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Micro coulometric titration in a liquid drop

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ABSTRACT

Miniaturized coulometric titration in a liquid drop has been investigated. Assays of ascorbic acid and thiosulfate with iodine titration were chosen as models. Constant volumes of falling liquid drops containing sample or reagent are manipulated via gravimetrical force to move along a slope hydrophobic path and directed to stop or to move out from an electrode. Such manipulation is useful for delivery of sample and reagents, in a way of flow without tubing. Electrochemical generation of titrant, in this case, iodine, is started at the electrode and micro coulometric titration can be performed in a drop by applying constant current. Timing in the titration can be made via naked eye with a stopwatch or via recording with a webcam camera connecting to a computer to detect the change due to the blue color complex of the excess iodine and starch.

1. Introduction

Attention has been paid to decrease chemical waste in analytical process. This leads to downscaling chemical analysis [1,2]. An interesting property of a liquid drop may refer to constant volume which should be due to adhesive force among the liquid molecules. A liquid drop which poses around 3–50 μL in volume should be readily available for micro scale chemistry manipulation. There have been some reports for such purpose, such as using a liquid drop as interfacing system for gas sampling and analysis [3–5], and a windowless spectrophotometric system [6]. Moreover, a falling liquid drop has been proposed to function as a sample introduction to a capillary electrophoresis system [7]. In addition, titration in liquid drop has been explored for acid–base neutralization [8] and complexometric titration of calcium [9,10].

Coulometric titration has gained interest in that an active reagent may be in situ generated. The known amount of reagent that reacts with the analyte can be electrogenerated precisely by the system itself with current–time control under Faraday's Law. This type of titration has shown benefit without conventional standard reagent preparation. Various analytical techniques including potentiometry, amperometry, and spectrophotometry have been used for end-point detection [11]. There have been various applications exploiting coulometric titration including pharmaceutical analysis such as butyrolactone determination employing glass electrode for end-point detection [12]. Some organic and pharmaceutical compounds were analyzed by electrogenerating gold(III) and biamperometric end-point detection [13]. CO_2 in water could be rapidly determined by coulometric titration [14]. Coulometric titration has been incorporated in flow analysis, also with various end-point detection systems including spectrophotometric (for acid–base) [15], potentiometric (for ascorbic acid determination) [16,17]. Air transported flow system or monosegmented flow coulometric titration was developed for, aniline determination with bromine generation and amperometric end-point detection [18], and bromine number in some petrochemical samples can be determined [19]. Micro coulometric flow cell was developed for oil/water interface study [20], and bromine generation in micro flow system with chemilumiescence detection was developed for hydrazine and ammonium analysis [21]. Coulometric titration in non-aqueous medium for acid number of biodiesel [22] and ethanol [23] samples was reported.

Recently, electrowetting-on-dielectric (EWOD) phenomena have been investigated by applying voltage between electrode and liquid droplet, leading to the interfacial surface of liquid droplet and surface to be modified to suit applications, including microfluidics as a sampling device for capillary electropherograph [24,25].

We present here micro coulometric titration in a liquid drop. With simple instrumentation, a drop of sample/reagent, via gravitation force, with practically precise constant volume can be handled for moving along a hydrophobic path or stop at an electrode system. Such manipulation leads to automation in

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delivery for a sample and reagents in a way of flow without tubings. Downscaling in iodometric titration can be performed. Thiosulfate and ascorbic acid were chosen as the model study. The developed system has been demonstrated to assay ascorbic acid in real samples of vitamin C tablets.

2. Experimental

2.1. Reagents and solutions

All reagents in this work were prepared by dissolving in deionized (DI) water. A solution of KI (0.1 M, 100 mL) was prepared from 1.66 g KI (BDH, UK.) which was added with 2% w/v starch with a volume ratio of 5:1. A stock solution of thiosulfate (0.1 M) was fleshly prepared from 1.58 g $Na_2S_2O_3$ (Sigma-Aldrich, USA) and standardized using the iodometric method [26]. A stock standard solution (0.100 × M, 100 mL) of ascorbic acid was freshly prepared from 1.76 g of $C_6H_8O_6$ (MERCK, Germany) and standardized using the 2, 6-dichloro indolphenol (2,6-DCIP) method [27]. A sample solution was prepared by weighing 3 tablets of a sample and followed by grinding in ceramic mortar. Then a portion of known weight (0.0500 g) of the ground sample was dissolved in water with a final volume of 100.00 mL solution. The sample solution was then filtered with filter paper (# 1 Whatman, UK).

