Talanta 79 (2009) 990–994

Contents lists available at ScienceDirect

Talanta

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

Simple lab-on-chip approach with time-based detection

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ARTICLE INFO

Article history:
Received 30 November 2008
Received in revised form 10 February 2009
Accepted 11 February 2009
Available online 20 February 2009

Keywords:
Lab-on-chip
Time-based detection
Ascorbic acid assay
Acetic acid
Iron determination
Cost-effective analysis
Migration time
Visual detection

ABSTRACT

A simple lab-on-chip approach with time-based detection is proposed. A platform is made from a piece of acrylic differently shaped channels for introducing sample and reagent(s) using flow manipulation. Time-based changes involving migration of the reaction zone are monitored. The changes can be visually monitored by using a stop-watch with naked eyes observation. Some applications for the determination of ascorbic acid, acetic acid and iron in real samples with different chemistries were demonstrated.

1. Introduction

"Lab-on-chip" is the state of the art analysis approach in analytical chemistry. All necessary parts of the analysis process, including sample introduction part (i.e. pump), reaction chamber and detection unit, are integrated into a small one-piece device. With this integrated downscaled system, chemical/biochemical analysis can be miniaturized performing. Advantages of the "lab-on-chip" include the reduction of chemical/sample consumption, ease of operation, rapidity of analysis and with possibility of portability [1,2].

When the word "lab-on-chip" is mentioned, one may assume that it is an invention that requires high technology and expensive fabrication involving a lot of new developments in materials science, electronics, physics and chemistry/biochemistry. However, "lab-on-chip" may simply be made based on the objective of performing an analytical process without bench space. The chip itself can be fabricated in a simple way with commonly found equipment. With a suitable detection method, the whole analysis process can be carried out on a chip without needing any extra-complicated devices.

In this work, we proposed an economic "lab-on-chip". A simple chip was made by drilling channels in a piece of acrylic plastic. Uncomplicated manual operation can be handled. We have demonstrated different uses of the chip with simple reactions, all involving color detection. To eliminate the use of any extra device such as a spectrophotometer, detection was done by bare eyes based on migration time of the reaction zone using a simple stop-watch.

2. Experimental

2.1. Fabrication of the simple chip

A piece of acrylic was cut into rectangular shape of the size 2.5 cm × 5.0 cm × 1.2 cm (width × length × thickness). It was secured on a drill press and channels were drilled through the side of the acrylic piece using a 1 mm drill bit. The whole volume of the channels was approximately 55 μL. The format of the channels, i.e. the crossing point of the vertical and horizontal channels, or the distance from the edges, can be varied according to the user's designs

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0039-9140/$ – see front matter.
doi:10.1016/j.talanta.2009.02.025

