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Microscale chemistry-based design of eco-friendly, reagent-saving and efficient pharmaceutical analysis: A miniaturized Volhard's titration for the assay of sodium chloride

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ABSTRACT

This work demonstrates the extended application of microscale chemistry which has been used in the educational discipline to the real analytical purposes. Using Volhard's titration for the determination of sodium chloride as a paradigm, the reaction was downscaled to less than 2 mL conducted in commercially available microcentrifuge tubes and using micropipettes for the measurement and transfer of reagents. The equivalence point was determined spectrophotometrically on the microplates which quickened the multi-sample measurements. After the validation and evaluation with bulk and dosage forms, the downsized method showed good accuracy comparable to the British Pharmacopeial macroscale method and gave satisfactory precision (intra-day, inter-day, inter-analyst and inter-equipment) with the relative standard deviation of less than 0.5%. Interestingly, the amount of nitric acid, silver nitrate, ferric alum and ammonium thiocyanate consumed in the miniaturized titration was reduced by the factors of 25, 50, 50 and 215 times, respectively. The use of environmentally dangerous dibutyl phthalate was absolutely eliminated in the proposed method. Furthermore, the release of solid waste silver chloride was drastically reduced by about 25 folds. Therefore, microscale chemistry is an attractive, facile and powerful green strategy for the development of eco-friendly, safe, and cost-effective analytical methods suitable for a sustainable environment.

1. Introduction

Due to an increasing environmental concern and conscience, the implementation of green philosophy and methodologies has gained more attention. In the analytical chemistry discipline, people have realized that the analytical process may have adverse impacts on the environment such as by the generation and release of toxic laboratory wastes. As a result, attempts have been made to develop and use analytical procedures with eco-friendly feature, in addition to other prerequisites, e.g. accuracy, sensitivity and precision. Among green analytical strategies, the reduction of reagents and minimization of waste have attracted wide interest and have been the subjects in a number of researches. For example, sequential injection analysis and multicommuted flow systems capable of dispensing microvolumes of samples and reagents into a reaction coil have been developed for the determination of several analytes [1–3]. Chromatographic separation time has been shortened by the use of capillary high performance liquid chromatography [4] or ultra-performance liquid chromatography [5], thereby lowering the amounts of mobile phase consumed and waste generated. Despite elegant analytical performance and dramatically green feature of these techniques, their drawback may come up with some laboratories with fiscal limitation because such operations sometimes require costly and sophisticated instrument acquisition or modification.

To solve these problems cost-effectively, we aimed to set up a facile downscaled titration that could be achieved in few milliliters of reaction by using equipment commonly available in the quality control laboratories. In this work, Volhard's titration for the determination of sodium chloride based on the analysis of chloride anion was used as a paradigm. The assay consists of the precipitation of chloride with a measured excess of silver nitrate in nitric acid medium and the titration of this excess with a standard solution of ammonium thiocyanate. Once the equivalence point has reached, the first excess of thiocyanate reacts with ferric ion indicator, forming red ferric thiocyanate complex. Usually, immiscible organic liquid such as nitrobenzene or dibutyl phthalate is added to coat silver chloride precipitates prior to the back titration,

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preventing them from reacting with thiocyanate. Although Volhard's method conforms with the characteristics required for titrimetric assay and is more rapid and less subject to interference by some anions compared with other methods such as Mohr's, it has some disadvantages. Firstly, as an argentometric titration, its drawback is the relatively high cost of silver nitrate which fluctuates widely with the world price of silver. Secondly, silver chloride and silver thiocyanate formed in the analysis produce milky background which masks the color of ferric thiocyanate, leading to a difficult and imprecise visual detection of the end point. Thirdly, these precipitates may turn to problematic solid wastes after the assay, causing clogging to the drainage system. Lastly, the method is undesired by the use of extremely carcinogenic nitrobenzene or environmentally harmful dibutyl phthalate.

