Droplet-counting Microtitration System for Precise On-site Analysis

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A new microtitration system based on the counting of titrant droplets has been developed for precise on-site analysis. The dropping rate was controlled by inserting a capillary tube as a flow resistance in a laboratory-made micropipette. The error of titration was 3% in a simulated titration with 20 droplets. The pre-addition of a titrant was proposed for precise titration within an error of 0.5%. The analytical performances were evaluated for chelate titration, redox titration and acid-base titration.

**Keywords** On-site analysis, titration, calcium, copper, acid consumption, river, tap water

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Introduction

A microscale analytical apparatus promises low-cost, safety, waste-saving and space-saving analysis.1 Miniaturized apparatuses are portable, and therefore they are useful for in-situ and on-site measurements evolving environmental monitoring.2,3 A microburette (0.1 mL) with a graduated glass tube and a screw-driven plunger was used for the titration of alkalinity, chloride and dissolved oxygen with errors of less than 1%.4 A micropipette connected to a micrometer-driven plunger was purchased from Gilmont Instruments, Co. This burette permits precise analysis with a smallest division of 0.002 mL for a 2-mL burette, but titration by turning the screw of a micrometer is bothersome compared to dropping by gravity. A microburette (0.15 mL, a division of 0.002 mL) used in Conway’s microdiffusion analysis5 is commercially available. The operation of this burette requires skill and calibration of the volume scale was required in our experience. A micro-flow titration was proposed,6,7 but was not suitable for on-site analysis. A compact photometric titrator with a semi-micro burette was developed for educational experiments.8 This titrator gave an error of 1 or 2% in the photometric titration of calcium and copper with EDTA. The titration was carried out without reading the graduation of the burette.6,8,10 The number of titrant droplets (each 0.05 mL) until the end point was counted for a semi-quantitative determination.9 Dropping bottles are used for this purpose in a commercial titration set, e.g., 5 μg Ca mL−1 corresponding to one droplet in the titration of calcium with EDTA.11,12 In a microplate concentration measuring method, the color change of a series of microwells with different amounts of titrant was used to obtain the end point of titration.11,12 This method is simple and suitable for on-site analysis. However, the analytical precision is restricted by the number of wells, and thereby this method is semi-quantitative.

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In the present work, a simple microtitration system was designed for precise on-site analysis. The titration was carried out by counting the titrant droplets until a visual identification of color change of indicator occurred. Analytical performances of the proposed method were compared with those of conventional titration methods. The proposed system was successfully applied to typical chelate, redox and acid-base titrations.

Experimental

Reagents and apparatus

All chemicals were of analytical reagent grade. Deionized and distilled water was purified to be ≥18 MΩ·cm by an Autopure WT101 UV ultra-purification unit (Yamato Scientific). An EDTA solution (10 mM) was prepared by dissolving ethylenediamine-N,N,N′,N′-tetraacetic acid (disodium salt, dihydrate) (Dojindo Laboratories) with water. Solutions with lower concentrations were prepared by diluting the 10-mM solution with water. All EDTA solutions were standardized against a Calcium Standard Solution (1 g L−1 of Ca(II) in 0.1 M HNO3) (Wako Pure Chemical Industries). The factor (f) of EDTA solutions was 1.000. A calcium solution (1 g L−1 of Ca(II) in 0.2 M HCl) was prepared by dissolving a CaCO3 reagent with HCl and diluting with water. The solution was standardized by EDTA titration and used for a performance test of titration. A 0.5% NN indicator (Dojindo Laboratories) was purchased and used by diluting a 5% solution (0.1 M Na2S2O3 in 0.5% 3-metyl-1-butanol) (Wako Pure Chemical Industries) with water. A Sodium Thiosulfate Standard Solution (0.1 M Na2S2O3) in 0.5% 3-metyl-1-butanol (Wako Pure Chemical Industries) was purchased and used by diluting with water. A copper solution (10 g L−1 of Cu(II) in 0.05 M H2SO4) was prepared by dissolving a CuSO4·5H2O reagent with dilute H2SO4 and diluting with water. The solution was standardized by thiosulfate titration and used for a performance test of titration. Hydrochloric acid (0.1 M) was prepared by
Titration procedure