2.2. Manipulation of liquid drop on tilt sheet

Fig. 1 illustrates the setup of the instrument. An acrylic sheet is attached with Teflon tape that becomes platform of the system. This could be fabricated by using easily available and low cost materials. The hydrophobic surface of Teflon tape provides the high contact angle of aqueous liquid drop on surface, while the glass auxiliary electrode provides the good hydrophilic surface for aqueous liquid drop.

Falling liquid drop from a disposable syringe through the end of 1/16″ O.D. peek tube (Upchurch scientific, WA) attached to the syringe, is manually dropped to the slope acrylic plane ($7 \times 13 \times 0.1$ cm^3) covered with Teflon tape ($1.9 \times 15 \times 0.1$ mm^3; normally used for plumbing). The end of the peek tube is set to have ~1 cm space above the Teflon plane. The liquid drop of sample, having a volume of ~25 μL, would move due to gravity force along this hydrophobic tape to the electrode system and would attach, due to adhesive force of aqueous drop and hydrophilic surface of glass, to the end of hydrophilic surface of disposable glass dropper of the auxiliary electrode which is set ~2 mm above Teflon tape. After a drop of the reagent (a mixture of iodide and starch) from another syringe falls down and moves along the path, it stops and merges with the sample drop. By this, the total volume would become ~50 μL. The whole drop still hangs on the glass auxiliary electrode. When, another drop (water from the third syringe) comes to merge, the gravitational force of the whole liquid drop becomes high enough to make the drop detach and moves out from the glass auxiliary electrode. One factor affecting manipulation of liquid drop in this format also involves the angle of tilt sheet. The tilt sheet in a higher slope position results in faster movement of the drop along the hydrophobic path. If it is in too high slope, it would not be easy to control the movement and also the drop will not be easy to stop at the electrode. The angle would be adjusted to meet the ability in manipulating the liquid drop. In this experiment, the suitable tile sheet angle was found to be ~50° from horizontal plane. The tilt sheet angle was estimated by applying basic trigonometry.

Positions of the syringe ends, for the sample and that for the reagent should be arranged to be above the Teflon plane for 3–4 mm to let liquid drop freely. The path for the liquid drop moving should not be too long. In this experiment, it was about 5–10 cm.

