

# Application of Feedback-Based Flow Ratiometry to Simultaneous Determination of Calcium and Magnesium Ions by Chelatometry Using Photometric and Potentiometric Detectors

Tursunjan Aydan<sup>1\*</sup>, Ayumi Kitagawa<sup>2</sup>, Masaki Takeuchi<sup>1</sup> and Hideji Tanaka<sup>1</sup>

<sup>1</sup> Institute of Health Biosciences, Graduate School of Tokushima University, Shomachi, Tokushima 770-8505, Japan.

<sup>2</sup> Faculty of Pharmaceutical Sciences, Tokushima University, Shomachi, Tokushima 770-8505, Japan.

## Abstract

We advanced the principle of feedback-based flow ratiometry to stepwise titration using two detectors set in tandem in a flow system. The method was applied to the simultaneous determination of calcium and magnesium ions ( $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , respectively) by a chelatometry. The flow rate ( $F_M$ ) of titrand containing  $\text{Ca}^{2+}$  and/or  $\text{Mg}^{2+}$  was varied in response to controller output signals ( $V_c$ ) having triangular wave profile, while the total (titrand + titrant + indicator solution) flow rate ( $F_T$ ) was held constant. Titrant solution (EDTA or EGTA, pH 10) was aspirated to the confluence point of the flow system at the flow rate of  $F_T - F_M - F_I$ , where  $F_I$  was a constant flow rate of indicator solution (EBT), and then mixed with the titrand. Downstream, the analytical signals of the mixed solution were monitored. First, a high-throughput photometric titration of a single analyte ( $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$ ) was examined by a feedback-based flow ratiometry coupled with a subsequent fixed triangular wave controlled flow ratiometry. Maximally 17 titrations could be done per minute with reasonable precision (RSD = ca. 1 %). Next, the algorithm of the former ratiometry was modified so that a photometric detector and a potentiometric detector ( $\text{Ca}^{2+}$ -selective electrode) could detect the total concentration of both ions and the concentration of  $\text{Ca}^{2+}$ , respectively, using EGTA as a titrant. The equivalence points of the photometry and the potentiometry were each estimated from the  $V_c$  values at the instant of the sensing of the corresponding equivalence signal in the course of upward and downward scans of  $V_c$ . Good throughput (ca. 31 s per determination) was attainable with allowable precision (RSD < 1.6 %).

**Keywords:** Flow ratiometry, flow titration, chelatometric titration, simultaneous determination, calcium ion, magnesium ion

## 1. Introduction

Titrimetry – one of the classical analytical methods – has never lost its importance despite the emergence of various new methods. Compared with other competing techniques, titrimetry exhibits excellent precision, convenience and affordability. Indeed, titrimetry can be operated as an absolute method. That is, as long as the titrant concentration is exactly known and volumetric wares are well calibrated, true titrations require no calibration curve. Conventional manual titrations using glassware are, however, tedious and time consuming. Although various techniques using flow systems had been developed for the automation of titrimetry, as reviewed elsewhere [1], their efficiencies still remained in the order of minutes per titration.

Tanaka *et al.* [2,3] proposed a feedback-based flow ratiometry for automated titration with high throughput rate. In their approach, titrant flow rate was linearly varied while the total (titrand + titrant) flow rate was held constant. The scan direction of the titrant flow rate was reversed from upward to downward, and *vice versa*, whenever the equivalence point was sensed. Thus the scan range is limited to just the range around the equivalence point. More recently, they further developed the flow ratiometry by combining it with a fixed triangular wave controlled flow ratiometry that scanned narrower range with higher scan rate [4,5]. Unprecedented high throughput rate (1.76 s per titration, corresponding to 34 titrations per minute) was achieved for photometric titrations of acids and bases.

Calcium and magnesium ions ( $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , respectively) are common cations in natural water as well as  $\text{H}^+$ ,  $\text{K}^+$ ,  $\text{Na}^+$  and  $\text{Fe}^{3+}$ . The quantities of these two cations have often been the

matters of concern because they closely relate to the hardness of water. Some papers have been published for the simultaneous determination of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  by flow analysis schemes. Alonso *et al.* [6] developed an FIA system with potentiometric and AAS detectors for the determination of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , respectively. Yamane *et al.* [7] reported an FIA with the strategy of multi-injections of two samples and a masking agent plugs. Themelis *et al.* [8] introduced an on-line masking technique to FIA for the spectrophotometric determination of the ions. Stepwise titration of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  using a single titrant and an indicator is, however, difficult because of the similarity in chemical properties of these cations.