detachable to aspirate a titration solution. After sucking the titrant solution, the microburette was set on air until over the upper part of the flow resistance tube (5). The solution was sucked into the flow-resistance tube using a dropper bulb. The stand, as shown in Fig. 1. Dropping was started by pushing the stand was set on it. The titrant solution was sucked in the microburette to a level of 1 mL by decompressing air in the dropper bulb (1). Dropping was prevented by sucking air until over the upper part of the flow resistance tube (5). After sucking the titrant solution, the microburette was set on the stand, as shown in Fig. 1. Dropping was started by pushing the solution into the flow-resistance tube using a dropper bulb. The number (n) of droplets until the end point was counted so as to calculate the titration volume by multiplying n by the volume (v) of one droplet. The value of v was preliminary measured gravimetrically by a commercial palmtop-size digital electrobalance.

For precise titration by the pre-addition of titrant, a preliminary test was carried out to determine the pre-addition volume (α ≥ 1.3 mL, see the section of Design of on-site titration) of the titrant. The titration volume was selected by changing the sample volume or the concentration of titrant added. A titrant solution containing a higher concentration of titrant is used in the preliminary test. In the pre-addition, a titrant of more than 1.3 mL was added by a micropipette into the vial with the sample and the reagent solutions. The vial was set as shown in Fig. 1. The number of droplets of the titrant until the end point was calculated to count the titration volume (α + nv).

In the on-site titration of calcium with EDTA, the volumes of water and reagent solutions were reduced to one-20th of those in the conventional titration method. An aliquot of the sample was taken into the glass vial and diluted to be about 3 mL with water. After adding 0.5 mL of a 5 M KOH solution, the solution was mixed, and then 0.1 mL of a 0.5 M hydroxyammonium chloride solution and about 0.01 g of the 0.5% NN indicator were added in this order. The titration was carried out with a 0.5 or 5 mM EDTA solution until the color change (blue from red-purple). In the pre-addition of the titrant, the titrant added was more than 1.3 mL by a micropipette before titration by dropping, e.g., α = 2 mL for the determination of 0.05 mg of calcium.

In the on-site titration of copper with thiosulfate, the volumes of water and reagent solutions were reduced to one-50th of those in the conventional titration method. An aliquot of the sample solution was taken into the glass vial and diluted to be about 1 mL with water. After adding 0.3 mL of a 20% KI solution and 0.5 mL of 2 M H$_2$SO$_4$, the solution was titrated with a 10 or 100 mM sodium thiosulfate solution until the color of the solution became pale yellow. After adding a 0.5% starch solution, the color of the solution became blue; the titration was then continued until disappearance of the blue, indicating the end point. In the pre-addition of the titrant, 10 mM sodium thiosulfate solution was used as the titrant. The titrant was added, e.g., α = 3 mL for the determination of 2 mg of copper, by a micropipette, as described concerning the titration of calcium.

The on-site measurement of acid consumption (pH 4.8) was based on acid-base titration with HCl. The volumes of water and reagent solutions were reduced to one-50th of those in the conventional titration. An aliquot of the sample solution was taken into a glass vial and diluted to be about 2 mL with water. After adding one drop of the BM indicator, the solution was titrated with 2 or 20 mM HCl until a color change (grey reddish purple from green). The acid consumption was indicated as the concentration of hydrogen ion (mM). In the pre-addition of the titrant, 2 mM HCl was used as the titrant. The titrant was added, e.g., α = 3.8 mL for the determination of 4 mM H$^+$ in a 2-mL sample, by a micropipette, as described concerning the titration of calcium.

Results and Discussion

Design of on-site titration

In the DCM method, the titration volume (V) is calculated by

\[ V = nv. \] (1)
The error or uncertainty (standard deviation, σ mL) of V includes the error (σα) caused by adding one droplet of titrant or not in the end point and the error (σγ) introduced by the reproducibility of v. From the propagation of the error (the law of propagation of uncertainty),15 σ is represented by

\[ σ^2 = v^2σ_α^2 + n^2σ_γ^2. \]  

(2)

In the DCM method, n = 20 was recommended to eliminate the counting miss and to titrate rapidly (2 min). The number of droplets was experimentally n ± 1 once in five measurement runs of V (N = 5), and thereby the standard deviation base on the unbiased variance was calculated as σα = 1/\sqrt{Nn} = 0.45. The values of other symbols obtained experimentally were v = 0.016 mL and σγ = 0.0002 mL (see the next section). The relative error (%) of σ is represented by

\[ X = 100σ/(nv). \]  