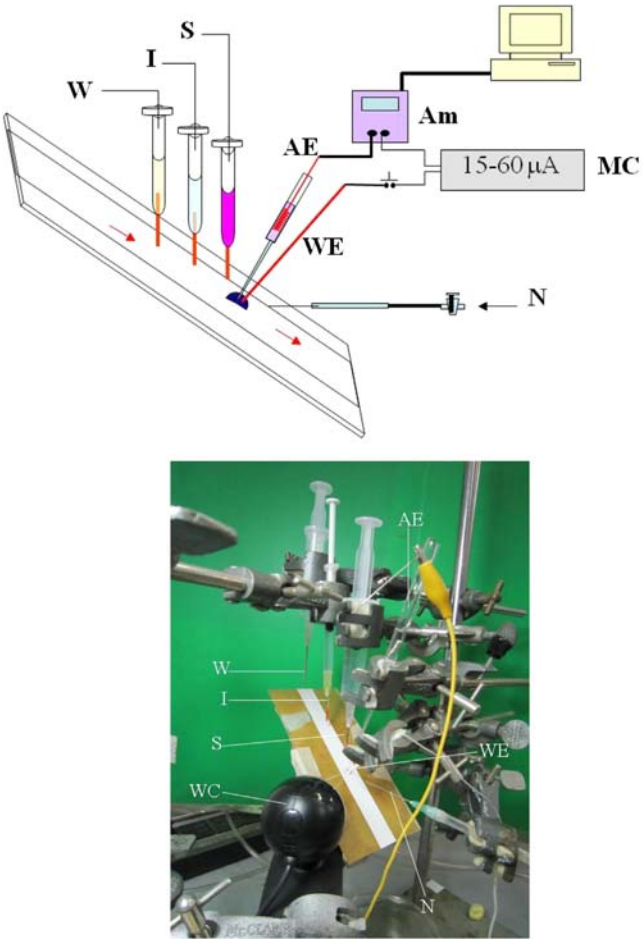

Fig. 1. Setup of micro coulometric titration of liquid drop on tilt sheet; W=DI water, I=iodide+starch, S=sample, WC=web camera, AE=auxiliary electrode, WE=working electrode, N=nitrogen gas through syringe needle, Am=digital ammeter, MC=micro coulometer.

The positions of the auxiliary and working electrodes would also affect the attachment of the liquid drop cell and effective surface area of the electrode for electrochemical generation of iodine. If the auxiliary electrode was arranged to be on the upper position relatively to working electrode, effective surface area of the working electrode was found to be better, since liquid drop cell prefers to attach to the hydrophilic glass body of auxiliary electrode.

2.3. Micro coulometric titration system and procedure

A liquid drop can behave as a micro coulometric cell ("coulometric drop cell"). The electrode system is composed of a working electrode (WE) made of platinum wire (0.5 mm diameter) or stainless steel and an auxiliary electrode (AE) fabricated from a disposable glass dropper. The auxiliary electrode contained 2% agar in 3 M KCl which had been boiled for ~5 min and filled to set as gel at room temperature at the end of the disposable glass dropper. An internal solution was 3 M KCl and the spiral shape of stainless steel wire (0.5 mm diameter) was dipped in the 3 M KCl solution. It was connected to the constant current source as shown in Fig. 1.

The constant current source was fabricated in-house by using 3-terminal adjustable voltage regulator, LM317 (ST microelectronics, Singapore). The operating current was designed in the range of 15–

60 μA. Number of moles of the electrogenerated iodine was calculated from the total charge through Faraday's constant. The total charge of coulometric operation (titration) was evaluated from the titration time (the time period when starting to apply the current until the appearing of blue color) and the current observed and recorded by a digital multimeter (UNI-T, model UT60A, China) interfacing to computer through serial port.

As described in the above section that a sample would drop and move along the hydrophobic Teflon path and would stop at the AE. A drop of the reagent (a mixture of KI and starch) would also fall down to the Teflon tape plane and would move along the plane before stopping at the AE to merge with the sample drop, previously stopped at the point. The sample and the reagents would blend to each other with the aids of nitrogen gas blowing (~300 mL/min) through a disposable hypodermic syringe needle (# 21, 0.8 mm O.D., Nipro Corporation, Japan). This would create coulometric drop cell as depicted in Fig. 1, and the VDO clip attached. By applying constant current, coulometric titration could be started.

The end point in the coulometric titration could be detected by the appearance of blue color due to excess iodine and starch. This could be observed by naked eye with a stopwatch or recording as VDO via computer through webcam camera (C200, Logitech, www.logitech.com) [28] of which driver and software are available for download on the website. The timing for the titration can be evaluated via the time appearing on the VDO recording. After the titration process completes, water from the third syringe (see Fig. 1) was dropped. It would move along the path to attach the coulometric drop cell at the AE, becoming a bigger drop and would leave out from the electrode system. The electrode system could be cleaned up in such a way.

3. Results and discussion

3.1. Falling liquid drop volume

As a liquid drop is formed by the adhesive force of liquid–liquid interface, the liquid prefers to interact themselves more than interacting with air. In case of the falling liquid drop which flows from, for example, end of tubing, it forms a pendant shape. The size expanding leads to increase in weight, and finally, it falls down. As a result, with the same tubing size, viscosity and liquid surface tension, the liquid mass which forms a falling drop would be the same for every falling liquid drop. Therefore, the falling liquid drop volume would be the same. This phenomenon is useful for miniaturizing an analytical system without the need for using complicate and/or expensive volume measuring device.

The volume of falling liquid drop from the end of tubing can be expressed through the Eq. (1) [29].

$$V = 2\pi r\sigma/\rho g \quad (1)$$

Where, V=volume of liquid drop, r=tubing radius, σ=surface tension of liquid, ρ=density of liquid, and g=gravitational force constant.