The purpose of the present study is to develop a stepwise chelatometric titration system based on a feedback-based flow ratiometry for the simultaneous determination of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . For this purpose, the feedback-based and subsequent fixed triangular wave controlled flow ratiometry [4,5] was firstly investigated to photometric titration of  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$ . The feedback-based flow ratiometry was further advanced to the simultaneous determination of both ions. Two detectors (*i.e.*, photometric and potentiometric detectors) were introduced in series in flow system and EGTA (ethylene glycol bis (2-amino-ethyl ether)-*N,N,N',N'*-tetraacetic acid), which has much higher affinity to  $\text{Ca}^{2+}$  than to  $\text{Mg}^{2+}$  was used as a titrant.

## 2. Experimental

### 2.1. Flow system

Figure 1 shows the schematic diagram of the flow system used in the present study. Three peristaltic pumps ( $P_1$ ,  $P_2$  and  $P_3$ ; Rainin Dynamax RP-1) were used for delivering solutions. The pump tubes used were 0.51 mm i.d. Pharmed<sup>®</sup> tubings. The flow rate ( $F_M$ ) of titrand solutions was linearly varied in

\*Corresponding author.

E-mail: c400741001@stud.tokushima-u.ac.jp

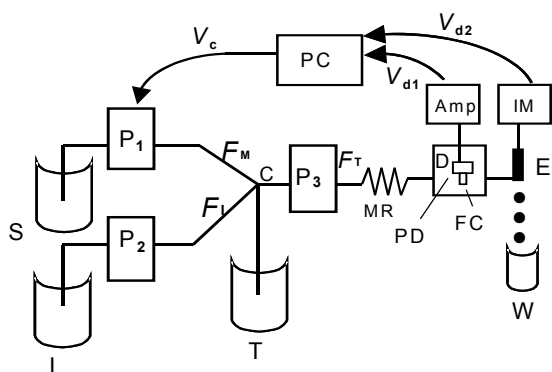


Fig. 1 Schematic diagram of the present flow system. S, sample solution; T, titrant (EDTA or EGTA); I, indicator (EBT); W, waste; P<sub>1</sub>, P<sub>2</sub> and P<sub>3</sub>, peristaltic pumps; C, confluence point; MR, mixing reactor; D, spectrophotometer; FC, flow cell; PD, photodiode; Amp, current amplifier; PC, notebook computer with an A/D-D/A converter; E, calcium ion selective electrode; IM, ion meter;  $V_c$  controller output voltage;  $V_{d1}$  and  $V_{d2}$ , detector output voltages.  $F_M$ ,  $F_I$  and  $F_T$  are the flow rate of sample, indicator and mixed solutions, respectively.

response to controller output voltages ( $V_c$ , 0 – 5 V) generated from a computer (PC; Toshiba Dynabook Satellite 1850 SA 120C/4) through an A/D-D/A converter (Measurement Computing PC-CARD-DAS16/12-AO). The total flow rate ( $F_T$ ) was held constant at *ca.* 2.91 cm<sup>3</sup> min<sup>-1</sup> by the P<sub>3</sub> pump. The titrant solution was, therefore, aspirated to the confluence point (C) at the flow rate of  $F_T - F_M - F_I$ , where  $F_I$  was a constant flow rate of indicator (*ca.* 0.31 cm<sup>3</sup> min<sup>-1</sup>). For adequate mixing of the merged solutions, a mixing reactor (knotted tubing; 0.51 mm i.d., 25 cm length) was introduced between the confluence point and a handmade flow cell (FC; 1 mm i.d., quartz tubing, *ca.* 1 mm optical aperture), mounted in the optical cell compartment of a Shimadzu UV-120-02 single beam spectrophotometer (D). The solution was radiated at the maximum absorption wavelength (625 nm) of the indicator (*i.e.*, EBT) in its free state (blue). The transmitted light was detected by a Hamamatsu S2281 photodiode. The resulting electric current was amplified with a Hamamatsu C2719 current amplifier and acquired in the computer as  $V_{d1}$  as a Microsoft Excel format at the frequency of 10 Hz. For stepwise titration using two detectors, the effluent from the photodetector was further introduced to a flow cell compartment covered the surface of a Horiba #7683 calcium ion selective electrode (E). The electrode potential was monitored with a Horiba F-53 ion meter and its output signals were acquired in the computer as  $V_{d2}$  in the similar manner to  $V_{d1}$ . A program written in Visual BASIC in house was used to generate  $V_c$ , acquire  $V_{d1}$  and  $V_{d2}$ , analyse them and graphically display the results automatically.

