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Talanta 67 (2005) 848–853
Talanta
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High throughput continuous titration based on a flow ratiometry controlled with feedback-based variable triangular waves and subsequent fixed triangular waves

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Received 24 February 2005; received in revised form 6 April 2005; accepted 6 April 2005

Abstract

We propose a new approach for high-throughput continuous titration based on a flow ratiometry. The method was applied to potentiometric titrations of acids and bases. A base solution, the flow rate F_B of which was varied in response to controller output voltage V_c, was merged with an acid solution under constant total (acid + base) flow rate. Downstream, the pH of the mixed solution was measured with a flow-through glass electrode. Initially, V_c, and thus F_B was increased linearly. At the instant the equivalence point (EP) was sensed, the ramp direction of V_c was reversed from upward to downward. The direction was reversed to upward when EP was sensed again. Such the feedback-based operation gives a triangular waveform of V_c, because there is a delay corresponding mainly to the transit time of merged solutions to reach the sensor. The value of V_c that gives EP composition, V_E, was estimated by averaging the most recent maximum and minimum values of V_c. Next, fixed triangular waves of V_c was used to control F_B. The amplitude and the scan rate of the waves were fixed narrower and faster, respectively, than those in the feedback-based operation in order to improve the throughput rate. The EP can be located as long as the scan range covers V_E. These automated processes limited the titration to just the narrow range around EP, and thus realized extremely high throughput rate of maximally 17.6 titrations per minute (=3.4 s per titration) at R.S.D. = 0.35%.

Keywords: Flow ratiometry; Continuous titration; Feedback-based control; Triangular wave control; High throughput titration

1. Introduction

Titrimetry is a classical analytical method, the origin of which can be traced back to the analysis of vinegars with K_2CO_3 by Geoffroy in 1729 [1]. Titrimetry is still in use widely, especially for the determination of major components in macro-scale samples, in virtue of its excellent advantages regarding precision, convenience, and so on. Conventional manual operations using glassware are, however, tedious and time-consuming. Various techniques using flow system have, therefore, been developed for the automation of titrimetry. Among them, flow injection titration [2] is probably the most famous method, where the peak width of sample zone typically at the equivalence level is proportional to the logarithm of analyte concentration. Such the principle was extended to a sequential injection analysis [3]. The groups of Nagy et al. [4,5] and of Spohn et al. [6] proposed triangle programmed coulometric titration. In their method, the concentration of titrant has a triangular profile by triangulating electrolysis current profile for titrant generation. The time elapsed between the two equivalence point, each attained in the course of rising and falling current, is decreased linearly with an increase in titrand concentration. Titration can also be performed by varying the flow rate ratio of titrant and titrand. Blaedel and Laessig [7,8] first introduced such a flow ratiometry in the middle of 1960s. Recently, Tanaka and Nakano [9] reviewed above-mentioned flow titrations, together with some other techniques.

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doi:10.1016/j.talanta.2005.04.007

Tanaka et al. [10,11] introduced a new concept named "feedback-based flow ratiometry" to flow ratiometric titration. In their approach, the scan direction of titrant flow rate was reversed from upward to downward, and vice versa, whenever the equivalence point (EP) was detected downstream. Thus, the titration was limited to just the range of interest (i.e., EP), resulting in considerably high throughput rate: typically six titrrations per minute for photometric titration [10] and five titrations per minute for potentiometric titration [11]. Jo and Dasgupta [12] applied the methodology to continuous cheletometric titrations.

In many applications such as process monitoring, the concentration of analyte does not change so drastically and remains in a certain range. This fact stimulated us to introduce a new concept to flow ratiometry. That is, once EP is located by a feedback-based flow ratiometry, the scan range of titrant flow rate can further be limited to just around EP. The EP can surely be sensed as long as the scan range covers EP, even though the scan direction is reversed before the sensing of EP by using fixed triangular waves. In the present study, therefore, such a combination of feedback-based and fixed triangular wave controlled flow ratiometries is investigated in order to get higher throughput. The validity of the concept was demonstrated by applying it to various acid–base titrations with potentiometric detection.