Here, we report our success on applying "microscale chemistry" for the development of a cost-effective miniaturized Volhard's titration with markedly reduced reagent consumption and waste generation, thereby was saver and safer for health and the environment. The general and specific consideration in the method design process was described and discussed. After the optimization, the analytical performance of proposed miniaturized titration was evaluated and compared with the macroscale pharmacopeial method to ensure its accuracy and precision for the assay of sodium chloride in bulk and pharmaceutical preparations. Lastly, greener and saver features of the proposed method were demonstrated.

2. Experimental

2.1. Materials and apparatus

Standard sodium chloride (Sigma, St. Louis, MO, USA) was of 99.9% purity. All other chemicals were purchased from Merck (Darmstadt, Germany). Deionized water obtained from Millipore Water System was used throughout the experiments. The sodium chloride raw material and commercial formulations were obtained from the local pharmaceutical manufacturers in Thailand. The volume-adjustable micropipettes, microcentrifuge tubes and pipette tips made from recyclable materials were of Eppendorf (Hamburg, Germany). The absorbance measurement was done on polystyrene 96-well microplates supplied by Corning (NY, USA) by using a plate reader (Packard Bioscience Company).

2.2. Protocols for the assay of sodium chloride

2.2.1. Proposed miniaturized photometric titration

Sodium chloride injection and dextrose–sodium chloride injection were directly subjected to the assay. Solid samples, i.e. standard sodium chloride powder, sodium chloride raw material and sodium chloride tablets were prepared as solutions consisting of about 0.9% sodium chloride by dissolving an accurately weighed about 180 mg of the powder with deionized water in a 20-mL volumetric flask. Throughout the assay, the measurement and transfer of solutions were done by means of calibrated micropipettes. Starting from the sample solutions, 550 μL aliquot was taken into a 2-mL microcentrifuge tube. After the addition of 200 μL of 6 M nitric acid, 200 μL of 5% ferric alum and 950 μL of standard 0.1 M silver nitrate, the solution was vortexed for 10 s and centrifuged at 10,000 rpm for 2 min to remove the precipitate. The supernatant was then transferred into five 1.5-mL microcentrifuge tubes, 200 μL each. Standardized 0.01 M ammonium thiocyanate titrant was added to titrate the residual silver nitrate in each tube with different volumes over the post-equivalence-point range (e.g. 110, 120, 130, 140 and 150 μL if the equivalence point was supposed to be 100 μL) and vortexed. After spinning down the silver thiocyanate precipitate at 10,000 rpm for 5 min, 200 μL of red colored supernatant from each tube was transferred into a 96-well microplate for absorbance (A) measurement at 450 nm. The absorbance of the reaction solution without titrant added was also read as a background value and used for further subtraction.

In order to obtain the equivalence point, a plot was drawn of titrant volume added in the X-axis. The Y-axis represented the absorbance values (A'') which were corrected for the dilution effect and then subtracted by the absorbance background read from the solution without the addition of titrant (A_{bg}) by using these formula;

$$\text{Dilution-corrected absorbance}\,(A') = \left(\frac{V_0 + v}{V_0}\right) A_{read\ \ from\,instrument}$$

$$A'' = A'_{at\,the\,volume\,added} - A_{bg}$$

where V_0 represented the initial volume of the sample solution (200 μL in this case) and v represented the volume of added titrant.

The volume of ammonium thiocyanate titrant used to reach the equivalent point was determined from the X-axis intercept as computed by using the linear regression equation. Finally, this volume was used for the calculation of the sodium chloride content based on the reaction stoichiometry and the dilution factors.

2.2.2. Pharmacopeial reference method

The macroscale titration was carried out for the comparison purpose by following the assay for sodium chloride tablets as described in The British Pharmacopoeia (BP) 2010 [6]. This method was also employed in this study for the determination of sodium chloride in raw material. The procedures which were entirely carried out in Erlenmeyer flasks, started with the dissolution of powder containing 0.2 g sodium chloride in 35 mL of water. Then, 15 mL of nitric acid, 5 mL of dibutyl phthalate and 50 mL of 0.1 M silver nitrate were added and the solution was vigorously shaken for 1 min. Five milliliters of 10% ferric alum was added and the solution was titrated with 0.1 M ammonium thiocyanate until a permanent reddish brown color was obtained.