In this work, the volume of solution could be estimated from the average weight obtained by weighing 5 drops (with 5 replications) using 4-decimal balance. Then the average volume of drops was estimated via density of 1 g/mL. With the surface tension of falling drop solution was assumed to be constant, the volume of droplet can be adjusted by changing the drop head orifice guided by Eq. (1). In this experiment, the droplet volume, when using 1/16″ O.D. capillary peek tube (or 1.588 mm), was found to be 25.8 μL (using the Eq. (1), the calculated volume would be 36.7 μL), while a falling drop volume will decrease to be 6.8 μL (the calculated value by the Eq. (1), being 8.33 μL), for using 0.35 mm O.D. fuse silica capillary.

3.2. Evaluation of some analytical parameters

There are 2 steps in coulometric titration, namely, (1) electrochemical generating for the titrant and (2) the chemical reactions of the generated titrant and sample. These 2 steps must be fast enough to provide the complete chemical reaction so that the end point can be observed easily and correctly. The operating current involves accuracy and precision of titration timing. Also the convection of sample and reagents in the coulometric drop cell is to be effective enough to provide such effective mixing and leading to complete reactions.

It was observed that employing higher operating current, such as 60 μA, would result in higher rate in producing the titrant and due to limitation in slower rate of mixing of the titrant with sample via convection, even applying nitrogen gas blowing to the drop. The not-yet reacted titrant in the drop would make the color change before reaching the equivalent point; leading to negative error result. Table 1 illustrates the effects of operating current and volumes of liquid drop (as a coulometric cell) to the time of color change of the indicator for a blank solution. The time for color change would take longer for the smaller operating current; the timing for the color change of indicator for the blank solution is taken into account for the blank value (or blank timing) in the analysis procedure.

It was observed that KI concentrations (0.01, 0.1 and 1.0 M) affected the indicator color change. The lower KI concentration (0.01 M) provides longer timing for change in indicator color, within ~2–3 sec, while 1 and 0.1 M KI provide faster timing (~1–2 sec). Since the higher KI concentration, the higher mass transfer rate of iodide to working electrode, although mass transfer rate was promoted with convection from nitrogen gas blowing.

The nitrogen gas blowing through syringe needle was employed to promote better convection in the coulometric drop cell. The higher flow rate of nitrogen gas makes liquid drop cell detach and spin out of electrode. The longer time for purging nitrogen gas to the liquid drop cell also makes water evaporate. It was found that for a total volume of coulometric drop cell of ~20 μL, blowing nitrogen gas longer than 5 min can make total volume of liquid drop cell decrease significantly. The decrease in coulometric drop cell would consequently affect the effective surface area of working electrode for electrochemical titrant generation. However, from previous investigation on using a liquid drop for gas sampling interface [30], it was found that the higher humidity atmosphere can decrease evaporation in coulometric drop cell. Therefore, purging of humidified nitrogen gas for convection in liquid drop cell would help to decrease the water evaporation from coulometric drop cell.

Hence, the suggested condition for further analysis should be 20–60 μA of applied current for 0.1 M KI and 1 liquid drop (V=27 μL) sample volume, 300 mL/min of nitrogen gas flow rate.

Table 1
Timing of color change of the indicator for a blank solution with different operating currents and volumes of the coulometric drop cells.

Volume of coulometric drop cell (μL)	Time of color change (s)							
	Operating current (μA)							
	20		30		40		60	
24	4.35	±0.40	2.25	±0.24	1.98	±0.14	0.45	±0.06
41	4.28	±0.44	2.27	±0.19	2.00	±0.11	0.88	±0.08
50	2.97	±0.14	2.03	±0.10	1.73	±0.09	1.28	±0.08

Triplicate results: mean ± SD.

3.3. Thiosulfate determination

The standard thiosulfate solution which was standardized by using standard iodate in presence of iodide [26] was used for verifying the proposed micro-coulometric titration in a drop. A change of color due to complex of starch and excess iodine was monitored by using a simple webcam camera [28]. The constant current source provides a simple system in microampere level (40 μA). The results of the thiosulfate determination by using the developed micro coulometric titration agree with those by the standard iodine titration with KIO_3 as shown in Table 2, having the correlation: $y=1.014x$ and R being 0.9987.