## 2.2. Reagents

Reagents of analytical grade, purchased from Dojindo, Katayama Chemical, Kanto Chemical, Nacalai Tesque or Wako Pure Chemical Industries were used without further purification. Sartorius Arium<sup>®</sup> 611DI deionised water was used throughout.

## 3. Principles

### 3.1. Fixed triangular wave controlled flow ratiometry

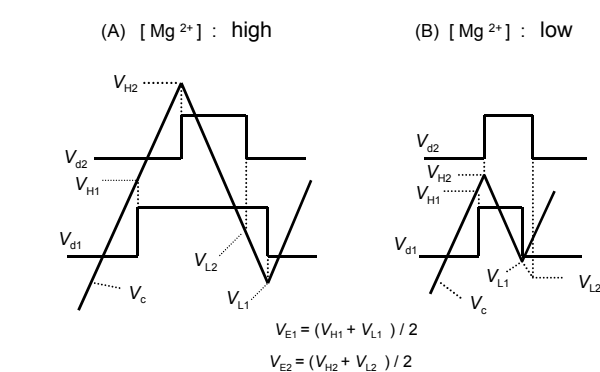


Fig. 2 Principle of simultaneous determination of Ca<sup>2+</sup> and Mg<sup>2+</sup> using photometric and potentiometric detectors. The meanings of symbols of  $V_c$ ,  $V_d$ ,  $V_H$ ,  $V_L$  and  $V_E$ : see the text.

Fixed triangular wave flow ratiometry [2], where  $V_c$  varied triangularly in a fixed range, was employed for the check of detector's signals and the determination of the levels of  $V_{d1}$  and  $V_{d2}$  each corresponding to the equivalence points of the photometry and potentiometry, respectively.

### 3.2. Feedback-based and subsequent fixed triangular wave controlled flow ratiometry

A flow ratiometry controlled by feedback-based variable triangular wave and subsequently by fixed triangular wave was applied to the high-throughput determination of Ca<sup>2+</sup> or Mg<sup>2+</sup> using EDTA as a titrant. Only the photodetector was used in this case by removing the potentiometric detector (E and IM in Fig. 1). In the feedback mode, the scan direction of  $V_c$  was reversed from upward to downward, and *vice versa*, at the instant of the sensing of the equivalence point. After the equivalence point being located three times, the fixed mode was applied so that the scan could be conducted in narrower range and at higher rate in order to further increase in efficiency. The principle of the method was described in detail before [4,5].

### 3.3. Simultaneous determination of calcium and magnesium ions by feedback-based flow ratiometry using photometric and potentiometric detectors

Figure 2 shows the principle of the simultaneous determination of calcium and magnesium ions by a feedback-based flow ratiometry using the two detectors. In this method, EGTA is used as a titrant instead of EDTA. Initially, the control output voltage ( $V_c$ ), and thus the flow rate ( $F_M$ ) of a sample solution is increased linearly;  $V_c$  determines the composition of the merged solution at the confluence point (C in Fig. 1). There is a lag time ( $t_{lag}$ ) between the merging and sensing of the solutions. The  $t_{lag}$ , which consists primarily of the physical transit time of the merged solution to reach the detector, is considered to be virtually constant because total flow rate ( $F_T$ ) is held constant.