In the cases of liquid samples, e.g. sodium chloride injection, the assay began with 25 mL of sample solution instead of the powder and was then run by the same procedures as those for the sodium chloride tablets.

2.3. Method validation

The accuracy of the miniaturized titration method was studied by the assay of sodium chloride solutions with known concentrations. These solutions were accurately prepared by dissolving pure standard sodium chloride in deionized water to obtain three levels of exact concentrations i.e. 0.855, 0.900 and 0.945% (w/v), which corresponded to 95%, 100% and 105% of 0.9% (w/v) sodium chloride, respectively. For each concentration, six replicate determinations within the same day were carried out. The accuracy was evaluated from the percent relative accuracy as calculated by using the equation;

$$\%\text{relative accuracy} = \frac{\text{concentration of NaCl found in the assay}}{\text{concentration of NaCl prepared}}$$

The % relative accuracy within 98–102% indicated an acceptable accuracy of the method.

The precision of the proposed method was evaluated in terms of repeatability (intra-day precision) and intermediate precision. The intra-day precision was determined using six independent 0.9% sodium chloride test solutions within the same day while inter-day precision was evaluated by the assays on three different days. The intermediate precision was also evaluated by using three different analysts and three different micropipettes ($n = 3$). The precision results were reported as the % of relative standard deviation (RSD)

where the value below 2.0% was recommended. In addition, the analysis of variance (ANOVA) was used as a tool to verify the internal validity of an analytical procedure.

3. Results and discussion

3.1. Method design and optimization

3.1.1. Rationale and general consideration

As one of the viable means in green chemistry, "microscale chemistry" aims to reduce the chemical use to the minimum level at which experiments can be effectively performed without compromising the quality of chemical applications [7,8]. In practical, this approach can be performed by several ways, i.e. (i) scaling down of reagent use, (ii) shift from large to miniature labware in transfer, storage and reaction devices and (iii) use of multi-sample observational tools. From the available information, microscale chemistry has been originally introduced and devoted mostly to academic activities for downsizing the experiments taught in chemistry classes [8–11]. However, its application for the real situations in quantitative analytical field is rare, probably due to the doubt on its validity after the operating scale is reduced. Among a few reports, the microscale Kjeldahl system designed by Campins-Falco et al. for nitrogen determination in environmental waters is an example showing that the procedure is faster, cheaper, eco-friendlier and easier than macroscale system, but giving the same level of the accuracy [12]. Thus, the results from our study extensively substantiate that the approach is not only valuable for educational activities but also practical for the industrial purposes, at least for the pharmaceutical quality control tasks.

In the present study, 1.5- and 2-mL polypropylene microcentrifuge tubes were chosen as the reaction devices because they were easily handled when vortex mixing or centrifugation was required. For the precise measurement and delivery of solutions at microliter level, calibrated commercial micropipettes were used. The spectrophotometric observation of the endpoint was used in lieu of visual method because human eyes cannot proficiently detect a little change of color in the titration with such small volumes. As a result, more accurate, more precise and unbiased equivalence point was obtained by the use of photometric titration. Ideally photometric titration was performed by continuously circulating the titrated solution through a flow-through cell and back to the titration vessel for data collection. Lacking this special equipment, one may carefully transfer a portion of the analyte to the absorption cell for each measurement. However, special care must be taken to return it without loss to the titration vessel before adding more titrant and repeating the procedure. In addition, the continuous titration was not suitable for the precipitate-forming reaction where the determination of the endpoint was based on the colored species soluble in the supernatant since the separation of precipitates must be done prior to the spectrophotometric measurement. Consequently, we set up the solutions in five separate tubes to which five different volumes of titrant were then added for the individual back titration. After removing the silver thiocyanate precipitates, the absorbance of red ferric thiocyanate formed in each tube was read on 96-well microplates, facilitating and quickening the measurement of multi-samples. Nevertheless, it was found that typical plastic micro-cuvettes could also be used and gave the satisfactory results of analysis (data not shown). Like other photometric titrations, the equivalence point was determined from a plot between the corrected absorbance values versus the volume of titrant added. Fig. 1 illustrates a plot example showing the *X*-axis intercept which corresponds to the equivalence point of the titration.

