Talanta 84 (2011) 1396–1400

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Exploiting green analytical procedures for acidity and iron assays employing flow analysis with simple natural reagent extracts

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ARTICLE INFO

Article history:
Available online 8 April 2011

Keywords:
Natural reagents
Flow based systems
Simple lab on chip
Reflective absorption detection
Green chemical analysis

ABSTRACT

Green analytical methods employing flow analysis with simple natural reagent extracts have been exploited. Various formats of flow based analysis systems including a single line FIA, a simple lab on chip with webcam camera detector, and a newly developed simple lab on chip system with reflective absorption detection and the simple extracts from some available local plants including butterfly pea flower, orchid flower, and beet root were investigated and shown to be useful as alternative self indicator reagents for acidity assay. Various tea drinks were explored to be used for chromogenic reagents in iron determination. The benefit of a flow based system, which allows standards and samples to go through the analysis process in exactly the same conditions, makes it possible to employ simple natural extracts with minimal or no pretreatment or purification. The combinations of non-synthetic natural reagents with minimal processed extracts and the low volume requirement flow based systems create some unique green chemical analyses.

1. Introduction

Economical and environmentally friendly chemical analysis has become an important aspect in sustainable development of modern chemical analysis systems [1,2]. The use of less toxic reagent will lead to less waste production but measurement systems would require integration of sufficiently sensitive detectors. The replacement of toxic and/or expensive chemicals with alternative less harmful and lower cost reagents is a way to create greener chemistries [2,3]. Utilizations of natural extracts from plants to replace synthetic chemical indicators/complexing agents [4–10] and high cost/commercially unavailable enzymes [11–16] have been of interest. It would be most beneficial if the extraction could be carried out in aqueous phase and could be used directly without purification. [17] Development of a system that can accommodate these requirements would be useful.

Flow injection analysis is a well known technique, adapting batch-wise wet chemistry, for simultaneously performing chemical/biochemical reactions and detections within small tubings. The main advantages of flow injection based techniques over the conventional batch-wise operation include low amount of reagent/sample consumption, low waste production, and more rapid analysis while the instrumentation set up can be cost effective. The components such as pump and valve can be adapted or built at low cost. Various types of detectors can be coupled with the flow injection technique to create almost endless numbers of possibilities for the development of new flow based analytical chemistry systems. An additional advantage of the flow system lies on the semi-automatic nature of the operation where standard solutions and samples are passed into the system in exactly the same manner and conditions via the control of valve and pump with constant flow rate. It is possible to use natural reagents with a flow injection system, even though the reagents may not be pure [5], as long as they do not contain substances that cause major side reactions or severely inhibit the reaction of interest. Down scaling of a flow system at economical price using common laboratory facilities to make a simple version of lab-on-chip (LOC) with bare eyes detection has been reported by our group [18]. A more effective detection method at low cost such as webcam camera detector was also described [19]. Combination of these cost effective flow based systems and natural reagents associates closely to some of those in the 12 principles of green chemistry that are related to the field of analytical chemistry [2], namely, prevention of high waste production (low

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doi:10.1016/j.talanta.2011.03.090

volume flow analysis), safer solvent and auxillaries (aqueous based natural reagent extracts), design for energy efficiency (rapid flow analysis), and inherently safer chemistry for accident prevention (closed system).

Here we present simple green analytical methods extended from previous reports [18–20] by utilizing a new lot of minimal processing natural resources as natural reagents, namely, dry tea leaves which are normally available for tea drink, beet root, butterfly pea flower, and orchid flower, to be used with flow systems: a simple FIA set up, LOC using modified webcam camera (LOC-CMOS) detector developed previously [19] and a newly developed simple lab on chip with fiber optic reflective absorption probe detector (LOC-RA).

2. Experimental

2.1. Preparation of natural reagents

Natural reagents were extracted, with water, from butterfly pea flower (*Clitoria ternatea*), orchid flower (*Dendrobium Sonia*), and beet root (*Beta vulgaris* subsp. *vulgaris*). Each was performed using a similar procedure to each other. Briefly, the sources of natural reagents were mixed and blended with deionized (DI) water at the following ratios of natural source (g):water (mL); dry butterfly pea flower 1 g:100 mL; fresh reddish purple orchid flower 50 g:200 mL; and skinned fresh beet root 515 g:300 mL. The materials from butterfly pea flower and beet root were filtered after blending, while material from orchid flower was boiled for 30 min before filtering for further use. Appropriate dilutions of the stocks were made.