2. Experimental

2.1. Flow system

Fig. 1 shows the flow system in the present study. Two peristaltic pumps (P_1 and P_2; Rainin Dynamax RP-1) were used for delivering solutions. The pump tubes used were 0.51 mm, i.d. Pharmed tubing. The flow rate of a base solution F_B was varied in response to controller output voltage V_c (0–5 V) generated from a computer (PC; Toshiba Dynabook Satellite 1850 SA120C/4) through a card type A/D-D/A converter (Measurement Computing PC-CARD-DAS16/12-AO). The total (acid + base) flow rate F_T was held constant at ca. 2.2 cm^3 min^{-1}. An acid solution was, therefore, aspirated to the confluence point (polypropylene tee) at the flow rate of $F_T - F_B$ and merged with the base solution there. The mixed solution was led to a Horiba (Kyoto, Japan) 6964-15C flow-through glass electrode (FC) equipped with a Horiba 2461A-15T reference electrode. Both the electrodes, wired to a Horiba F-13 pH meter (D), were calibrated daily before the measurements with commercial standard pH buffer solutions in flow mode. Output signal (V_d) from the pH meter (−0.7 to +0.7 V for pH 0–14) was digitized by the converter at the frequency of 20 Hz, and the resulting data were acquired in PC as Microsoft Excel format. Code written in Visual BASIC in-house was used to generate V_c, acquire data, analyze them and graphically display the results automatically.

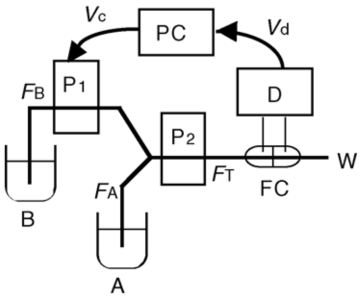

Fig. 1. Schematic diagram of the present flow system. A, acid solution; B, base solution; W, waste; P_1 and P_2, peristaltic pump; FC, flow-through glass electrode equipped with a reference electrode; D, pH meter; PC, notebook computer with a card type A/D-D/A converter; V_c, controller output voltage; V_d, detector output voltage. F_A, F_B and F_T are the flow rate of acid, base and mixed solutions, respectively.

2.2. Reagents

Reagents of analytical reagent grade purchased from Kanto Chemicals (Tokyo, Japan), Nacalai Tesque (Kyoto, Japan) or Wako Pure Chemical Industries (Osaka, Japan) were used without further purification. Milli-Q SP grade deionized water was used throughout.

3. Principles

3.1. Algorithm for operating the system

The principle of the proposed method is schematically shown in Fig. 2A. Initially, the controller output voltage V_c, and thus the flow rate of base solution F_B increases linearly. The V_c determines the composition of the merged solution at the confluence point of both the solutions. The pH measured downstream increases after a delay. This lag time t_{lag} consists primarily of the physical transit time of the merged solution to reach the flow cell and minutely of the response time of the sensor. In the present configuration, t_{lag} is considered virtually constant because the total flow rate F_T is held constant. At the instant (α) the detector senses equivalence pH (pH_E), where the slope of the titration curve becomes maximal; the ramp of V_c is reversed to downward direction. The pH of the mixed solution continues to increase because of t_{lag}, and then decreases after reaching a maximum. When the pH_E is sensed again (β), the ramp of V_c is reversed to upward direction. Such the feedback-based control (FB) gives the triangular waveform of V_c which has an amplitude of $2(dV_c/dt)t_{lag}$ and a frequency of $(4t_{lag})^{-1}$, where (dV_c/dt) is the scan rate of V_c. The maximum and minimum values of V_c (V_H and V_L, respectively) are higher and lower, respectively, than the value of controller output voltage (V_E) that gives the true equivalence composition at the confluence point of both the solutions, as expressed as follows [10,11].

