Unprecedented High Throughput Titration by Feedback-Based and Subsequent Fixed Triangular Wave-Controlled Flow Ratiometry and Its Application to Quantification of Japanese Pharmacopoeia Drugs

Aiko Miyazaki[1], Naoya Kakiuchi[2], Kazumasa Okamoto[1], Masaki Takeuchi[1,2,3] and Hideji Tanaka[1,2,3,*]

[1] Faculty of Pharmaceutical Sciences, Tokushima University, 1-78-1 Shomachi, Tokushima 770-8505, Japan
[2] Graduate School of Pharmaceutical Sciences, Tokushima University, 1-78-1 Shomachi, Tokushima, Japan
[3] Institute of Biomedical Sciences, Tokushima University, 1-78-1 Shomachi, Tokushima 770-8505, Japan

Abstract

Throughput rate of flow ratiometric titration has further been enhanced by shortening the lag time from the confluence of solutions upstream to the sensing of signal downstream and by optimizing analytical parameters. Feedback-based upward and downward scans of titrand/titrant flow ratio were repeated in order to offset the effect of the lag time and thus to locate the equivalence point. Subsequent faster fixed triangular wave-controlled scans in narrower range further increased the throughput rate. Analytical parameters such as scan rate and scan range were optimized. Maximally, 46.9 titrations/min was realized with reasonable precision (RSD = 1.79%). Applicability of the method to the quantitation of the Japanese Pharmacopoeia drugs (furosemide, isoniazid and prochlorperazine maleate) was verified, where the latter two drugs were determined by nonaqueous titrations.

Keywords Flow titration, flow ratiometry, high-throughput titration, nonaqueous titration, Japanese Pharmacopoeia drug

1. Introduction

Titrimetry is one of the classical analytical methods still in use widely. It has advantages over rather new instrumental analyses, especially, in the respect of versatility and high precision. In addition, titrimetry has traceability to SI units (kg and mol) because it is based on a stoichiometric chemical reaction and does not need calibration curve (*i.e.*, absolute method). In the field of pharmaceutical sciences, about half of the drugs listed in the 17th ed. Japanese Pharmacopoeia [1] are specified to be quantified by titrimetry.

Conventional manual titrations using glassware such as burette are, however, labor-intensive and time-consuming, and not suitable for large number of samples. Various flow titration methods have, therefore, been studied, as reviewed by Tanaka and Nakano [2]. Flow injection titration [3] and sequential injection titration [4], for example, can follow wide range of titrand concentration. However, high precision cannot be expected because not titrand concentration but logarithm of the concentration gives linear relationship with analytical signals (peak widths). It takes typically several minutes per titration. “Continuous automated, buretteless titrator” reported by Blaedel and Laessig [5,6] is considered as a prototype of the present flow ratiometry, where titrand of constant flow rate was merged with titrant delivered at various flow rate. The flow ratio of titrand and titrant was converged to the equivalence point level within 5 min. This time was limited by the lag time between the merging of solutions upstream and the sensing downstream.

Feedback-based flow ratiometry originated by Tanaka and Dasgupta [7,8] is a sophisticated concept for flow titration. The effect of the lag time was compensated for by repeating rapid upward and downward scan of titrand/titrant flow rate. Maximally 18.8 titrations/min (*i.e.*, 3.2 s/titration) can be performed by their approach. Recently, Fais *et al*. [9] constructed a sensor-controlled flow apparatus for online titration based on this method.

Tanaka *et al*. further enhanced the throughput rate of flow ratiometry by combining the feedback-based control with subsequent fixed triangular wave-control [10,11]. As high as 34.1 titrations/min was realized by this approach [11]. The method was applied to the analyses of commercial vinegar samples [12]. Magnesium and calcium ions were simultaneously determined with ion sensor and photo sensor set in tandem in a flow system [13].

In the present study, we have challenged for unprecedented high throughput titration by a feedback-based and subsequent fixed triangular wave-controlled flow ratiometry. For this purpose, reacted solution was aspirated from the most downstream of the flow conduit [7] in order to shorten the lag time between merging and sensing of solutions. In addition, the scan range and scan rate in the fixed mode were respectively set as narrow and fast as possible. As high as 46.9 titrations/min can be achieved by the proposed method. It has been applied to various acid-base titrations including nonaqueous titrations of the Japanese Pharmacopoeia drugs.