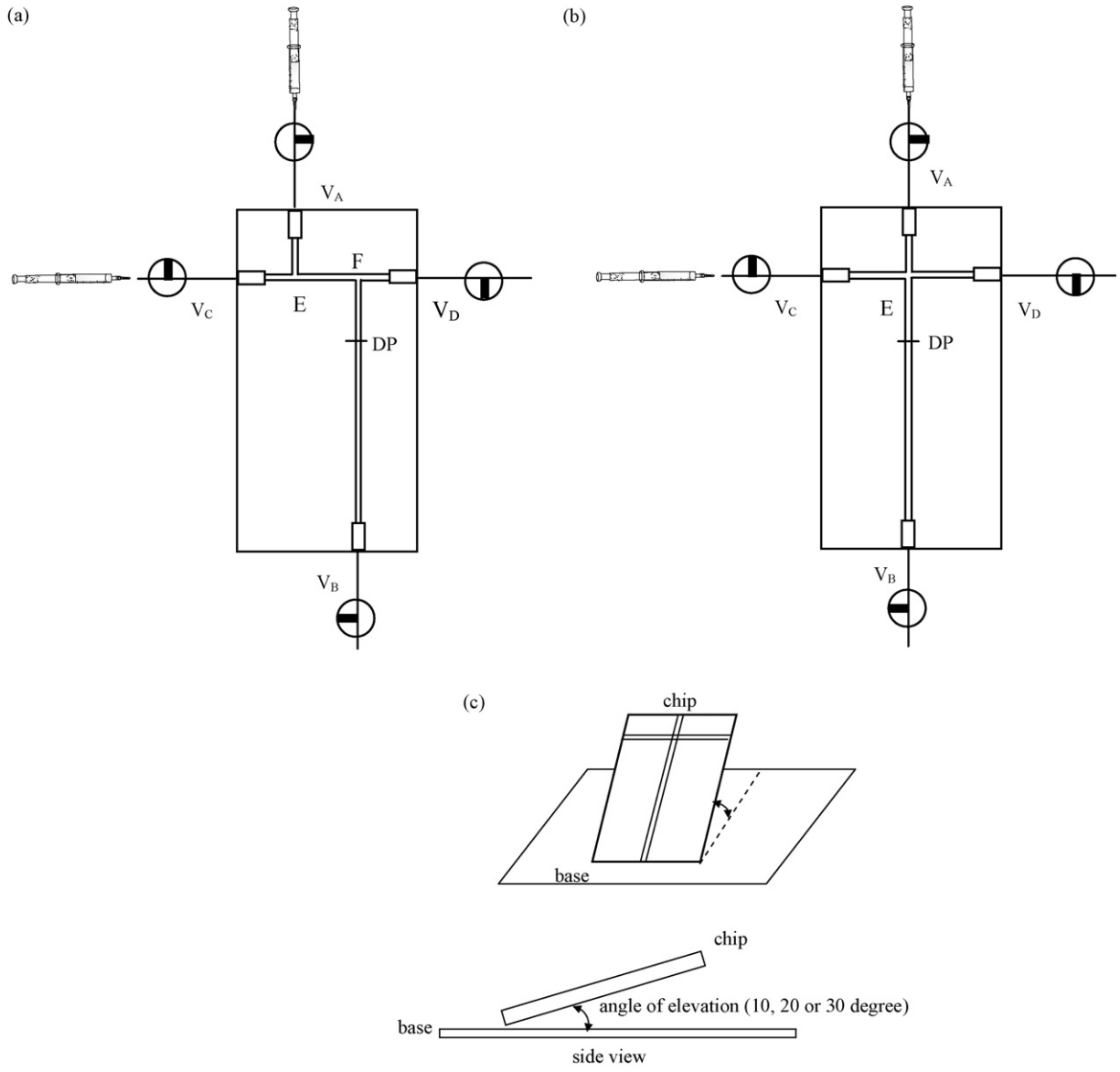

Fig. 1. (a) and (b) Two designs of the simple chip (V: three-way valve, E and F: the points where analyte and reagent having the first contact, i.e. start the reaction zone, DP: mark for detection point) and (c) illustration for the chip tilted on a base.

and applications. Here two different designs, see Fig. 1(a) and (b), were proposed to demonstrate the different migration patterns of the reaction zone.

A larger diameter was made at each channel opening and threads were made to perfectly fit a normal nuts using in flow injection assembles. Each three-way valve was connected to each FI nut with a piece of 0.03 in. i.d. PTFE tubing. These valves act as injection and exit ports of the chip. One of the ports on the vertical channel and one of the ports on the horizontal channel were injection ports where syringes were placed. The rest of the openings were exit ports, connected to the waste container.

2.2. Determination of ascorbic acid in vitamin C

Determination of ascorbic acid in vitamin C tablet was demonstrated through two different reactions, one with $KMnO_4$ and another with NH_4VO_3 [3,4]. In the experiments with $KMnO_4$, standard ascorbic acid solutions were prepared at 2, 4, 6 and 8 mM in 0.2 M H_3PO_4. The $KMnO_4$ solution was also prepared in 0.2 M H_3PO_4 at 1×10^{-4} M. In the experiments with NH_4VO_3, standard ascorbic acid solutions were prepared at 0.6, 0.8, 2, 4, 6 and 8 mM in water. The NH_4VO_3 solution was prepared in 0.2 M H_2SO_4 medium at the concentration of 6.84×10^{-2} M.

Three commercial vitamin C tablet samples with different additives were used in this study. Their compositions are as follows: sample C1 contains 500 mg vitamin C; sample C2 contains 1000 mg ascorbic acid with bioflavonoid complex lemon, acerola and rose hip; sample C3 contains 400 mg ascorbic acid with 350 mg sodium ascorbate, 400 mg calcium ascorbate, 50 mg bioflavonoids, 50 mg rutin, 50 mg hesperidin, 250 mg rose hip, 50 mg acerola, 150 mg tapioca starch, 30 mg magnesium stearate and 30 mg soy polysaccharide.