$$V_H = V_E + \left(\frac{dV_c}{dt}\right) t_{lag} \qquad (1)$$

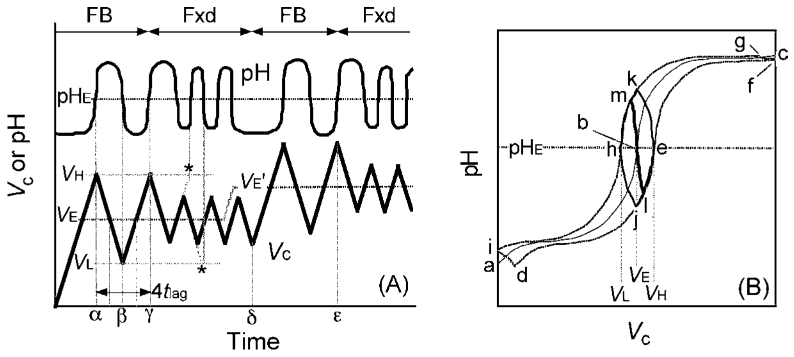

Fig. 2. Principle of continuous titration based on feedback-based and fixed triangular wave controlled flow ratiometry. (A) Temporal profile of titration. (B) Titration curves. V_c, controller output voltage; V_E, controller output voltage that gives equivalence composition at the confluence point of acid and base solutions; V_H and V_L maximum and minimum values of V_c obtained through a feedback-based control; EB, feedback-based operation; Fxd, fixed triangular wave operation.

$$V_L = V_E - \left(\frac{dV_c}{dt}\right) t_{lag} \tag{2}$$

The second terms in the right sides of both the equations correspond to the overshoot and undershoot of V_c, respectively, from the V_E due to the t_{lag}. The V_E can, therefore, be obtained by averaging the most recent V_H and V_L values.

$$V_E = \frac{V_H + V_L}{2} \tag{3}$$

When pH_E is detected third time (γ), a fixed triangular wave control (Fxd) is put into operation, where the amplitude of the wave is fixed narrower (half, in the present study) than that in FB in order to further improve the throughput rate of the measurement. Although the scan direction of V_c reverses before the detection of pH_E in this fixed mode, pH_E will surely be sensed after the reversal, as long as the V_c scan range covers V_E; the fixed triangular wave controlled operation can be applied as long as this condition is satisfied, even if the analyte concentration is varied. The V_H and V_L (denoted by the asterisks), such as those obtained by FB, can be estimated by extrapolating V_c before the reversal to the time pH_E is just detected. These estimated values are used for computing V_E according to Eq. (3) in the same way as in the feedback-based mode.

If V_E moves outside of the scan range due to the considerable change in analyte concentration, pH_E comes to be unable to be detected during the period corresponding to one temporal period of the fixed triangular wave. In this case, a feedback-based control is started again (δ) in order to search for new equivalence point (V'_E in Fig. 2A) and then a fixed triangular wave control is applied (ε) to a new V_c range in a similar way as described above.

3.2. Titration curves

Fig. 2B shows the theoretical titration curve for the present approach together with those for other methods. The titration curve clearly shows the feature and advantage of the proposed method over other titrimetries. In a conventional flow titrimetry [13], V_c is changed discontinuously and each V_c value is kept long enough to obtain corresponding invariable pH. Titration curve of a–b–c, similar in shape to that in conventional batch titrimetry, would be obtained by connecting data points with smooth curve. This method is time-consuming (typically several minutes per titration) because it needs waiting time at each V_c.

On the other hand, if V_c is varied continuously and triangularly so that it can cover entire flow ratio ($F_B/F_A = 0–\infty$) [10], counterclockwise loop titration curve of d–j–l–e–f–g–k–m–h–i–d would be obtained. The reason for the loop shape of the titration curve is in the time lag between the merging of solutions at the confluence point (upstream) and their being measured with the sensor (downstream). Although this continuous method seems more efficient than the discontinuous method described above, it still spends large amount of time to scan needless range far from the equivalence point.