2. Experimental

2.1. Reagents

The reagents used in the present study were purchased from Kanto Chemicals (Tokyo, Japan), Nacalai Tesque (Kyoto, Japan) or Wako Pure Chemical Industries (Osaka, Japan). The reagents were used without further purification. Fine granule of 4% furosemide (Lasix®), Bulk powder of isoniazid (ISCOTIN®) and prochlorperazine maleate (Novamin®) tablets were the 17th ed. Japanese Pharmacopoeia drugs, and were purchased from Nichi-Iko Pharmaceutical Co., Daiichi Sankyo Company, and Kyowa Pharmaceutical Industry Co., respectively. The drugs were respectively dissolved in *N,N*-dimethylformamide, acetic acid-acetic anhydrate (5:1 in volume ratio) and acetic acid. As for the latter two drugs, undissolved excipients were removed by filtration with Advantec No. 1 filter paper prior to the analyses. Zartorius Ariumm 611 DI grade deionized water was used throughout.

* Corresponding author.
E-mail: h.tanaka@tokushima-ac.jp

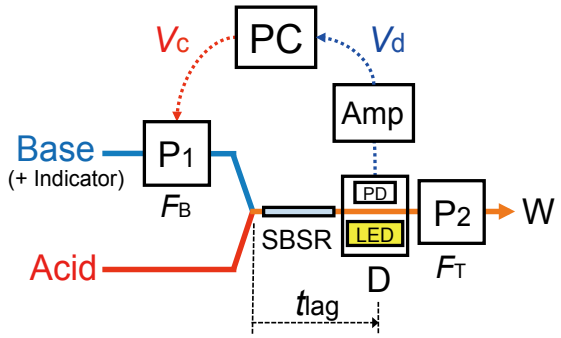

Fig. 1 Flow system. P_1 and P_2, peristaltic pump; SBSR, single-bead string reactor; D, light emitting diode (LED)-photodiode (PD) based detector; Amp, current amplifier; PC, laptop computer with A/D-D/A converter; V_c, controller output voltage; V_d, detector output voltage; F_B, flow rate of base solution (0 − 1.39 cm^3 min^{-1}); F_T, total flow rate (1.80 cm^3 min^{-1}); W, waste; t_{lag}, lag time between merging solutions (upstream) and sensing solution (downstream).

2.2. Flow system and procedures

Figure 1 shows the flow system of the present study. Two peristaltic pumps (P_1: Rainin Dynamax RP-1, USA; P_2: Gilson Minipuls 3, USA) were used for delivering solutions. Pharmed® tubings (3.7 mm o.d., 0.5 mm i.d.) were used as pump tubes. Other conduits were configured with PTFE tubings of 1.58 mm o.d. and 0.5 mm i.d. Flow rate (F_B) of a base solution containing an acid-base indicator was varied in accordance with a control signal (V_c) supplied from a laptop computer (PC; IBM ThinkPad 1843-BLJ, China) *via* an A/D-D/A converter (Measurement Computing PC-CARD-DAS16/12-AO, USA). Later in this study, some experiments were carried out with a new set of computer (Dell 45176/SDPPI/2016 5100, China) and A/D-D/A converter (CONTEC USB I/O Terminal AIO-160802GY-USB, Japan) because of the breakdown of the above mentioned converter (discontinued product). The base solution was merged with an acid solution at the confluence point, while the total flow rate was held constant. Mixing of the solutions was facilitated by a single-bead string reactor (SBSR; PTFE tubing of 2.0 mm o.d., 1.0 mm i.d. and 8.8 cm length packed with glass beads of 0.7 mm diameter) installed just downstream of the confluence point. Without SBSR, precise results could not be obtained because of insufficient mixing of titrand and titrant. Thus mixed solution was led to an optical flow cell (3.0 mm o.d., 1 mm i.d. quartz tubing) of an LED-photodiode based photometer (D) newly fabricated in-house according to a previous study [12]. The light from the yellow LED is made incident in the quartz tubing from the right angle. The intensity of transmitted light was measured with a Hamamatsu S2281 photodiode (PD). The resulting electric current was amplified with a Hamamatsu C9329 current amplifier (Amp) and the output voltage (V_d) was acquired in PC as Microsoft Excel format. In the present study, P_2 was set at the most downstream of the flow system in order to shorten the lag time (t_{lag}) between the merging of solutions and the sensing of the corresponding signal, and thus to increase the throughput rate of titration.