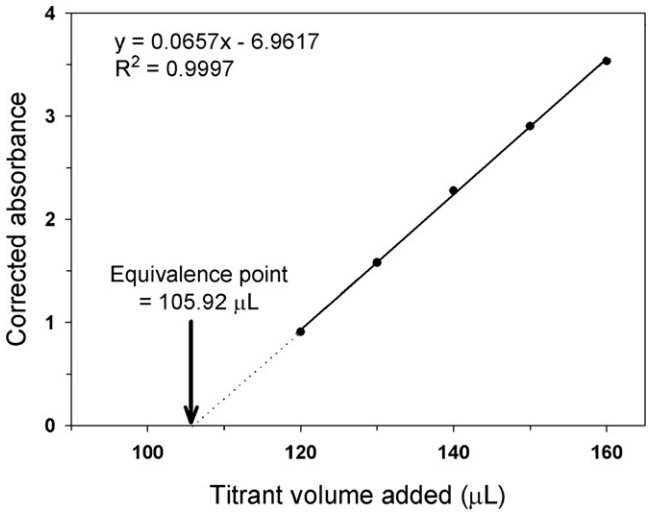

Fig. 1. Example of plot between the corrected absorbance values and titrant volumes added, showing the *X*-axis intercept from the graph extrapolation which represents an equivalence point of titration. In this titration, 0.0998 M silver nitrate, 0.0101 M ammonium thiocyanate and 200 μL of 5% ferric alum indicator were used.

3.1.2. Selection of the volumetric solutions' concentration and volume

The amounts (a function of concentration and volume) of silver nitrate and ammonium thiocyanate solutions exerted a remarkable effect on the correctness of the results since sodium chloride content was calculated from the difference between total moles of silver ion added and the excess moles of silver ion titrated by thiocyanate ion. By this reason, their concentrations and volumes for the protocol were delicately chosen based on the following criteria. First, the used amount was kept to the minimum, but it must not be too small to cause significant pipetting errors. Second, the concentration of solutions could be lowered, e.g. by using 0.01 M instead of 0.1 M so that larger volumes were used to obtain equivalent moles. Third, the total volume of reaction must not exceed 1.5 or 2 mL which is the maximum capacity of microcentrifuge tubes used and must be enough for the subsequent individual back-titrations. Forth, if possible, 200 μL was a preferred volume since pipetting this volume by the use of 200-μL micropipette caused the least systematic and random errors compared with 1000-μL micropipette as guided by the manufacturer. And last, the excess of silver nitrate added should be sufficient for the titration of samples containing 110% labeled amount, covering the maximum pharmacopeial purity rubric of sodium chloride raw material and all preparations. To meet all these criteria, volumetric solutions were used in the concentration and volume as described in the standard protocol. With these quantities, the equivalence point of the titration was in the range of 20–200 μL for the samples with 90–110% labeled amount.