In the present approach, the feedback-based control limits the scan of V_c just the range of interest (i.e., the equivalence point) by reversing the scan direction at the instant the equivalence point is detected. The small counterclockwise loop titration curve of j–l–e–k–m–h–j would be obtained from this feedback-based operation. The scan range of V_c can further be limited by the proposed fixed triangular wave control, where the scan direction is reversed in premature fashion without waiting for the detection of the equivalence point. The titration curve obtained through this approach would be a form of short reciprocation of l–b–m–b–l if the amplitude of the triangular wave were fixed half of that in the feedback-based operation.

4. Results and discussion

4.1. Optimization of the system

Basic analytical parameters were examined by using $0.1\,mol\,dm^{-3}$ HCl and NaOH as a titrand and titrant, re-

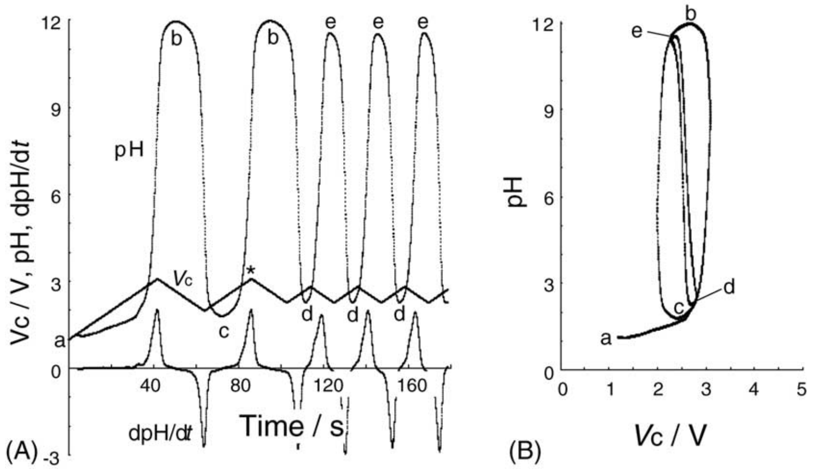

Fig. 3. Typical results for the proposed titrimetry. A 0.1 mol dm^{-3} HCl was titrated with a 0.1 mol dm^{-1} NaOH. (A) Temporal profile of titration. (B) Titration curve. The symbols of (a–e) in both the figures correspond to each other.

spectively. As for the feedback-based approach, shorter t_{lag} is desirable in order to increase efficiency because the time required per titration equals to $2t_{\text{lag}}$. In a preliminary experiment, an active mixing by the rollers of P_2 pump was found to be sufficient to mix the solutions and, therefore, no mixing reactor was inserted between the P_2 pump and the flow cell. The scan rate of V_c for feedback-based control was set at 50 mV s^{-1} by refereeing to the results in the previous works on potentiometric titrations in flow mode [11,14]. A moving average filter was used to smooth detector output signals; consecutive 25 raw pH values were averaged and thus obtained data were processed a second time, and then third time in a similar way. The equivalence point was located as the point where the slope (|dpH/dt|) of the pH curve became maximal.