The results were compared with the standard titration method using blue 2,6-dichlorophenol indophenol (DCIP), prepared in $NaHCO_3$, as a titrant. End point was observed when the titrand changed from colorless to pink.

2.3. Determination of acetic acid in vinegar

Acetic acid standard solutions were prepared at the concentration of 0.04, 0.06, 0.08 and 0.1 M by dilution of concentrated glacial acetic acid with DI water. Reagent used for this study was

the mixture of 2×10^{-4} M NaOH and phenolphthalein. The vinegar samples were diluted 10-fold with DI water. The performance of the system was demonstrated using three commercial vinegar samples with approval quality from the Thai Food and Drug Administration. Amounts of acetic acid found were compared to the reported values on the products' labels.

2.4. Determination of iron in nail

This application was demonstrated with high iron content nail. A 0.6436 g nail sample was digested with HNO_3 acid and heated for 30 min. The final volume of the digested nail solution was adjusted to 250 mL with deionized water. The sample solution (0.3 mL) was diluted to 10 mL prior to use.

A 17.9 mM stock Fe^{3+} solution was prepared from $NH_4Fe(SO_4)_2{\cdot}12H_2O$. Working solutions were diluted to the concentrations of 0.18, 0.54, 0.90, 1.25 and 1.61 mM. The complexing agent was 1 M KSCN solution.

The performance of this system was evaluated by comparing the results with two standard methods; batch molecular spectrometry and atomic absorption spectrometry (AAS). For batch spectrometry, standard Fe^{3+} solutions were prepared at the concentrations of 3.58×10^{-3}, 7.16×10^{-3}, 1.07×10^{-2}, 1.43×10^{-2} and 1.79×10^{-2} mM. The intensity of the red complex was monitored using a spectronic 21. Since the working range is different from the proposed lab-on-chip system, the nail sample solution was prepared at lower concentration by diluting the digested sample solution to obtain concentration in the working range of the standard calibration curve (3.58×10^{-3} to 1.79×10^{-2} mM). For AAS, the procedure was followed the ASTM method [5].

3. Results and discussion

3.1. Important parameters

3.1.1. Planar/angle of elevation

Migration occurred from the difference in concentration of the reagent and sample zones. Capillary action due to small channel also helps accelerate the migration. In addition, gravimetric force should also help to promote migration. This could be due to differences in the densities between the analyte and reagent solutions. This effect was illustrated by the determination of ascorbic acid using $KMnO_4$, in which the de-colorization is due to redox reaction [3], the effect of elevation was demonstrated using the chip A format. $KMnO_4$ was introduced in the vertical channel and ascorbic acid was introduced in the horizontal channel. The chip was tilted at various angles (0°, 10°, 20° or 30°) with respect to the horizontal plane, see Fig. 1(c).

It was found that with different tilting angles, reaction zone migrated downward at different rates. Time-based calibration graphs obtained from various angles are shown in Fig. 2. For this particular reaction, the 10° angle gave the best sensitivity but required more detection time. The 20° angle offered better R^2 value with more rapid detection time. Migration was faster with 30° arrangement, but sensitivity and R^2 value were decreased. The selection of the chip's elevation angle should compromise sensitivity and analysis time. Therefore, the 20° angle was chosen for further experiments. However, other reactions or different matrices with different densities and viscosities may yield different results. In some cases, the degree of the angle may not be critical, but it may slightly change sensitivity and working range of the analysis. The selection of the chip's angle should be consistent.

3.1.2. Detection point

The detection is time-based detection. It was done by recording the time that the front of the reaction zone took to reach the detection point. If the zone migrates vertically, the detection point (see Fig. 1: DP) was set down from the intersection. The shorter the distance between the intersection to the detection point, the better sample throughput. However, the longer the distance offers the better precision in timing. The detection point should be located based on compromising between analysis time and precision.