Extending the previous work [20], as tea was another natural reagent demonstrated for iron determination using the simple FIA system, some commonly found commercial tea drinks in the forms of powder and dry leaf were tried. Chinese jasmine tea (Ra-Ming brand), Japanese green tea (Green tea) and black tea (Lipton) solutions were prepared by simple perfusion with hot water. After cooling down, they were used directly to demonstrate their potential to be used as a simple chromogenic reagent for assay of iron.

2.2. Preparation of standard solutions

Standard acetic acid solutions were prepared at concentrations of 2–7% (w/w). Iron stock solution was prepared from $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ (Merck) at a concentration of $1000\,mg\,L^{-1}$ with DI water in the presence of 0.01 M H_2SO_4. By diluting the stock solution with DI water, the working solutions were obtained.

2.3. Samples

Four samples of cooking vinegar commercially available in local convenient stores were used to demonstrate the performance of the systems.

2.4. Instrumentation

Three different instrumentation set ups were employed.

A single line FIA was assembled using fiber optic (P400,Ocean Optics Inc., USA) detector having Z-cell (10 mm path length) coupled with a LS-1-LL light source, USB2000 spectrophotometer; peristaltic pump (Ismatec, ISM796B, Switzerland) and six port injection valve (Takasago, Takasago Electric, Inc. Nagoya, JAPAN). The FIA-gram was recorded using software FIA lab 5.0 for Windows (FIAlab Instruments USA).

A simple lab-on-chip coupled with a webcam camera (CMOS) detector was set up as previously reported [19].

A simple lab-on-chip with fiber optic reflective absorption probe detector (LOC-RA) Fig. 1) was developed further by replacing the CMOS detector with a reflective absorption fiber optic probe (R200-7-UV–vis) with tungsten halogen light source (LS-1-LL) and a spectrophotometer unit (USB 2000). The chip body was made as described in previous report [19]. Then it was mounted with 2 pieces of acrylic placed on top and bottom of the chip. The center of the top acrylic piece was drilled to fit the size of the fiber optic head (6.35 mm OD). Before securing, the top acrylic piece was positioned in order to place the fiber optic directly on the selected detection point on the chip. The whole chip unit was painted black to protect it from interference due to the outside light. Then the unit was secured on the angle adjustable holder.

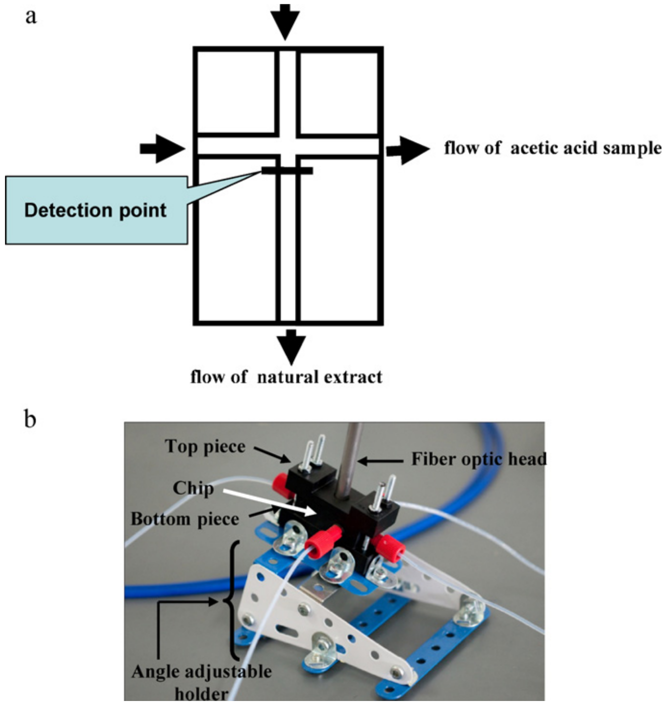

Fig. 1. Simple lab-on-chip with fiber optic reflection probe detector: (a) diagram, (b) photograph

3. Results and discussion

3.1. Absorption spectra of natural reagents

Extracts from butterfly pea flower, orchid flower and beet root were diluted with water at the ratios of 1:1, 1:3, 1:9 (v/v) to ensure that the absorbance signals would not exceed the limit that can be measured by the spectrophotometer. Their pH values were adjusted with either CH_3COOH or NaOH to the entire pH ranges of 1–14 before scanning their absorption spectra with a spectrophotometer (Shimadzu, UV-1800, Japan) in the visible wavelengths 400–800 nm. It was found that butterfly pea flower and orchid flower govern two similar pKa(s); 3.9 and 6.3 for butterfly pea flower, and 3.0 and 6.7 for orchid. Beet root has only one pKa of 9.3 shown in the visible region.