4.2. Basic performance of the developed system

Fig. 3A shows a typical temporal profile of V_c, pH and dpH/dt of the proposed titrimetry, where 0.1 mol dm^{-3} HCl was titrated with the same concentration of NaOH. The scan rate of V_c for the fixed triangular wave control was set at 50 mV s^{-1}, which was the same as that for the feedback-based control. Operation mode is changed at the time of 86.1 s (denoted by the asterisk) from the feedback-based control to the fixed triangular wave control. Fig. 3B shows the titration curve for the same results, where the symbols of (a–e) correspond to those in Fig. 3A. The large loop of c–b–c and narrow loop of d–e–d, both in counterclockwise, correspond to the titration curves obtained through the feedback-based and fixed triangular wave operations, respectively. The plots in Fig. 3A and B agreed well with the respective theoretical plots in Fig. 2A and B. The reason for the narrow loop form of the curve d–e–d in Fig. 3B, in contrast to the reciprocation of l–b–m–b–l in Fig. 2B, is attributed mainly to the delay of phase of pH curve caused by the moving average filtering. The values of V_E obtained from both the operations agreed well with each other: the V_E and its relative standard deviation (R.S.D.) were 2.543 V and 0.2% for the feedback-based operation and 2.545 V and 0.3% for the fixed triangular wave operation, respectively. The time needed for one titration was 21.9 and 11.0 s per titration for the respective operations.

4.3. Effect of V_c scan rate for fixed triangular wave control

As for the fixed triangular wave control, the throughput rate of titration can be increased by adopting narrower scan range and higher scan rate of V_c. The time needed for one titration is considered to be proportional to the scan range and inverse proportional to the scan rate. In the present study, the scan range was fixed half as the latest scan range in the feedback-based operation, as described in Section 3.1, because even subtle change in analyte concentration might shift V_E to uncovered region if too narrow scan range were used. The effect of the scan rate (dV_c/dt) of V_c in fixed triangular wave control was, therefore, examined in the range of 50–200 mV s^{-1} in order to improve the efficiency. The efficiency of the measurement, expressed as the time needed for one titration, is plotted as a function of dV_c/dt in Fig. 4A. As expected, the time is greatly and then gradually decreased with an increase in dV_c/dt. When 175 mV s^{-1} was set as dV_c/dt, the efficiency of 3.4 s per titration was realized, which means 17.6 titrations can be done per minute. The relative standard deviation of V_E was plotted against dV_c/dt in Fig. 4B. The R.S.D. increased with dV_c/dt. The measurement became unstable at the dV_c/dt of 200 mV s^{-1}. By taking both the efficiency and precision into account, 75 mV s^{-1} was selected as the optimum dV_c/dt for fixed triangular wave control. The efficiency at this condition was 7.4 s per titration corresponding to the throughput rate of 8.1 titrations per minute, which seems to have sufficient speed for most practical use.

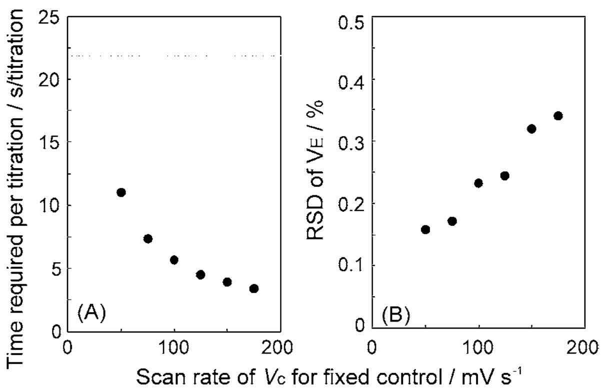

Fig. 4. Effect of scan rate of V_c for the fixed triangular wave control on the efficiency (A) and on the precision (B) of titration. Titrand and titrant are the same as those in Fig. 3. The efficiency is expressed in terms of the time required for one titration. Horizontal dotted line represents the corresponding data for feedback-based control.