3.1.3. Substitution of toxic coating reagent with the precipitate removal step

Since the metathesis of silver chloride to silver thiocyanate ($AgCl + SCN^- \rightarrow AgSCN + Cl^-$) causes an over-consumption of titrant, resulting in lower value for the sodium chloride content in the sample, various methods have been devised to prevent this error. In the past, nitrobenzene was added to coat the silver chloride precipitates. However, this practice is normally discouraged because nitrobenzene is extremely carcinogenic. Later, BP has replaced this reagent by the use of dibutyl phthalate. Nevertheless, this chemical is still harmful for the operators and aquatic organisms. A safer and environmentally friendlier procedure is to simply remove solid silver chloride from solution prior to performing the

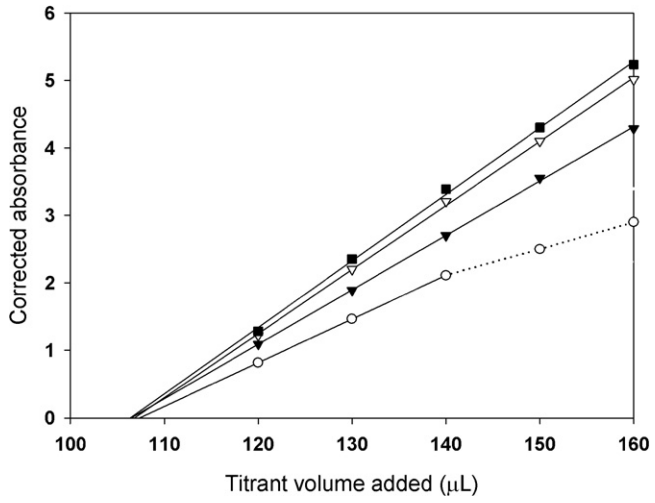

Fig. 2. Effect of indicator concentration on the analysis, as investigated by the use of 200 μL of 2.5% (○), 5% (▼), 10% (▽) and 20%(■) ferric alum for the titration.

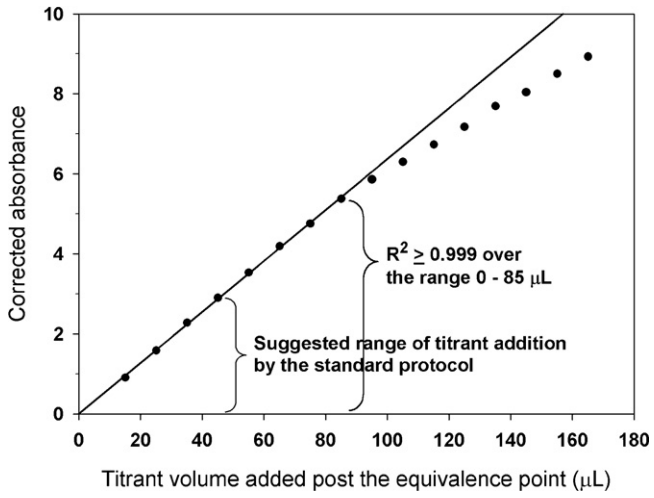

Fig. 3. Linearity of the plot between the corrected absorbance values and titrant volumes added post the equivalence point. In this titration, 0.0998 M silver nitrate, 0.0101 M ammonium thiocyanate and 200 μL of 5% ferric alum indicator were used.

back titration. In our experiments, the separation of precipitates from the solution could be done with ease by centrifugation at a sufficient speed for a few minutes. It was found that, in comparison with the use of large conventional Erlenmeyer flasks, microcentrifuge tubes were superior devices for this purpose. Mutually, the favorable condition was adjusted by the addition of nitric acid to the solution in the early step of the assay to increase the ionic strength and facilitate the coagulation of the colloidal precipitates. A further advantage was that protons from the nitric acid helped to displace adsorbed silver ions. This event rendered the ions remaining in the supernatant; otherwise they would be lost along with the silver chloride, contributing to an error in the amount of excess silver ions when determined in the subsequent titration. From the experiments, the assay of 0.9% sodium chloride employing dibutyl phthalate-coating step versus direct removal of precipitate (no dibutyl phthalate added) yielded the results without significant difference (data not shown). Hence, these toxic reagents were no longer used in our proposed procedure.