The ratio of  $[M] / [EGTA]$ , where  $[M]$  is the sum of the concentrations of Ca<sup>2+</sup> and Mg<sup>2+</sup>, is increased with an increase of  $V_c$ . At the equivalence point of  $[M] = [EGTA]$ , the color of EBT is changed from red to blue (thus,  $V_{d1}$  increased steeply);  $V_{H1}$ , the  $V_c$  at this first equivalence point is recorded in the computer. After the first equivalence point, the reaction of Ca<sup>2+</sup> + Mg<sup>2+</sup>-EGTA → Mg<sup>2+</sup> + Ca<sup>2+</sup>-EGTA occurs, because the

formation constant of  $\text{Ca}^{2+}$ -EGTA ( $\log \beta = 11.0$ ) is much higher than that of  $\text{Mg}^{2+}$ -EGTA ( $\log \beta = 5.2$ ) [9]. At the second equivalence point ( $[\text{Ca}^{2+}] = [\text{EGTA}]$ ), the electrode potential ( $V_{d2}$ ) is increased steeply;  $V_{H2}$ , is the  $V_c$  at this point. At the instant of the sensing of the second equivalence point, the ramp direction of  $V_c$  is changed from upward to downward. That is, for the upward scan, the ramp direction is changed when the potentiometer senses the equivalence point. The  $V_c$  values ( $V_{L2}$  and  $V_{L1}$ , for potentiometry and photometry, respectively) at the equivalence points in the course of downward scan are also recorded. The ramp direction is changed from downward to upward when not the potentiometer but the photodetector senses the equivalence point (see Fig. 2A). When the concentration of  $\text{Mg}^{2+}$  is not so high,  $V_{L1}$  might be detected earlier than  $V_{L2}$ . In this case, the value of  $V_{L2}$  is automatically estimated through the extrapolation of  $V_c$  to the time when the equivalence point is detected by the electrode (see Fig. 2B). The  $V_c$  values that gives the first and second equivalence points on the mixing at the confluence point ( $V_{E1}$  and  $V_{E2}$ , respectively) are obtained as the averages of  $(V_{H1} + V_{L1}) / 2$  and  $(V_{H2} + V_{L2}) / 2$ , respectively [4,5].

#### 4. Results and Discussion

##### 4.1. Feedback-based and subsequent fixed triangular wave controlled flow ratiometry for high-throughput chelatometry with photodetector

The effect of the species (single bead string reactor (SBSR) and knotted tubing) and their length of mixing reactors were investigated in order to attain adequate mixing of solutions. Longer reactor is preferable for better mixing, but would decrease the efficiency because  $t_{lag}$  would become longer. In the preliminary experiments using  $0.1 \text{ mol dm}^{-3} \text{ CaCl}_2$  and  $0.1 \text{ mol dm}^{-3} \text{ EDTA}$  as titrand and titrant, respectively, and photodetector alone as detector, no noticeable differences were observed in the  $V_E$  values obtained using different reactors. Consequently, a knotted tubing (0.51 mm i.d., 25 cm length) that gave 5.2 s for  $t_{lag}$  was selected by taking the stability of the signals into account.

Effect of  $V_c$  scan rate of feedback-based mode on  $V_E$  was examined in the range of  $25 - 250 \text{ mV s}^{-1}$ . It took longer time

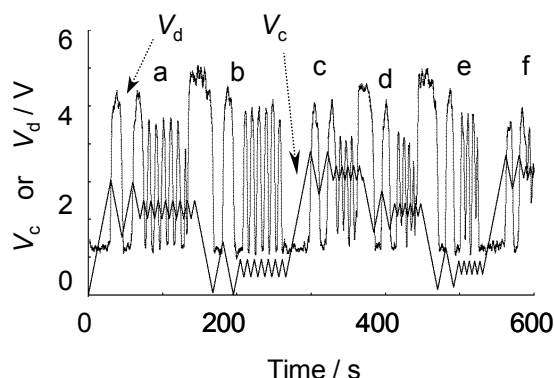


Fig. 3 Continuous titrations of a stream changing composition. Titrant:  $0.1 \text{ mol dm}^{-3} \text{ EDTA}$  ( $\text{pH} = 10$ ). Indicator: EBT. Scan rate for both of feedback and fixed modes was  $100 \text{ mV s}^{-1}$ . Titrand ( $\text{CaCl}_2$ ) concentration: (a) 0.1, (b) 0.5, (c) 0.05, (d) 0.1, (e) 0.5 and (f) 0.05  $\text{mol dm}^{-3}$ .