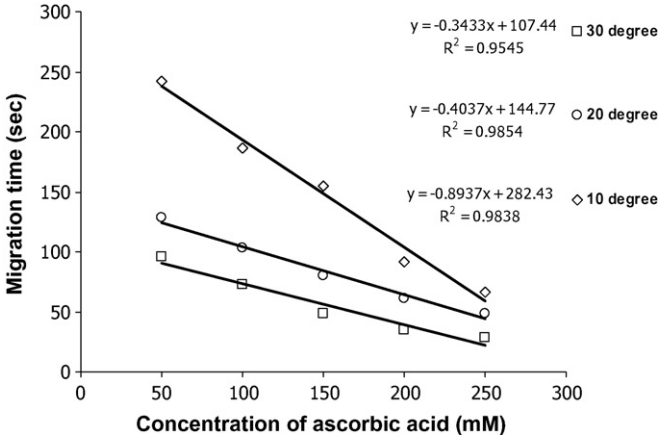

Fig. 2. Time-based calibration graphs of ascorbic acid with various elevation degrees.

3.2. Demonstration of the system with various reactions

3.2.1. Determination of ascorbic acid in vitamin C tablets

In this study, two reactions were carried out using two different chip formats and both were placed at a 20° angle with respect to the horizontal plane. Chip A was used for the reaction of ascorbic acid with $KMnO_4$. First, valves V_A and V_B were opened while valves V_C and V_D were closed. $KMnO_4$ was injected through valve V_A to fill all the channels. Then, valves V_A and V_B were closed, and valves V_C and V_D were opened. Standard ascorbic or sample solution was injected into valve V_C and it could flow out through valve V_D, replacing the reagent in the horizontal channel. Both valves V_C and V_D were immediately closed and timing was started. The purple color of $KMnO_4$ began fading away from the intersection point F in the vertically downward direction. The detection point was set at 2.0 cm from point F. The migration was faster with the higher concentration of ascorbic acid and therefore, a shorter time was used to reach the detection point.

Chip B was used for the reaction of ascorbic acid with NH_4VO_3. The operation procedures were similar to that described above for chip A. However, here the standard or sample solution was introduced vertically from valve V_A to V_B. The reagent NH_4VO_3 was injected horizontally from valve V_C to V_D. After immediately closing all the valves and starting the timing, the change in color (from pale yellow to blue) of the detection zone was observed starting at the intersection E. The migration of the detection zone was on both left and right horizontally. The detection time was recorded when all the solution in the horizontal channel turned blue. The migration direction of the observed detection zone was different from the above system, even though the chip was also at 20° tilt. This is likely due to the nature of the vanadium comproportionation reaction which can be explained based on the standard reduction potential values of ascorbic acid [6] and vanadium of various oxidation states [7]. When V^{5+} in the form of VO_2^+ in the acidified NH_4VO_3 came into contact with the reducing agent, ascorbic acid at the intersection E, it was likely reduced to V^{3+}, as the reducing power of the ascorbic acid is not strong enough to change V^{5+} to V^{2+}. This V^{3+} started the auto-redox of vanadium contained in the horizontal channel, changing the rest of yellow colored V^{5+} to blue