(3)

Based on Eqs. (2) and (3), using σα = 0.45 and σγ = 0.0002, the effect of n on X was simulated; curves with solid lines in Fig. 2. An increase of n decreases X. A larger value of ν gives a smaller error. The titration error is about 3% in n = 20. In the conventional titration method, a smaller error of 0.5% was estimated in titration with 10-mL of the titrant solution, when one droplet (v = 0.05 mL) added or not in the end point is the main error for a 50-mL glass burette. Therefore, regarding the analytical precision, the above DCM method is inferior to the conventional titration method. In this study, we decreased the value of X by increasing V, i.e., by the pre-addition of the titrant, in which the titrant with a constant volume (α) was added by a micropipette and then n drops of it were added by the microburette. In this case,

\[ V = nv + α, \]  

(4)

and thereby

\[ σ^2 = v^2σ_α^2 + n^2σ_γ^2 + σ_α^2, \]  

(5)

where σα is a pipetting error. The value of σα was 0.0003 mL for the 1-mL micropipette used. The relative error (%) of σ is represented by

\[ X = 100σ/(nv + α). \]  

(6)

From Eqs. (5) and (6), using v = 0.016, σα = 0.45, σγ = 0.0002 and σα = 0.0003, X < 0.5 was estimated by pre-addition of the titrant more than 1.3 mL in n = 20. The simulation results (Fig. 2) for different values of ν and n in α = 0 or 1.3 suggest that the pre-addition technique is useful to minimize the relative error (<1%). In addition, broken lines for α = 1.3 suggest that the smaller volume of ν is effective to reduce the relative error for a smaller n (n < 45).

Minimization of volume of one titrant droplet

The effects of the opening and thickness of the front end of the burette were investigated so as to minimize the volume of a single droplet. Water was dropped as the titrant, and its dropped volume was measured gravimetrically. The results (A and B in Table 1) indicate that a smaller inner diameter of the opening effectively decreases the volume of one droplet; a smaller thickness of the wall is also effective (C and D). The reproducibility (N = 5) in the titration with 20 droplets was less than 1%. On the other hand, a polytetrafluoroethylene (PTFE) tube with a small thickness was not recommended to use, because the shape of the opening deformed easily, and such a tube often released a droplet due to its swaying, when an analyst touched the DCM system. Commercially available micropipette tips were tested because of their robustness. For these tips (E and F in Table 1), the standard deviations in the measurement of the volume of 20 droplets were smaller than those for the PTFE tube. In the proposed DCM system, a 100-μL polypropylene micropipette tip (6 in Fig. 1) was used as the tip giving a smaller volume of one droplet (E in Table 1). For the micropipette tip, without controlling the dropping rate, the dropping rate was too high to count the droplets. In this study, the flow resistance of a narrow PTFE tube (5) was introduced to adjust the rate.

### Table 1  Effects of the inner diameter and thickness of the burette tip on the reproducibility of the dropping and volume of the droplet

|                | PTFE tube | Polypropylene pipette tip |
|----------------|-----------|---------------------------|
| Inner diameter/mm | A   | B   | C   | D   | E   | F   |
| Thickness/mm     | 0.5  | 0.3  | 0.25 | 0.25 | 0.5  | 1   |
| Volume of 20 droplets, V/μL | 0.571 ± 0.007 | 0.418 ± 0.012 | 0.503 ± 0.002 | 0.284 ± 0.002 | 0.3271 ± 0.0004 | 0.3714 ± 0.0005 |
| Volume of one droplet/μL | 0.029 | 0.021 | 0.025 | 0.014 | 0.016 | 0.019 |

a. Micropipette tip for 100 μL. b. Micropipette tip for 1 mL. c. Average and standard deviation (N = 5) obtained by weighing 20 droplets of water dropped successively. d. Calculated by V/20.
Table 2  Determination of calcium, copper and acid consumption by the proposed DCM method with and without the pre-addition (PD)