to find the first  $V_E$  when too low scan rate was applied to. On the other hand,  $V_c$  reached to the upper or lower limit (5 or 0 V) before the sensing of the equivalence point, when too high scan rate ( $250 \text{ mV s}^{-1}$ ) was adopted. Almost the same  $V_E$  values ( $2.90 \pm 0.01 \text{ V}$ ) were obtained irrespective of the scan rate in the range of  $25 - 200 \text{ mV s}^{-1}$ . The relative standard deviation (RSD) of  $V_E$  was, however, increased from 0.95 to 1.71 % with the scan rate. As a compromise,  $100 \text{ mV s}^{-1}$  was selected by taking both efficiency and precision ( $\text{RSD} = 1.24 \%$ ) into account.

In the feedback-based mode, the time required for one titration is  $2 \times t_{lag}$ . On the other hand, in a fixed mode, the ramp direction of  $V_c$  was changed from upward to downward and *vice versa* before the sensing of the equivalence point. In this mode, therefore, narrower scan range of  $V_c$  is preferable for higher throughput rate. However, in such condition,  $V_c$  might not be able to cover  $V_E$  when titrand concentration was changed. The effect of scan range ratio (the ratio of scan range for fixed mode and that for the last feedback-based mode) on  $V_E$  was examined. Almost constant  $V_E$  values were obtained irrespective of the scan range ratio examined (1/2, 1/3 and 1/4). The 1/3 was selected as the optimum ratio because the most stable signals were obtained at this condition.

The present system can continue fixed triangular wave operations as long as the scan range of  $V_c$  covers the equivalence point  $V_E$ , even if the analyte concentration is varied. When the analyte concentration is greatly changed beyond the covered range, the developed system was programmed to follow such change by restarting the feedback-based operation [4,5]. We therefore tested this function by drastically changing the titrand concentration. That is, 0.1, 0.5, 0.05, 0.1, 0.5 and 0.05  $\text{mol dm}^{-3} \text{ CaCl}_2$  were successively aspirated to the flow system as titrand, and were measured by using the present system. The results are shown in Fig. 3. The initial feedback-based scans were followed by fixed triangular wave scans at the time of 60.00 s (a) for  $0.1 \text{ mol dm}^{-3} \text{ CaCl}_2$ . When  $0.5 \text{ mol dm}^{-3} \text{ CaCl}_2$  came into the flow system, the  $V_c$  scan range in the fixed triangular wave control became too high to cover the new equivalence point. A feedback-based scan therefore began to the downward direction at the time of 143.55 s (b). This control was followed by a fixed triangular wave control at a time of 195.35 s. Such a series of operations were repeated over automatically when the titrand concentration was greatly changed. It took 33.1 s (time = 266.00 – 299.10 s) to locate the new equivalence point when the titrand was changed from 0.5 to  $0.05 \text{ mol dm}^{-3} \text{ CaCl}_2$ . Although the measurement included such extreme case, 50 titrations were nevertheless made during whole period of the operations (596.6 s), resulting in an average titration time requirement of as low as 11.9 s per measurement.

The present method is an absolute method, in principle, meaning no calibration curve is required as long as the titrant concentration is well standardized and the flow rate is accurately calibrated. It is, however, more practical and convenient to obtain calibration curve, because the flow rate calibration seems troublesome. In this titration, the following relationship is held at the equivalence point,

$$C_M k V_E = C_Y (F_T - F_1 - k V_E) \quad (1)$$

where,  $k$  is proportionality constant ( $= F_M / V_c$ ; ca.,  $0.37 \text{ cm}^3 \text{ min}^{-1} \text{ V}^{-1}$ ) and  $V_E$  is the value of  $V_c$  that gives the equivalence point;  $C_M$  and  $C_Y$  are the concentration of analyte and that of titrant, respectively. From Eq. (1), the following equation can