3.1.4. Optimization of ferric indicator concentration

Scaling down the reagent use to the minimum level offers several benefits; less waste, less exposure to toxic chemicals and less cost on chemical purchase and waste management. However, it must be assured that the methods still achieve the same level of analytical rigor. Here, we investigated the optimal concentration of ferric alum by adding fixed 200 μL of the indicator solutions whose concentrations were varied (2.5, 5, 10, 20 and 30%) into the reactions. This resulted in $[Fe^{3+}]$ of 0.007–0.079 M at the equivalence point. As shown in Fig. 2, the absorbance owing to ferric thiocyanate and the slope of plots increased with increasing ferric concentration. Nonetheless, *X*-intercepts which indicated the volume of titrant used at the equivalence point for all $[Fe^{3+}]$ tested were not statistically different. Two-hundred microlitres of 5% indicator solution gave the satisfactory endpoint coloration that could be directly measured without extra tedious dilution step. An excellent linearity ($r^2 = 0.9997$) between the absorbance values and the volumes of titrant added was observed over at least 50-μL range post the equivalence point. In another aspect, it was the most economical quantity. Thus, this indicator concentration was used in the standard assay protocol.

Table 1
The results of accuracy studies ($n = 6$) performed at three concentration levels of standard sodium chloride solutions.

Actual concentration (% w/v)	Found concentration (% w/v)	Relative accuracy (%)	RSD (%)
0.855	0.854	99.91	0.089
0.900	0.899	99.85	0.140
0.945	0.946	100.16	0.062

3.1.5. Linear relationship between the absorbance and the volume of titrant added

For a successful photometric titration it is necessary that the measured species obey Beer–Lambert's law, maintaining the relationship $A = kbc$. With the optimized reagent amount and operating condition, the relationship between the volume (or mole) of ammonium thiocyanate added after the equivalence point of back titration and the absorbance of red ferric thiocyanate after corrected for the dilution and background effect was remarkably linear over a broad initial range (Fig. 3). In fact, even the titrant addition of about 50 μL post the equivalence point was enough for the plot as suggested in the proposed protocol, it was found that the excellent linearity ($r^2 > 0.999$) extended up to 85 μL of titrant addition.

Table 2
The method precision results as determined by the assay of 0.900% standard sodium chloride solution.

Intra-day and inter-day precision	Day 1	Day 2	Day 3	Inter-day
Mean of % relative accuracy	99.85	99.83	99.92	99.87
%RSD	0.140	0.142	0.178	0.051
n	6	6	6	3
Inter-analyst precision	**Analyst 1**	**Analyst 2**	**Analyst 3**	**Inter-analyst**
Mean of % relative accuracy	100.39	100.21	100.13	100.24
%RSD	0.196	0.176	0.256	0.133
n	3	3	3	3
Inter-equipment precision	**Pipette 1**	**Pipette 2**	**Pipette 3**	**Inter-equipment**
Mean of % relative accuracy	100.37	99.94	100.12	100.14
%RSD	0.153	0.251	0.260	0.213
n	3	3	3	3

Table 3
Assay results of sodium chloride in raw material and pharmaceutical preparations ($n = 6$).

Samples	Label claim for sodium chloride content	BP 2010 acceptable % labeled amount	% labeled amount found ± SD	
			Miniaturized method	BP macroscale method
Raw material	–	99.0–100.5	100.19 ± 0.12	100.10 ± 0.22
NaCl Injection	0.9%	95.0–105.0	103.60 ± 0.10	103.66 ± 0.15
Dextrose–NaCl injection	0.9%	95.0–105.0	102.37 ± 0.12	102.26 ± 0.03
NaCl tablets	50 mg/tablet	95.0–105.0	102.42 ± 0.16	102.13 ± 0.13

Table 4
Comparison of the amount of reagents consumed and wasted generated in the BP method and the proposed miniaturized method, as determined from the analysis of one sample.