2.3 Principle

The principle of the feedback-based and subsequent fixed triangular wave-controlled flow ratiometry was described before [10-12]. In the feedback-based control flow ratiometry, scan direction of V_c is changed from upward to downward and *vice versa* at the instant when the detector senses the pre-determined V_d that corresponds to the equivalence point. The V_c that gives the equivalence composition, V_E, can be estimated by averaging adjacent V_c maximum and minimum. Concentration of titrand (either Acid or Base in Fig. 1) can be obtained from V_E by employing the other as a titrant (*i.e.*, standard solution). After repeating preset times (5–10 times) of feedback-based scans, fixed triangular wave-control is applied. In this control, the scan rate and scan range are respectively faster and narrower than those in the preceding feedback-based control in order to increase the throughput rate. An Excel VBA program that was reported in the previous study [3] was employed for controlling the system. However, later in this study, an in-house program written in Visual Basic was newly developed, out of necessity for controlling the new set of computer and converter. Both programs contain source code for automatic removal of air signals [14] as a measure against air bubbles accidentally come in the optical flow cell.

3. Results and Discussion

3.1. Optimization of analytical parameters

Analytical parameters were studied using 0.1 mol dm^{-3} NaOH containing 0.2 mmol dm^{-3} Bromothymol Blue (BTB) and 0.1 mol dm^{-3} HCl. In the feedback-based flow ratiometry, the time needed per one titration is $2t_{lag}$ [7]. The t_{lag} consists principally of the transit time of the solution from the confluence point to the detector and very slightly the response time of the detector. In the present study, 3.67, 3.75, 3.78, 3.83 and 3.82 s/titration were obtained at the V_c scan rate of 0.1, 0.2, 0.3, 0.4 and 0.6 V s^{-1}, respectively. The slight increase of the time is considered to be due to the delay of the response time, because the oscillation of the composition of mixed solution from the equivalence composition became larger with the V_c scan rate. Obtained V_E values were almost constant irrespective of the scan rate except 0.6 V s^{-1}; 1.70 ± 0.06, 1.69 ± 0.05, 1.72 ± 0.07, 1.72 ± 0.06 and 2.04 ± 0.07 (n = 50) at the scan rate of 0.1, 0.2, 0.3, 0.4 and 0.6 V s^{-1}, respectively. Therefore, 100 mV s^{-1} was selected as the V_c scan rate for the feedback-based control.

The effects of the scan range and scan rate of the fixed triangular wave controlled flow ratiometry were investigated. In this mode, the time needed for one titration is inversely proportional to the scan rate and proportional to the scan range of V_c [10,11]. When 50% or 40% of the latest scan range in the feedback-based mode was set as V_c scan range (center: V_E) for the fixed mode, the time needed for one titration was 1.69 and 1.44 s, respectively at the V_c scan rate of 0.2 V s^{-1}. However, RSD of V_E became worse from 0.75% to 1.10% (n = 50) with the decrease of the scan range. Therefore, 50% was selected as the scan range for triangular wave-controlled flow ratiometry by taking the repeatability into account. As for the effect of V_c scan rate in this mode, the time needed per titration could be reduced with the increase of the rate: 3.15, 1.76, 1.59, 1.28 and 1.20 s/titration at the scan rate of 0.1, 0.15, 0.2, 0.25 and 0.3 V s^{-1}, respectively. Corresponding V_E values were almost constant at 2.40, 2.41, 2.37, 2.39 and 2.40 V, but their RSD increased with the scan rate (0.69%, 1.18%, 1.38%, 1.79% and 3.51%, respectively). Although the scan rate of 0.3 V s^{-1} can give as high as 50.0 titrations/min, 0.25 V s^{-1} was selected as the optimum V_c scan rate as a compromise of the throughput rate and repeatability. The throughput rate was 46.9 titrations/min at this condition. This rate is the highest ever reported, as far as we have searched.