Absorption spectra of each reagent in different pH media helped to determine the most suitable detection wavelengths for each flow based system. Fig. 2(a)–(c) shows the selected absorption spectra of the water extracts of butterfly pea flower, orchid flower and beet root, respectively. Butterfly pea flower extract has increased absorbances in the 450–650 nm range with the pH increase of 2–6. Orchid flower extract showed a similar trends in the 560–610 nm range with a pH increase of 3–5 while for pH 5–8 there was a slight decrease of absorbance. Beet root extract also gave the same trends in the 450–590 nm range with the pH increase of 3–5 (spectra not

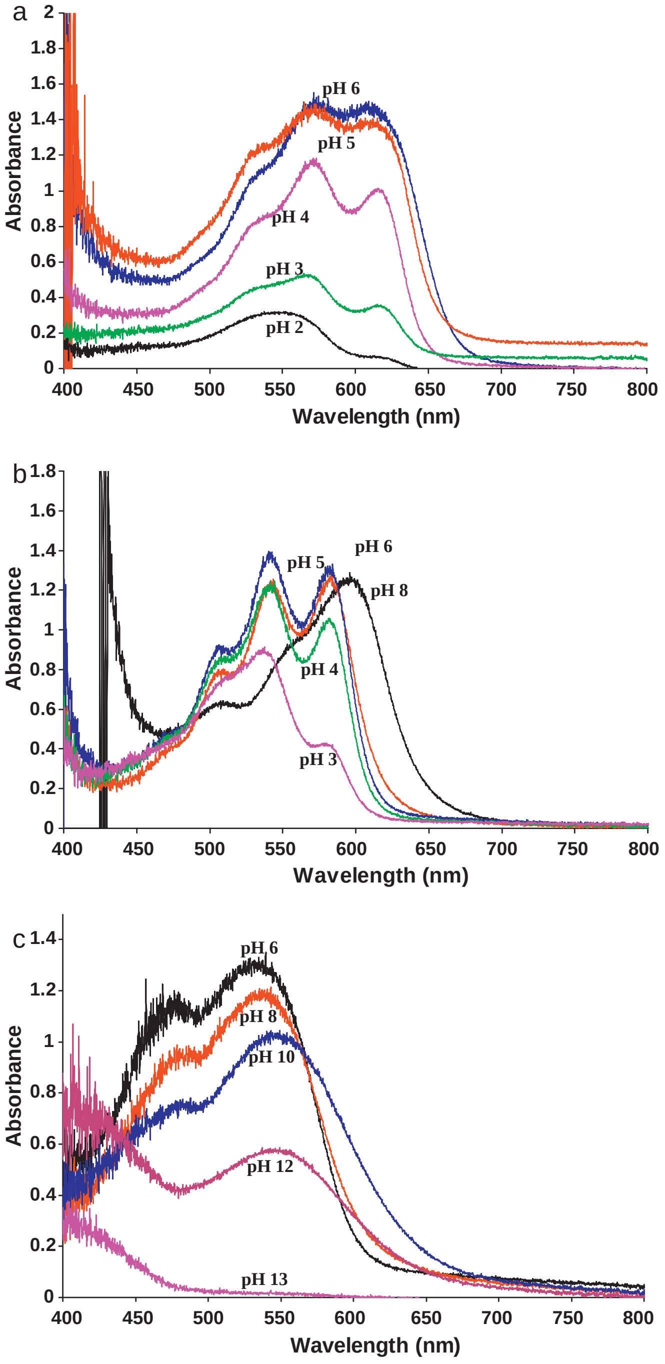

Fig. 2. Absorption spectra of various pHs of natural indicator extracts from (a) butterfly pea flower, (b) orchid flower and (c) beet root.

shown) while increasing pH in the range of 6–13, opposite trends of decreasing absorbance were observed.

