EP}$. The pH of the mixed solution continues to decrease because of hisp, and then increases after reaching a minimum. The $V_o$ is sensed again in the course of this downward scan (b in Figs. 2 and 3). The $V_o$ at this point constitutes $V_{EP}$. In the previous system [13], $Fr$ and thus $t_{MEP}$ were varied with titrant flow rate. On the other hand, in the present system, the influence of $t_{MEP}$ can be offset simply by averaging the most recent $V_{EP}$ and $V_{EP}$ [11, 12], assuming that the electrode response time is constant. The $V_{EP}$ can, therefore, be expressed as follows.

$$V_{EP} = \frac{V_{EP}^* + V_{EP}^*}{2}$$

This ramp mode is succeeded by the hold mode. The titrant/titrant flow ratio is fixed at the EPₕₜ level (described in detail in the section 3.2) for bare minimum period for obtaining an invariable pH corresponding to EPₕₜ. In one approach (Approach 1), $V_o$ is changed to the level $V_{EP}^*$ corresponding to EPₜ and held there under invariant $V_o$, as shown in Fig. 2. In the other approach (Approach 2), $V_o$ and thus the total flow rate are changed to the voltage $V_{EP}^*$ that corresponds to EPₜ while $V_o$ is fixed at $V_{EP}$, as shown in Fig. 3. The first 13 - 16 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 pHₜ corresponding to EPₜ and the next 1 s is then devoted to signal integration and data acquisition. The pHₜ is thus obtained by using the $pH_{EP}$ of analyte.

The next ramp mode is started from $V_o$ (c in Figs. 2 and 3) to upward direction, without waiting for the completion of the data processing for $pH_{EP}$. This scheme contributes to the improvement in the throughput rate of the measurement. When the second $V_o$ is located, the hold mode is applied again to obtain the value of $pH_{EP}$ in the same way. The third ramp mode is started not from $V_o$, but from $V_o$ (d in Figs. 2 and 3), in this case, to downward direction.
These processes are repeated over automatically.

3.2 Estimation of the half equivalence point

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

$$nC_tF_{a} = C_hF_{r}$$  \hspace{1cm} (2)

In the present configuration, $F_h$ corresponds to the difference of $F_r$ and $F_{sh}$ as expressed by Eq. 3.

$$F_h = F_r - F_{sh}$$  \hspace{1cm} (3)

Assuming that $F_h$ and $F_r$ are proportional to the respective control voltage completely in the same manner (i.e., $F_h = k_hV_h$, $F_r = k_rV_r$, and $k_h = k_r = \text{const}$) because the same model of pump and pump tubing were used for $P_h$ and $P_r$, Eq. 2 is rewritten for the $n$-th equivalence point.

$$nC_t(V_{sh} - V_{a}) - C_hV_h$$  \hspace{1cm} (4)

In the hold mode of Approach 1, the following relationship holds for the half equivalence point $EP_{1a}$.

$$nC_t(V_{a} - V_{a'}) = \frac{2n}{2n-1}C_tV_{a'}$$  \hspace{1cm} (5)
From Eqs. 4 and 6, $V_{el}$ (the half equivalence composition at the confluence point) is expressed by Eq. 6.

$$V_{el}^* = \frac{(2n - 1)V_{el} - V_E}{2n - 1 - 1}$$  \hspace{1cm} (6)

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

$$nC_3(V_{el}^* - V_E) = \frac{2n - 1}{2n - 1}$$  \hspace{1cm} (7)

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

$$V_{el}^* = \frac{2nV_{el} - V_E}{2n - 1}$$  \hspace{1cm} (8)

Thus computed $V_{el}$ and $V_{el}^*$ were used to keep the solution at the half 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 $E_{ME}$, 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 $pH_{ME}$ 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 $H^+\cdot$ (e.g., CH$_3$COOH).

$$K_a = \frac{a_{H^+} a_{A^-}}{a_{HA}} = \frac{\alpha_{H^+} \gamma_{A^-} C_{HA}}{C_{HA}}$$  \hspace{1cm} (9)

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

$$pK_a = p\text{H}_{H^+} - \log \gamma_{A^-}$$  \hspace{1cm} (10)

Similarly, the $pK_a$ value of $BH^+$ (e.g., NH$_3^+$) was calculated from the equation of $pK_a = p\text{H}_{H^+} + \log \gamma_{BH^+}$ for diprotic acid (H$_3$A$^+$) (e.g., H$_2$CO$_3$), $pK_{A1} = p\text{H}_{H^+} - \log \gamma_{A_{1}}$ and $pV_{A1} = p\text{H}_{H^+} - \log \gamma_{A_{1}} + \log \gamma_{A_{2}}$. Activity coefficient of the ion is calculated according to the Davies equation [16]:

$$\log \gamma = -0.51 \frac{1}{1 + 1/(1 + 0.3 \lambda)}$$  \hspace{1cm} (11)

where $n$ is the charge magnitude of the ion and $I$ is the ionic strength of the medium at the half equivalence point.