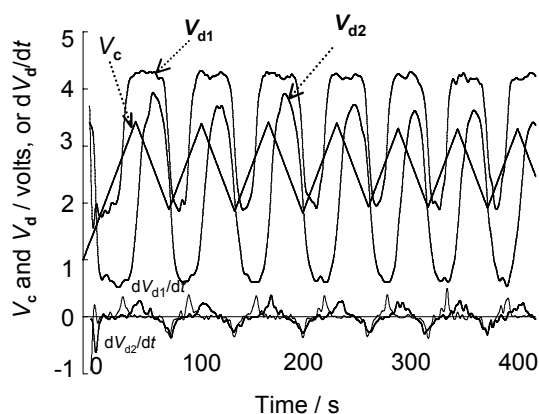


Fig. 4 Typical analytical signals for proposed stepwise titration. Titrand:  $0.1 \text{ mol dm}^{-3} \text{ Ca}^{2+}$  and  $0.01 \text{ mol dm}^{-3} \text{ Mg}^{2+}$ ; Titrant:  $0.1 \text{ mol dm}^{-3} \text{ EGTA}$ . As for  $V_{d1}$  and  $V_{d2}$ , relative values after amplification were shown here for easy comparison between them. The  $V_c$  is real control voltage signals.

be derived,

$$V_E^{-1} = k C_M / C_Y (F_T - F_1) + k / (F_T - F_1) \quad (2)$$

The linearity of the plots based on Eq. (2) was examined by titrating  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  ( $0.025 - 0.5 \text{ mol dm}^{-3}$ ) with  $0.1 \text{ mol dm}^{-3} \text{ EDTA}$ ;  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  ( $0.005 - 0.05 \text{ mol dm}^{-3}$ ) with  $0.01 \text{ mol dm}^{-3} \text{ EDTA}$ . Straight regression lines ( $r^2 > 0.999$ ) were obtained for all of the measurements.

#### 4.2. Simultaneous determination of calcium and magnesium ions by feedback-based flow ratiometry

Feedback-based flow ratiometry was applied to the stepwise titration of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  with  $0.1 \text{ mol dm}^{-3} \text{ EGTA}$  using photometric and potentiometric detectors. Figure 4 shows a typical profile of  $V_c$ ,  $V_{d1}$  and  $V_{d2}$ , for the titration of a titrand containing  $0.1 \text{ mol dm}^{-3} \text{ Ca}^{2+}$  and  $0.01 \text{ mol dm}^{-3} \text{ Mg}^{2+}$ . The slopes of  $V_{d1}$  and of  $V_{d2}$  ( $dV_{d1}/dt$  and  $dV_{d2}/dt$ , respectively) were also plotted in this figure as references. Compared with the profiles of  $V_{d1}$  in the absence of  $\text{Mg}^{2+}$ , the  $V_{d1}$  signals around their bottoms became higher and noisier probably due to the competition between EGTA and EBT for the complex formation with  $\text{Mg}^{2+}$ . The  $V_{d2}$  signals became less steep, which meant the response of the  $\text{Ca}^{2+}$ -selective electrode became slower in the presence of  $\text{Mg}^{2+}$ . As a result, the throughput rate of the measurement was lowered with an increase of  $[\text{Mg}^{2+}] / [\text{Ca}^{2+}]$  ratio. In order to eliminate such effects by  $\text{Mg}^{2+}$ , the levels of  $V_{d1}$  and  $V_{d2}$  that correspond to the respective equivalence points had carefully been determined by a fixed triangular wave controlled flow ratiometry. The determined values were  $3.0 \text{ V}$  and  $1.5 \text{ V}$ , respectively. The results shown in Fig. 4 agreed well with the theoretical plot shown in Fig. 2. The  $V_E$  obtained were  $2.349 \pm 0.016 \text{ V}$  ( $n = 12$ ) and  $2.559 \pm 0.018 \text{ V}$  ( $n = 12$ ) for the photometry and potentiometry, respectively. The time needed for one titration was  $31.0 \text{ s}$ . The  $0.1 \text{ mol dm}^{-3} \text{ Ca}^{2+}$  solutions containing different concentration of  $\text{Mg}^{2+}$  ( $0 - 0.05 \text{ mol dm}^{-3}$ ) were each titrated with  $0.1 \text{ mol dm}^{-3} \text{ EGTA}$ . The equivalence points,  $V_{E1}$  and  $V_{E2}$ , were plotted against the concentration of