Absorption spectra in the range of 400–700 nm of tea and Fe(II)–tea complex were compared. It was found that tea reagents show higher absorbances than Fe(II)–tea complexes at low wavelengths. These low wavelengths were avoided for use as detection wavelengths because they will cause the up-side-down FIA-gram and at various complex concentrations, there is the possibility of having both normal peaks and up-side-down peaks in the same FIA-gram, which would make it difficult to process the data. The differences in absorbances between tea reagents and Fe(II)–tea complexes were smaller at higher wavelengths and finally no differences were observed at particular wavelengths which are 500, 475, and 540 nm when using the water extracts of jasmine tea, green tea and black tea as reagents, respectively. Fe(II)–tea complexes can absorb light better than tea extracts alone at the wavelengths higher than these particular wavelengths. In other words, various detection wavelengths were possible at wavelengths higher than these particular wavelengths of each tea reagent, but for simplicity, the detection wavelength at 550 nm was used for each of the three tea reagents. The observation results were similar to previous study of green tea leaf [20].

3.2. Using natural reagents with LOC-RA system

Selection of pH of reagent media was done based on characteristics of the spectra. The starting pH must be selected where the trend in change of absorbance could be seen when the pH was lowered during the acid assay process. It was found that the suitable starting pHs of the extracts from butterfly pea flower, orchid flower and beet root were pH 6, 6, and 12, respectively. This is because at lower pHs, the decrease or increase of absorbance could be observed. Therefore, the extracts were adjusted to their suitable starting pHs prior to use.

A natural reagent was introduced into the longer channel and the acetic acid standard/sample solution was injected into the shorter channel of the lab-on-chip (see Fig. 1(a)). Mixing of the acetic acid into the natural reagent line changed the absorbance of the natural extract. Ideally, the point of detection should be located at the cross (at the confluence of the acid and reagent streams) but it was observed that a point beneath the cross (see Fig. 1(a)) would provide a better precision in monitoring (recording spectra *vs* time, not shown here).

Although this instrumentation set up can record spectrum at every preset time, i.e. the absorbance at various scanned spectra, only particular wavelengths for each natural reagent and a suitable detection time can be selected. It is based on the best discrimination among different acid sample concentrations. At higher acidic concentration, more neutralization occurred causing more change in the colored reagent. By fixing detection time, monitoring of changes in absorbance would also minimize the effects due to dispersion of the product in the channel. By this way of manipulation, it provides fast analysis time. The ability of this novel manipulation offers additional advantages. Optimization procedures for operation could be minimized and leading to less sample and reagents consumption. This flow manipulation provides means for sample and reagents delivery to a reactor.

Therefore, calibration graphs (absorbance *vs* log concentration of acetic acid) were constructed from data recorded at selected wavelengths and fixed detection times of 618 nm (at 45 s), 584 nm (at 60 s), and 575 nm (at 45 s) for butterfly pea flower, orchid flower and beet root, respectively. Results obtained from the three natural extracts are summarized in Table 1. The results obtained by using beet root extract were observed to be higher values than the others. The cause of this has not yet been fully investigated.

3.3. Using natural reagents with LOC-CMOS system

Natural indicators extracted with water from butterfly pea flower, orchid flower and beet root could be directly employed with the LOC-CMOS system after filtration with no need of pH adjustment because the system can monitor real time change of color (based on %RBG) for the whole migration distance. This is the benefit of this system whereas solution used in the previous system must be adjusted to a suitable pH that will show the trend change of absorbance at the particular detection point.

When introducing acetic acid into the channel that crosses with the channel in which reagent has been previously filled, the acetic acid migrated from the intersection point into the reagent

Table 1
Determination of acetic acid in vinegar (labeled as 5% w/w) employing natural reagents using LOC-RA system as compared to standard titration method.

Standard/ Sample	Concentration of acetic acid (%w/w)#			
	Natural reagents			Standard titration method [21]
	Butterfly Pea (618 nm at 45 s)	Orchid (584 nm at 60 s)	Beet root (575 nm at 45 s)	
Calibration graphs* of standard acetic acid 2-7% w/w	$Y = -0.93X + 1.4$ $R^2 = 0.997$	$Y = -1.13X + 1.25$ $R^2 = 0.986$	$Y = -0.72X + 1.12$ $R^2 = 0.981$	-
S1	5.2	5.3	6.7	5.1
S2	5.3	5.3	6.3	5.0
S3	5.7	5.4	6.7	5.3
S4	5.6	5.4	6.7	5.1

means of triplicate results.
* Y is absorbance signal, X is log concentration of acetic acid.

line, causing the reduction of the color of the reagent. The signal recorded by this system is the continuous migration time used for the diffusion zone to reach various distances (represented as relative distance) [19]. The most suitable relative distance that gave the best differentiation among various acid concentrations in the shortest time was selected. Calibration graph was constructed from the migration times at this selected relative distance *vs* log acid concentrations.