4. Results and Discussion

4.1 Parameters for operating the system

The scan rate of $V_1$ 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 EF 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 the 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

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Titrant</th>
<th>Approach 1</th>
<th>Approach 2</th>
<th>Literature values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$pK_a$</td>
<td>S.D. (mol)</td>
<td>$pK_a$</td>
<td>S.D. (mol)</td>
</tr>
<tr>
<td>Formic acid (HCOOH)</td>
<td>NaOH</td>
<td>3.754</td>
<td>0.017 (16)</td>
<td>3.754</td>
</tr>
<tr>
<td>Acetic acid (CH$_3$COOH)</td>
<td>NaOH</td>
<td>4.765</td>
<td>0.005 (7)</td>
<td>4.765</td>
</tr>
<tr>
<td>Pyruvic acid (C$_3$H$_4$COOH)</td>
<td>NaOH</td>
<td>4.895</td>
<td>0.01 (9)</td>
<td>4.895</td>
</tr>
<tr>
<td>$\alpha$-Alanine (C$_4$H$_4$COOH)</td>
<td>NaOH</td>
<td>4.835</td>
<td>0.012 (15)</td>
<td>4.835</td>
</tr>
<tr>
<td>$\alpha$-Ferulic acid (C$_7$H$_6$COOH)</td>
<td>NaOH</td>
<td>4.885</td>
<td>0.016 (15)</td>
<td>4.885</td>
</tr>
<tr>
<td>Phenol (C$_6$H$_5$OH)</td>
<td>NaOH</td>
<td>9.935</td>
<td>0.005 (18)</td>
<td>10.105</td>
</tr>
<tr>
<td>Phosphoric acid (H$_3$PO$_4$)</td>
<td>NaOH</td>
<td>2.125</td>
<td>0.11 (17)</td>
<td>2.187</td>
</tr>
<tr>
<td>$pK_{A1}$</td>
<td>NaOH</td>
<td>7.305</td>
<td>0.15 (6)</td>
<td>7.214</td>
</tr>
<tr>
<td>Oxalic acid (C$_2$O$_4$H$_2$)</td>
<td>NaOH</td>
<td>3.226</td>
<td>0.07 (11)</td>
<td>3.231</td>
</tr>
<tr>
<td>Ammonium ion (NH$_3^+$)</td>
<td>HCl</td>
<td>9.186</td>
<td>0.02 (27)</td>
<td>9.186</td>
</tr>
<tr>
<td>Carbonic acid (H$_2$CO$_3$)</td>
<td>HCl</td>
<td>6.358</td>
<td>0.03 (14)</td>
<td>6.317</td>
</tr>
<tr>
<td>$pK_{A1}$</td>
<td>HCl</td>
<td>10.247</td>
<td>0.03 (15)</td>
<td>10.247</td>
</tr>
</tbody>
</table>

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

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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 \( V_0 \) (pH) values were averaged and thus obtained data were processed a second time in the same way. When thus obtained \( V_0 \) came in a preset range including \( EP \) (pH 3 – 7 for NH\(_4\) and pH 6 – 10 for CH\(_3\)COOH, for example), recent 4 data points at the interval of 0.16 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\(_3\)PO\(_4\) and NH\(_4\)\(_2\)CO\(_3\), their conjugate bases, CO\(_2\)\(_3\)\(_-\) (taken as Na\(_2\)CO\(_3\)) and NH\(_3\), were titrated with 0.1 mol dm\(^{-3}\) HCl. For H\(_3\)PO\(_4\) and HCO\(_3\)\(_-\), 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\(_3\)PO\(_4\). 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 \( pK_a \) 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 (26.42 s was needed per measurement at R.S.D. of 0.99 – 1.51 (0.29); for Approach 2, the values were 22.35 – 38.00 (28.89 s per titration) at the R.S.D. of 0.99 – 2.01 (0.25); 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 (\( \epsilon \)) was tested by adding an organic solvent to both the titrant and titrate. Acetonitrile (MeCN) was selected for this purpose because the solvent freely mixed with water and the \( \epsilon \) 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 (\( \epsilon \)-pH) 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].

\[
\epsilon \text{-pH} = \epsilon \text{-pH} + 3.81 X_{MeCN}^2
\]  

where \( \epsilon \)-pH means the pH in an MeCN-H\(_2\)O medium, referred to the standard pH buffers containing MeCN at the same \( X_{MeCN} \) as in the sample. The \( \epsilon \)-pH at EP 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 \( \epsilon \) 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 \( \epsilon \) of the MeCN-H\(_2\)O media were estimated from the Eq. 13 [21].

\[
\epsilon^{-4} = 0.015477 X_{MeCN} + 0.001305
\]  

The values obtained in the present study agreed well with the literature values; the \( pK_a \) values for CH\(_3\)COOH increased with the decrease of \( \epsilon \). 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 ratioimetry constitutes 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 [12]. 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\(_T\). 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 \( V_a \) at relatively lower value in the ramp mode by taking the margin for its rise in the subsequent hold mode into account. This makes the lag time \( \tau_{2} \) 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 \( V_a \) (5 V) can be used throughout the measurement.
References


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