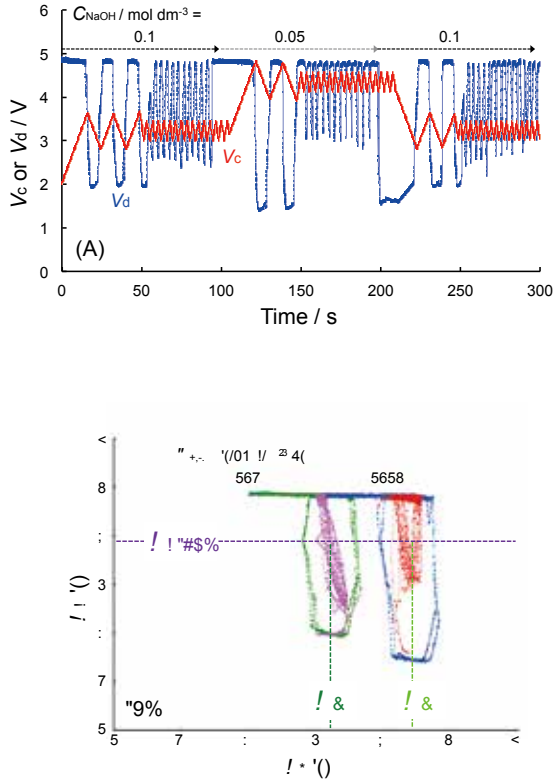

Fig. 2 Continuous titrations of a changing concentration. Temporal profile of V_c and V_d (A) and titration curve (B). Titrant: 0.1 mol dm^{-3} HCl. Indicator: BTB. Titrand (NaOH) concentration is changed from 0.1 mol dm^{-3} to 0.05 mol dm^{-3}, and then again to 0.1 mol dm^{-3}.

3.2. Ability to follow concentration change in sample stream

Our program holds the fixed triangular wave-control mode as long as the V_c scan range covers V_E, even if titrand concentration is varied. However, when V_E moved outside of the scan range due to the considerable change in titrand concentration, feedback-based scans are applied again. We tested this function by successively introducing 0.1, 0.05 and again 0.1 mol dm^{-3} NaOH as titrands. The temporal profile of the results is shown in Fig. 2A. Initial feedback-based scans were followed by fixed triangular wave scans at 49.15 s. When the titrand concentration was changed from 0.1 mol dm^{-3} to 0.05 mol dm^{-3}, the V_c scan range in the fixed mode became too low to cover the new equivalence point (*i.e.*, new V_E). When the equivalence V_d signal, $V_{d(eq)}$, is not detected during 3 periods of V_c (= 6 scan range [V] / scan rate [V s^{-1}]), feedback-based scans started again at 105.25 s. This mode was followed by a fixed mode at 147.65 s. Similarly, feedback and fixed operations were respectively applied at 209.60 and 246.60 s for 0.1 mol dm^{-3} NaOH. Figure 2A clearly shows that our system has enough ability to follow the change in titrand concentration. The V_E values for the first and the second introductions of 0.1 mol dm^{-3} NaOH were 3.22 ± 0.05 (n = 26, t = 20.5 − 93.8 s) and 3.26 ± 0.11 (n = 31, t = 227.0 − 298.2 s), respectively, indicating reasonable reproducibility. Good results were also obtained for the continuous titrations of 0.1, 0.3 and 0.1 mol dm^{-3} HCl with 0.1 mol dm^{-3} NaOH (data are not shown here). Although it took long time to locate new V_E when titrand concentration drastically changed, overall throughput rate of 16.5 titrations/min was obtained for the entire period of 0 − 300 s.

Figure 2B shows the relationship between V_c and V_d in the period of 0 – 200 s, which corresponds to the titration curve in conventional batch titration because the amount of titrant fed is proportional to V_c. The curves are of loop-shape because of t_{lag} [7]. For each NaOH concentration, larger clockwise and smaller anti-clockwise loops respectively correspond to the data obtained by feedback-based control and by fixed triangular wave-control. Narrow scan ranges of V_c clearly illustrates the ability of the present system for performing high throughput titration.

3.3. Application to titrations of various acids and bases including Japanese Pharmacopoeia drugs

The present system was applied to various acid-base titrations including nonaqueous titrations of drugs listed in the 17th ed. Japanese Pharmacopoeia. Regardless of titrand or titrant, acid and base solutions were fed from the channels denoted by Acid and Base, respectively, in Fig. 1. Analytical parameters such as the scan range and scan rate were the same as those described in Section 3.1. Only the equivalence V_d values (3.5 – 1.4 V) were determined individually for respective sets of titrand, titrant and indicator. Flow ratiometry is an absolute method in principle as long as the flow rate is accurately calibrated. However, daily