Examples of signals obtained from migration of acetic acid (2–5% w/w) into beet root extract line are illustrated in Fig. 3. Similar signals were obtained when using the butterfly pea flower and orchid flower extracts as reagents. The selected relative distances for construction of calibration graphs (migration time vs log acid concentration) are 9, 9.5 and 8.5 for the butterfly pea flower, the orchid flower and the beet root extracts, respectively. Table 2 summarizes the calibration graphs and results of determination of acetic acid contents in various vinegar samples.

3.4. Using the natural reagents with a simple FIA system

Natural reagents for acidity assays were prepared as previously described, but the extracts of butterfly pea flower, orchid flower and beet root were diluted prior to use with DI water to the concentration of 5%, 5%, and 0.5% v/v, respectively. Each natural reagent was used as a carrier without adjusting pH prior to use. Standard/sample solutions of various concentrations of acetic acid were injected into a natural extract carrier stream. The changes in absorbance were monitored at various wavelengths selected within the working wavelength range from the absorption spectra in Fig. 2.

The best detection wavelengths that provide good peak shapes and constant baselines when using the extracts from butterfly pea flower, orchid flower and beet root were found to be 575, 583 and 480 nm, respectively. Example of FIA-gram of a series of standard acetic acid solutions with the butterfly pea flower extract is shown in Fig. 4. The calibration graphs (peak height of absorbance *vs* concentration of acetic acid) and results of real sample determination obtained when using natural reagents were compared with those obtained when using a commercial phenolphthalein indicator together with NaOH standard solution and when using a standard batch titration method, see Table 3.

Precision, in terms of %RSD, was estimated from 5 replicate injections of 4% acetic acid standard. They were found to be in acceptable range of 1.2%, 1.6% and 2.7% for butterfly pea flower, orchid flower and beet root extracts, respectively.

Employing the FIA system also demonstrated that various tea drinks have the potential to be used without any treatment as reagents for the determination of iron. Primary studies without optimization showed that linear calibration graphs and working range of Fe (II) could be obtained as $Y = 0.092X - 0.129$, $R^2 = 0.999$ (5–20 mg L^{-1}) for the jasmine tea, $Y = 0.032X - 0.095$, $R^2 = 0.998$ (5–20 mg L^{-1}) for the green tea, and $Y = 0.037X - 0.296$, $R^2 = 0.996$ (10–25 mg L^{-1}) for the black tea, respectively (*Y* is absorbance and *X* is concentration of Fe(II)). The linear calibration graphs obtained indicate that it should be worthwhile to investigate the use of such tea infusion in more detail so that it would be supplement to those previously study [20].

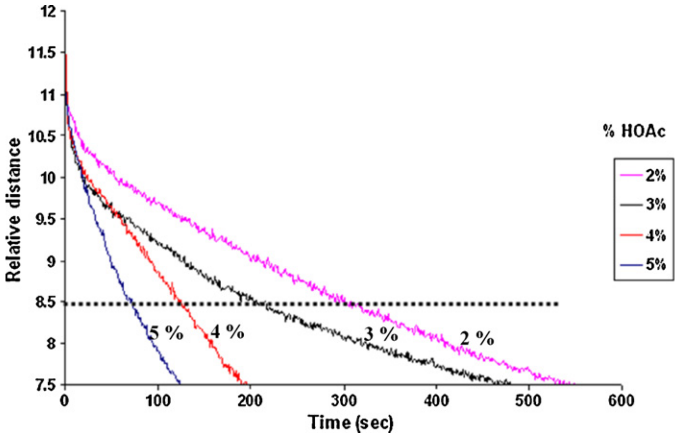

Fig. 3. Analytical signals recorded using the LOC-CMOS system for neutralization by diffusion of acetic acid at various concentrations into beet root reagent.