| Amount or concentration tested, β | Without PD | With PD | Without PD | With PD | Without PD | With PD |
|-----------------------------------|------------|--------|------------|--------|------------|--------|
| Analytical conditions             |            |        |            |        |            |        |
| Concentration of titrants/mM      |            |        |            |        |            |        |
| Factor of titrants                | 5          | 0.5    | 100        | 10     | 20         | 2      |
| Volume of one titrant droplet/mL  | 0.017      | 0.017  | 0.0092     | 0.0155 | 0.017      | 0.017  |
| Titrant volume equivalent to amount of β/mL | 0.2495 | 2.495 | 0.3139     | 3.1379 | 0.4013     | 4.013  |
| Analytical results and performances |            |        |            |        |            |        |
| Titrant volume added until the endpoint/mL | 0.252 ± 0.008 | 2.507 ± 0.008 | 0.316 ± 0.004 | 3.140 | 0.411 ± 0.008 | 4.007 ± 0.008 |
| Amount or concentration determined, γ | 0.050 ± 0.001 mg | 0.0502 ± 0.0002 mg | 2.01 ± 0.03 mg | 2.00 ± 0.014 mg | 4.10 ± 0.08 mM | 3.994 ± 0.008 mM |
| Reproducibility of γ, %            | 2          | 0.4    | 1          | —d     | 2          | 0.2    |
| Analytical error, %               | 0          | 0.4    | 0.5        | 0.05   | 2          | 0.2    |

a. Acid-consumption was calculated as concentration of hydrogen ion (mM) in titration of 2 mL of 2.00 mM Na2CO3. b. Average and standard deviation in each five titration runs. c. Pre-addition volume of titrant solution. d. The same analytical values were obtained.

Fig. 3 Effects of the length of the flow resistance tube (5 in Fig. 1) on the dropping rate (●) and the volume (○) of one droplet.

Figure 3 shows the effect of the length of the resistance tube on the dropping rate and the volume of one droplet. The length of tube did not affect the volume, and was effective to adjust the dropping rate in the range of 0.0022 – 0.0033 mL s⁻¹. The dropping rate for a length of 30 mm was suitable for droplet counting, and therefore this length was adapted to the DCM system. In the initial dropping, the dropping rate changed from 0.0026 mL s⁻¹ (the first drop) to 0.0029 mL s⁻¹ (the fifth drop), but the volume of one droplet did not change significantly (0.0162 ± 0.0002 mL, n = 20).

Evaluation of titration performances

The reproducibility and analytical error of the proposed DCM method with or without the pre-addition was evaluated concerning the determination of calcium, copper and acid consumption (Table 2). In the determination of calcium, the DCM method with the pre-addition gave a desirable reproducibility (<0.5%). The DCM method with 0.5 or 1 mM EDTA was applied to a Certified Reference Material (JSAC 0302-3, [Ca] = 13.0 ± 0.1 mg L⁻¹) of river water. Without the pre-addition, the same analytical values (12.6 mg L⁻¹ corresponding to 19 droplets of 1 mM EDTA) were obtained in the analysis (N = 2) of 1-mL sample. The resulting analytical error of 3% against the certified value was the same as that estimated (n = 19 and v = 0.016 in Fig. 2). With the pre-addition (α = 1.7 mL), the same analytical values (13.1 mg L⁻¹ corresponding to 16 droplets of 0.5 mM EDTA) were obtained in the analysis (N = 2) of a 3-mL sample. The analytical values agreed with the certified value within an error of 1%. An on-site analysis of river water was carried out with the pre-addition. The same analytical values ([Ca] = 30.00 mg L⁻¹) were obtained for N = 3, and agreed with that by the conventional titration with an error of 1%.

The determination of copper by the proposed DCM method was carried out in a laboratory in order to demonstrate the on-site quality test of the CuSO4·5H2O reagent. In this case, 3-methyl-1-butanol added in the titrants probably decreased the viscosity of titrant solutions, and thereby decreased the volume of the droplet (Table 2). The analytical performances of the pre-addition satisfied those required in the quality test (purity ≥99.5%).

In the measurement of acid consumption as the concentration of hydrogen ion, the resulting values of the reproducibility and analytical error indicate that the pre-addition was useful for precise analyses within an error of 0.5% (Table 2). An on-site measurement was applied to tap-water sample collected in an office kitchenette. The acid consumption was measured with 2 mM HCl. Without the pre-addition, The analytical result ([H⁺] = 0.32 ± 0.01 mM , N = 3) agreed with that obtained by the conventional titration with an error of 3%.

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