Table 1 Application to various acid-base titraions

Titrand	Titrant	Indicator[a]	Titrant concentration / mol dm^{-3}	Range / mol dm^{-3}	Linearity, r^2
HCl	NaOH	BTB	0.1	0.04 – 1.0	0.999
CH_3COOH	NaOH	TB	0.1	0.03 – 1.0	0.999
H_3PO_4, 1st	NaOH	BCG	0.1	0.04 – 0.8	0.995
2nd	NaOH	TP	0.1	0.05 – 0.8	0.998
NaOH	HCl	BTB[d]	0.1	0.03 – 0.6	0.999
NH_3	HCl	BCG[d]	0.1	0.03 – 0.6	0.999
Na_2CO_3, 1st	HCl	TB[d]	0.1	0.03 – 0.4	0.996
Furosemide[b]	NaOH	BTB	0.01	0.001 – 0.01	0.999
Isoniazid[b]	$HClO_4$[c]	PNB	0.1	0.05 – 0.2	0.999
Prochlorperazine Maleate[b]	$HClO_4$	PNB	0.001	0.0001 – 0.0008	0.997

a. BTB, Bromothymol Blue; TB, Thymol Blue; BCG, Bromocresol Green; TP, Thymolphthaleine; PNB, *p*-Naphtolbenzein
b. 17th ed. Japanese Pharmacopoeia drug.
c. Dissolved in acetic acid.
d. Indicator was added in base solution (*i.e.*, titrand).

calibration of the flow rate is troublesome and is not realistic. Therefore, absolute calibration method is adopted, where V_E^{-1} is plotted against base concentration or the reciprocal of acid concentration [7]. Table 1 lists the results. As for furosemide and prochlorperazine maleate, titrations in lower concentration range were investigated because the amounts of the drugs in pharmaceutical preparations were very low (4% in fine granule and 5 mg in a *ca.* 50 mg tablet, respectively). In the titration of furosemide, not a few bubbles were generated in the conduit, probably because the dissolved air in the nonaqueous titrand became saturated when its being mixed with aqueous titrant (Regarding to CO_2, its Henry's constant in *N,N*-dimethylformamide and water mixtures were reported in a literature [15]). Our program having software-based deaeration algorism [14] could completely remove bubble signals, resulting in the data listed in Table 1. The titration of photosensitive prochlorperazine maleate could successfully be carried out by shielding the system from ambient light with aluminum foil. The linearity of the calibration curve was acceptable ($r^2 > 0.995$) for all the titrands examined.

4. Acknowledgements

The present study is partly supported by the Grant-in-Aid for Scientific Research (C) (15K07889) from the Japan Society for the Promotion of Sciences (JSPS).

References

[1] The Japanese Pharmacopoeia, 17th ed., Part. 1, "*Official Monograph*", **2016**, The Ministery of Health, Labor and Welfare, Tokyo.
[2] H. Tanaka and S. Nakano, *J. Flow Injection Anal*., **21,** 123 (2004).
[3] A.U. Ramsing, J. Ruzicka, E.H. Hansen, *Anal. Chim. Acta*, **129**, 1 (1981).
[4] J.F. van Staden, H. du Plessis, *Anal. Commun*., **34**, 147 (1997).
[5] W.J. Blaedel, R.H. Laessig, *Anal. Chem*. **36**, 1617 (1964).
[6] W.J. Blaedel, R.H. Laessig, *Anal. Chem*. **37**, 332 (1965).
[7] H. Tanaka, P.K. Dasgupta, J. Huang, *Anal. Chem*., **72**, 4713 (2000).
[8] P.K. Dasgupta, H. Tanaka, K.D. Jo, *Anal. Chim. Acta*, **435**, 289 (2001).
[9] M. Fais, C. Schwarz, F.C. Leinwebr, *Chem. Ing. Tech*., **88**, 793 (2016).
[10] H. Tanaka, T. Baba, *Talanta*, **67**, 843 (2005).
[11] H. Tanaka, T. Baba, *Anal. Sci.,* **21**, 615 (2005).
[12] N. Kakiuchi, A. Miyazaki, A. Fujikawa, M. Takeuchi, H. Tanaka, *J. Flow Injection Anal*., **34**, 11 (2017).
[13] T. Aydan, A. Kitagawa, M. Takeuchi, H. Tanaka, *J. Flow Injection Anal*., **25**, 65 (2008).
[14] T. Ogusu, K. Uchimoto, M. Takeuchi, H. Tanaka, *Talanta*, **118**, 123 (2014).
[15] M. Jödecke, Á. P.-S.Kamps, G.Maurer, *J. Chem. Eng. Data.*, 57, 1249 (2012).

(Received July 16, 2019)
(Accepted November 24, 2019)