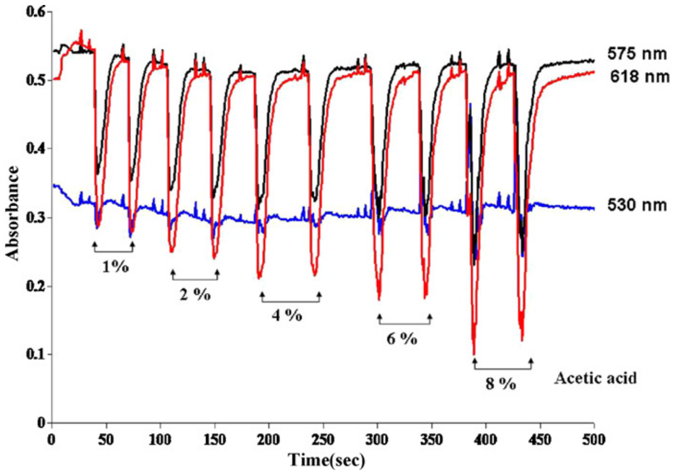

Fig. 4. FIA -grams of a series of acetic acid of various concentrations obtained from the simple single line FIA set up using extract from butterfly pea flower as a reagent.

Table 2
Determination of acetic acid in vinegar (labeled as 5% w/w) employing natural reagents using LOC-CMOS system as compared to standard titration method.

Standard/ Sample	Concentration of acetic acid (%w/w)#			
	Natural reagents			Standard titration method [21]
	Butterfly Pea relative distance** 9	Orchid relative distance** 9.5	Beet root relative distance** 8.5	
Calibration graphs* of standard acetic acid 2-7% w/w	$Y = -105.7X + 101.6$ $R^2 = 0.974$	$Y = -563.3X + 507.7$ $R^2 = 0.996$	$Y = -581.6X + 478.6$ $R^2 = 0.998$	-
S1	6.0	4.4	5.0	5.1
S2	6.2	5.0	4.7	5.0
S3	4.8	5.2	4.4	5.3
S4	5.6	5.1	4.8	5.1

\# mean of triplicate results.

* Y is absorbance signal, X is log concentration of acetic acid.

** relative distances were chosen according to the suitable discrimination of analytical signals obtained from various sample concentrations (see example in Fig.3, Y axis).

Table 3
Determination of acetic acid in vinegar (labeled as 5% w/w) using a simple single line FIA system and the standard titration method.

Standard/ Sample	Concentration of acetic acid (%w/w)# Butterfly Pea (575 nm)	Orchid (583 nm)	Beet root (480 nm)	Commercial indicator phenolphthalein (530 nm)	Standard titration method [21]
Calibration graphs* of standard acetic acid 2-7% w/w	$Y = -0.009X - 0.17$ $R^2 = 0.990$	$Y = -0.01X - 0.12$ $R^2 = 0.994$	$Y = -0.01X - 0.05$ $R^2 = 0.959$	$Y = -0.013X - 0.05$ $R^2 = 0.972$	-
S1	6.6	4.7	6.6	6.3	5.1
S2	6.4	4.9	5.6	5.4	5.0
S3	6.8	4.2	6.2	6.3	5.3
S4	6.7	3.9	5.8	6.1	5.1

\# mean of triplicate results.

* Y is absorbance signal, X is concentration of acetic acid.

4. Conclusion

Uses of various simply prepared natural reagents have been demonstrated with different flow based systems. Extracts from butterfly pea flower, orchid flower, and beet root were used for the assays of acidity. Preliminary experiments with various tea infusion extract which led very convenient in reagent preparation, indicated their potential use for iron determination, in addition to green tea extract, as previously reported [20]. The natural extracts in aqueous phase are less toxic, easy to prepare, and would be appropriate alternatives in some places. Apart from others, the newly developed LOC-RA system offers addition advantages. The flow based set ups are simple and have many possibilities of adaptation with various approaches which should lead to minimize cost of instrumentation and analysis. The developed procedures would offer novel approaches in green analytical chemistry, although at this stage, the green analytical systems are suitable for applications with less stringent requirements.

Acknowledgements

The Thailand Research Fund (TRF) and the Commission on Higher Education (CHE) (through RG grant and through the Center for Innovation in Chemistry (PERCH-CIC)) are acknowledged for support. The Multidisciplinary Science Research Center is acknowledged. The authors also thank Prof. G.D. Christian for his useful comments.

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