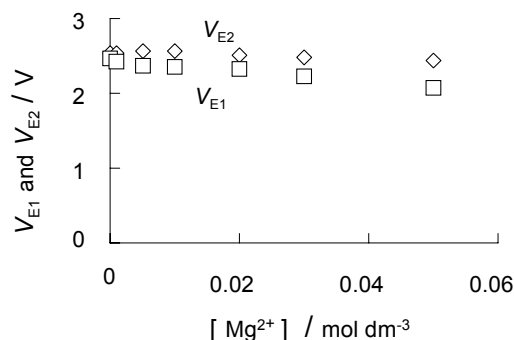


Fig. 5 The relationship between the concentration of  $\text{Mg}^{2+}$  in titrand and the equivalence points,  $V_{E1}$  and  $V_{E2}$ , obtained from photometry and potentiometry, respectively, using  $0.1 \text{ mol dm}^{-3} \text{ EGTA}$  as a titrant. The concentration of  $\text{Ca}^{2+}$  in the titrands was kept constant at  $0.1 \text{ mol dm}^{-3}$ .

$\text{Mg}^{2+}$ , as shown in Fig. 5. Almost constant values of  $V_{E2}$  were obtained irrespective of the  $\text{Mg}^{2+}$  concentration. On the other hand,  $V_{E1}$  decreased with the increase of  $\text{Mg}^{2+}$  concentration, as expected. Various concentration of  $\text{Ca}^{2+}$  ( $0.025 - 0.5 \text{ mol dm}^{-3}$ ) containing fixed concentration of  $\text{Mg}^{2+}$  ( $0.01 \text{ mol dm}^{-3}$ ) were also titrated with  $0.1 \text{ mol dm}^{-3} \text{ EGTA}$ . The reciprocals of  $V_{E1}$  and  $V_{E2}$  were plotted against the concentration of  $[\text{Ca}^{2+}] + [\text{Mg}^{2+}]$  and  $[\text{Ca}^{2+}]$ , respectively. The regression lines obtained were  $V_{E1}^{-1} = 2.24 ([\text{Ca}^{2+}] + [\text{Mg}^{2+}]) + 0.18$  and  $V_{E2}^{-1} = 2.49 [\text{Ca}^{2+}] + 0.15$ , respectively, the linearity of which were fairly good ( $r^2 > 0.997$ ).

In conclusion,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  could simultaneously be determined by a stepwise chelatometric titration with a single titrant (EGTA) and no masking agents. The method was based on a feedback-based flow ratiometry, the algorithm of which was written in house for automation. The system, which consists of two detectors set in series (*i.e.*, photometric and potentiometric detectors), was rather simple and affordable. Good throughput (*ca.*  $31 \text{ s}$  per determination) was attainable with allowable precision ( $\text{RSD} < 1.6 \%$ ).

#### References

- [1] H. Tanaka, S. Nakano, *J. Flow Inject. Anal.*, **21**, 123 (2004).
- [2] H. Tanaka, P.K. Dasgupta, J. Huang, *Anal. Chem.*, **72**, 4713 (2000).
- [3] P. K. Dasgupta, H. Tanaka, K. D. Jo, *Anal. Chim. Acta*, **435**, 289 (2001).
- [4] H. Tanaka, T. Baba, *Talanta*, **67**, 848 (2005).
- [5] H. Tanaka, T. Baba, *Anal. Sci.*, **21**, 615 (2005).
- [6] J. Alonso, J. Bartoli, J.L.F.C. Machado, *Anal. Chim. Acta*, **179**, 503 (1986).
- [7] T. Yamane, E. Goto, *Talanta*, **38**, 139 (1991).
- [8] D.G. Themelis, P.D. Tzanavaras, A.V. Trellopoulos, M.C. Sofoniou, *J. Agric. Food Chem.*, **49**, 5152 (2001).
- [9] The Japan Society for Analytical Chemistry (ed.), *Bunseki Kagaku Binran (Handbook of Analytical Chemistry, in Japanese)*, 5<sup>th</sup> ed., Maruzen, Tokyo, 2001, p. 654.

(Received February 25, 2008)

(Accepted April 25, 2008)