4.4. Continuous titrations of a stream of changing composition

The developed algorithm provides the function to follow considerable change in analyte concentration by re-applying the feedback-based control, as described in Section 3.1. Such performance was tested by feeding 0.1, 0.05, 0.5 and 0.1 mol dm^{-3} HCl successively as titrands. The results are shown in Fig. 5. (i) Initial feedback-based operation mode was followed by the fixed triangular wave operation mode at the time of 71 s. (ii) When 0.05 mol dm^{-3} HCl came in the flow line, following to the initial 0.1 mol dm^{-3} HCl, the V_c range of fixed triangular wave became too high to cover the equivalence point for the new titrand. Thus, the feedback-based scan to downward direction began at the time of 155 s. (iii) This control was followed by a fixed triangular wave control at the time of 223 s. Such the processes were repeated over when the titrand concentration was greatly changed and the fixed scan range of V_c no longer covered the equivalence point. These drastic changes in titrand concentration might be an extreme case in real applications, but the results shown in Fig. 5 clearly demonstrate the capability of the developed system to follow the change of titrand concentration. Totally 52 titrations were made during the whole period (720 s) including the time elapsing for finding new equivalence point by restarted feedback-based scan; average titration time requirement was, therefore, 13.8 s per titration. The values of V_E were 1.720 ± 0.005 ($n = 10$), 1.035 ± 0.014 ($n = 9$), 3.719 ± 0.017 ($n = 12$) and 1.728 ± 0.017 ($n = 21$) V for 0.1 (a), 0.05 (b), 0.5 (c) and 0.1 (d) mol dm^{-3} HCl, respectively, where the data after plus or minus sign mean the standard deviation. Ideally, the first and the last V_E values (1.720 and 1.728 V, respectively) described above should have been equal to each other because the same concentration (0.1 mol dm^{-3}) of titrand was measured. The difference between them is, however, very small (0.008 V) and the Student's t-test does not detect significant difference between the two mean values: calculated t values was 1.446, which is less than the tabulated t value (1.699 at the 90% confidence level) at the degrees of freedom of 29.

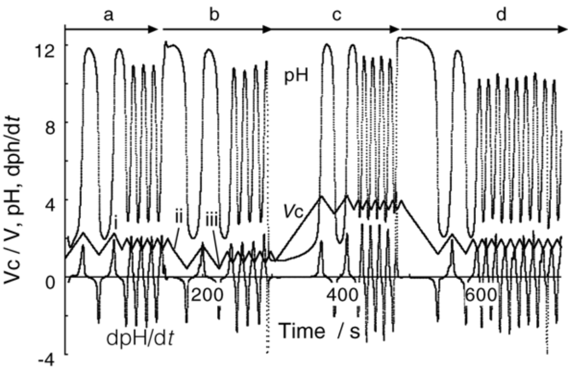

Fig. 5. Continuous titrations of a stream of changing composition. Titrant: 0.1 mol dm^{-1} NaOH. Titrand: 0.1 (a and d), 0.05 (b) and 0.5 (c) mol dm^{-3} HCl. Scan rate of V_c was 50 and 75 mV s^{-1} for feedback-based control and fixed triangular wave control, respectively.

4.5. Application to various acid–base titrations

The present method is an absolute method, in principle, which means no calibration curve is required as long as the titrant concentration is well standardized and the pump speed is accurately calibrated. It is, however, convenient to obtain a calibration curve by using standard solutions containing known concentration of analyte, because such the process may be more practical than the standardization and calibration mentioned above. In the present configuration, the following relationship is held at the equivalence point.

$$n_A C_A (F_T - kV_E) = n_B C_B k V_E \tag{4}$$

Here, n and C mean the valence and concentration, respectively, of the species denoted by the subscripts (A: acid; B: base), k is a proportional constant ($k = F_B/V_c$). From Eq. (1), the following equation is derived.

$$\frac{1}{V_E} = \frac{k n_B C_B}{n_A C_A F_T} + \frac{k}{F_T} \tag{5}$$

Therefore, the plot of the reciprocal of V_E (V_E^{-1}) against that of the acid concentration (C_A^{-1}) becomes linear when the acid solutions are titrated with the base titrant, and the plot of V_E^{-1} against the base concentration (C_B) becomes linear when the base solutions are titrated with the acid titrant.