3.4. Ascorbic acid determination

The ascorbic acid was determined by employing the proposed method compared to the standard method of ascorbic acid determination (the 2, 6-dichloroindolphenol titration) [27] for synthetic samples (Table 3) and real vitamin C samples (Table 4). Working electrodes of a platinum wire as well as a stainless steel wire (as to serve cost effective purpose) were employed. It could be observed that the results obtained by using the stainless steel having higher values than that using the platinum and that of the standard method. This could be due to the addition oxidation reactions of some components of the stainless steel producing higher charge.

It could be noted that webcam camera could be optional recording the change of the indicator color. Higher degrees for automation could be made by connecting the current source to the computer and employing automatic end point detection (such as potentionmetric, amperometric or optical detection). In such a way, the computer would be able to start the titration, to count the titration time and to continuously acquire the generated current and the detection signal (potential, current or absorbance, for instance), allowing the acquisition of titration curve (charge versus detection signal) and better precision would be obtained.

Table 2
Determination of thiosulfate using the micro coulometric titration in a liquid drop and the conventional iodometric titration.

Experiment number	Thiosulfate concentration (mM)			
	The proposed micro coulometric titration[a]		Conventional titration	
1.	1.0	± 0.0	0.98	± 0.01
2.	1.9	± 0.1	1.92	± 0.01
3.	2.9	± 0.0	2.93	± 0.08
4.	4.0	± 0.1	3.86	± 0.04
5.	4.8	± 0.0	4.80	± 0.06
6.	5.6	± 0.0	5.63	± 0.03
7.	6.9	± 0.0	6.73	± 0.12

Triplicate results: mean ± SD.
[a] Detection with the naked eye.

Table 3
Determination of ascorbic acid in synthetic sample solution employing both platinum and stainless steel as working electrode and the standard 2,6-DCIP titration method [27].

Synthetic sample	Ascorbic concentration (mM)					
	The proposed method[a]				2,6-DCIP method	
	Platinum electrode		Stainless steel electrode			
1	0.5	± 0.0	–	–	0.46	± 0.01
2	1.0	± 0.0	1.0	± 0.0	0.96	± 0.02
3	1.4	± 0.1	1.6	± 0.1	1.49	± 0.01
4	1.9	± 0.1	–	–	1.81	± 0.03
5	2.6	± 0.0	2.5	± 0.1	2.57	± 0.02
6	–	–	3.2	± 0.1	2.92	± 0.16

Triplicate results: mean ± SD.
[a] Detection with the naked eye.

Table 4
Determination of ascorbic acid contents in commercial vitamin C tablet samples using the proposed and the 2, 6-DCIP titration [27] methods.

Sample	Ascorbic acid content (g/tablet)					
	The proposed method[a]				2,6-DCIP titration	
	Platinum electrode		Stainless steel electrode			
Brand 1.	0.95	± 0.02	0.95	± 0.05	0.931	± 0.002
Brand 2.	0.98	± 0.01	1.00	± 0.10	0.954	± 0.001
Brand 3.	0.97	± 0.01	1.49	± 0.34	0.968	± 0.002
Brand 4.	1.02	± 0.01	1.28	± 0.07	0.954	± 0.002

Triplicate results: mean ± SD.
[a] Detection with the naked eye.

4. Conclusion

Micro coulometric titration in a liquid drop with electrochemical generation for iodine was proposed as an approach for downscaling analysis. Thiosulfate and ascorbic acid were chosen to be models to test the performance of the proposed systems. This system is simple and cost effective. It consumes very low sample and reagent(s) volumes, in microliter levels, with a simple falling drop phenomenon. This simple manipulation of liquid drop on a slope hydrophobic path by gravity provides a novel analytical system that is similar to a flow system but without tubing. The proposed system should be useful to various applications such as the total antioxidant compound determination in human serum which needs a low volume of sample solution [31,32], gas sampling interface on liquid drop [30]. Further development with some other types of detection and to provide higher degrees of automation has been in progress.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.04.039.

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