	BP 2010 method	Miniaturized method	Reduction (folds)
Reagents consumed (mmoles)			
Nitric acid	30.000	1.200	25
Dibutyl phthalate	18.862	0	∞
Silver nitrate	5.000	0.095	53
Ferric alum	1.037	0.021	50
Ammonium thiocyanate	1.578	0.007	216
Wasted generated			
Solid wastes, i.e. AgCl, AgSCN (g)	0.517	0.021	25
Liquid wastes (mL)	125.78	2.63	48

3.1.6. Color stability of ferric thiocyanate complex

There are evidences from the previous works about the fading of the ferric thiocyanate color, particularly when the solution is exposed to light, because of the reduction of ferric ion by thiocyanate or its decomposition products [13]. In our experiment which was carried out by exposing the red supernatant obtained from the titration to ambient light, its color faded significantly with time, especially after 1 h. The fading undesirably brought about smaller values of *X*-intercept and thereby the positive error in the analytical results. In contrast, by simply keeping the solution away from light, the color remained unchanged for at least 4 h. As a whole, it is recommended to read the absorbance values of the supernatants immediately once they were obtained after the removal of silver thiocyanate precipitates; otherwise these solutions should be well-protected from light until the measurement.

3.2. Method validation results

Once the operational conditions of the method were established, a set of assays to verify its overall performance was carried out. As shown in Table 1, the % relative accuracy obtained from the analysis of pure sodium chloride solution at three different concentration level indicated the reasonable accuracy of the miniaturized method. The intra-day and inter-day precision data are shown in Table 2. The intra-day and inter-day % RSD were in the ranged of 0.051–0.178% which were much lower than 2%, thus demonstrating satisfactory and acceptable precision. Statistical *t*-test analysis also showed no significant difference of the analysis results over a period of three days at 95% confidence level. Table 2 also showed the results of intermediate precision studies performed by using three different pipette models and by three different analysts who were previously trained to properly use micropipettes. The inter-instrumental RSD and the inter-personal RSD values were 0.213 and 0.133%, respectively. Based on these low RSD values and as confirmed by statistical analysis at 95% confidence level, it can be concluded that the proposed method showed high intermediate precision in the aspect of using different pipettes or analysts.

3.3. Application to the analysis of real samples

The proposed procedure has been applied to real samples in bulk and dosage forms in the comparison with BP macroscale titration; the mean values of % content or % labeled amount found with the standard deviation (SD) are shown in Table 3. In all cases, the samples complied with the official content requirement with the statistically similar results from these two methods at 95% confidence level. Therefore, this study confirmed that the analytical performance of the downsized photometric titration was comparable to the pharmacopeial macroscale titration.

3.4. Reagent-saving and environmental compatibility aspect of the proposed method

Evaluation of reagent-saving feature and environmental compatibility of the downscaled method was performed by the comparison of quantities of chemicals consumed and wastes produced from the macro- and microscale procedures. As clearly seen in Table 4, all the amounts of chemical reagents used in the proposed method, including costly silver nitrate were drastically lowered by the factor of 25–216 times. The use of environmentally dangerous dibutyl phthalate was absolutely abolished. Furthermore, the method produced significantly reduced liquid and solid wastes. Therefore, the miniaturized method not only offered the benefits to operators and environment, but also saved costs on chemical purchase and waste management.

4. Conclusions

The application of microscale chemistry, formerly used as an educational tool, is successfully extended to the real circumstances for the industrial or analytical purposes. Using the Volhard's titration as a paradigm, the rational miniaturization design with the aid of spectrophotometry could overcome all drawbacks and limitation of this titrimetric method in a large scale. The developed assay could reduce the consumption of reagents and the generation of wastes at satisfactory levels. In addition, the procedure was suitable for the faster multi-sample assays and required commonly available and unsophisticated instrumenta-

tion. Importantly, the results obtained from this proposed assay were of adequate accuracy and precision compared with the macroscale method. Therefore, microscale chemistry is an attractive, facile and powerful strategy for the development of green, safe and cost-effective analytical methods suitable for a sustainable environment.

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