Based on theses backgrounds, various concentrations of acids (HCl, CH_3COOH, H_3PO_4 and $(COOH)_2$) and bases (NaOH, NH_3 and Na_2CO_3) were titrated with NaOH and HCl, both in 0.1 mol dm^{-3}, respectively. As for the titrations of H_3PO_4, $(COOH)_2$ and Na_2CO_3, the first and second equivalence points could independently be determined, by setting the range of pH for the judgment of pH_E as a software parameter. The ranges were, for example, pH 3–6 and 9–11 for the first and second equivalence points of H_3PO_4, respectively. The results are summarized in Table 1. The linearity of the

Table 1
Application to various acid–base titrations

Titrand	Titrant	Measurement range ($mol\,dm^{-3}$)	No. of individual concentrations examined	Linearity, r^2
HCl	NaOH	0.025–0.500	5	0.9999
CH_3COOH	NaOH	0.025–0.500	5	0.9972
H_3PO_4				
(1st)	NaOH	0.025–0.500	5	0.9995
(2nd)	NaOH	0.025–0.500	5	0.9980
$(COOH)_2$				
(1st)	NaOH	0.025–0.250	5	0.9997
(2nd)	NaOH	0.025–0.250	5	0.9972
NaOH	HCl	0.025–0.500	6	0.9999
NH_3	HCl	0.050–0.500	6	0.9995
Na_2CO_3				
(1st)	HCl	0.025–0.500	6	0.9985
(2nd)	HCl	0.025–0.500	6	0.9990

Concentration of titrant: $0.1\,mol\,dm^{-3}$. Scan rate of V_c: $50\,mV\,s^{-1}$ for feedback-based control and $75\,mV\,s^{-1}$ for fixed triangular wave control.

plots based on Eq. (2) is listed in the rightmost column as the coefficient of determination r^2. Within the range of concentration examined, the linearity of the plots for the compounds tested was satisfactory.

5. Conclusion

We have proposed a high throughput continuous titrimetry based on a flow ratiometry. A fixed triangular wave control to cover very narrow range just around the equivalence point was applied after a feedback-based control. The performance of the proposed method was demonstrated by applying it to various acid–base titrations with potentiometric detection. The developed system can locate the equivalence point precisely at the unprecedented speed of maximally 17.6 titrations per minute. We are applying the proposed approach to photometric titrations and the results will be published elsewhere in near future.

References

[1] F. Szabadváry, History of Analytical Chemistry (Japanese translation), Uchida-Roukakuho, Tokyo, 1988 (Chapter 3).
[2] A.U. Ramsing, J. Ruzicka, E.H. Hansen, Anal. Chim. Acta 129 (1981) 1.
[3] J.F. van Staden, H. du Plessis, Anal. Commun. 34 (1997) 147.
[4] G. Nagy, K. Tóth, E. Pungor, Anal. Chem. 47 (1975) 1460.
[5] G. Nagy, Z.S. Fehér, K. Tóth, E. Pungor, Anal. Chim. Acta 91 (1977) 87, 97.
[6] U. Spohn, G. Nagy, E. Pongor, Anal. Sci. 2 (1986) 423, 431.
[7] W.J. Blaedel, R.H. Laessig, Anal. Chem. 36 (1964) 1617.
[8] W.J. Blaedel, R.H. Laessig, Anal. Chem. 37 (1965) 332.
[9] H. Tanaka, S. Nakano, J. Flow Inject. Anal. 21 (2004) 123.
[10] H. Tanaka, P.K. Dasgupta, J. Huang, Anal. Chem. 72 (2000) 4713.
[11] P.K. Dasgupta, H. Tanaka, K.D. Jo, Anal. Chim. Acta 435 (2001) 289.
[12] K.D. Jo, P.K. Dasgupta, Talanta 61 (2003) 131.
[13] H. Katsumata, N. Teshima, M. Kurihara, T. Kawashima, Talanta 48 (1999) 135.
[14] H. Tanaka, T. Tachibana, R. Oda, P.K. Dasgupta, Talanta 64 (2004) 1169.