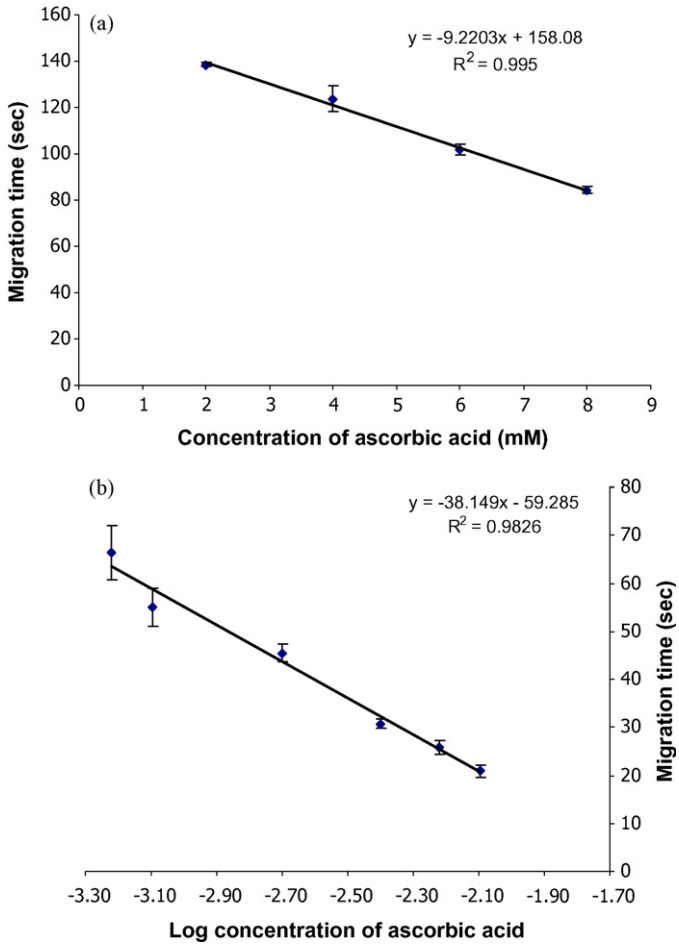

Fig. 3. (a) The time-based calibration graphs for ascorbic acid using (a) $KMnO_4$ as and (b) NH_4VO_3 as oxidizing agent.

colored V^{4+} (in the form of VO^{2+}), $V^{3+} + V^{5+} \rightarrow 2V^{4+}$ [8]. The higher the concentration of ascorbic acid, the more V^{3+} was produced and the faster the auto-redox reaction occurred. This reaction took place at a faster rate as compared to the migration of V^{5+} into the ascorbic acid line. Therefore, the change in color intensity was observed in the horizontal direction rather than in the vertical direction.

Fig. 3(a) and (b) shows the time-based calibration graphs of the ascorbic acid standards, when using $KMnO_4$ and NH_4VO_3 as a reagent, respectively. Both have linear working range in the concentration ranges used in this study. Vitamin C tablet samples were also analyzed with these two systems. It was found that the proposed systems yielded results that agreed with the standard batch titration method [9], as shown in Table 1.

The results from both systems indicated that this simple lab-on-chip approach with time-based detection could be applied for analysis of vitamin C tablets of various matrices without the need of dilution or other sample pretreatments. This would help to reduce the errors due to dilution.

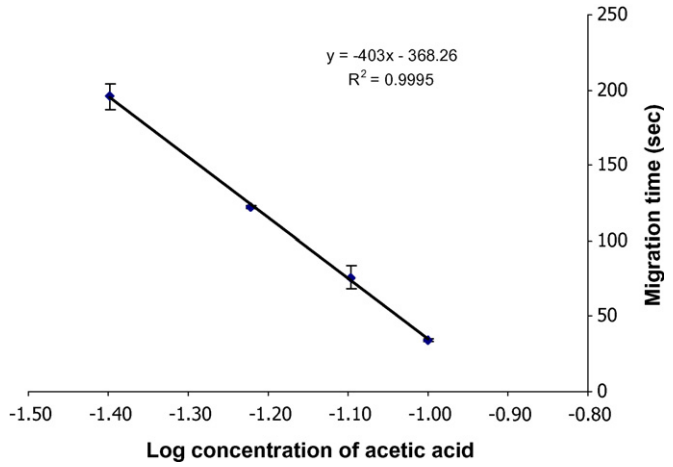

Fig. 4. Time-based calibration graph of acetic acid.

Table 2
Amount of acetic acid in vinegar samples.

Vinegar sample	Labeled amount (%, w/v)	Lab-on-chip (%, w/v)
Sample A1	5	4.8
Sample A2	5	4.8
Sample A3	5	4.2

3.2.2. Determination of acetic acid in vinegar

Chip A was used in this study. It was placed at a 20° angle with respect to the horizontal plane. The mixture of NaOH and phenolphthalein was injected vertically through valves V_A and V_B. The acetic acid standard solution or vinegar sample was injected horizontally through valves V_C and V_D. The detection point was set at 0.5 cm down from point F. When acid migrated down into the reagent line, the pink color faded away. The detection time was recorded and plotted against log the concentration of acetic acid standard. The time-based calibration graph of standard acetic acid is shown in Fig. 4. Amounts of acetic acid in the vinegar samples were determined and the results agreed well with the value reported on the labels, as shown in Table 2.

3.2.3. Determination of Fe in nail

Chip A was used by introducing standard or sample solution through vertical valves V_A and V_B and injecting KSCN through horizontal valves V_C and V_D. The chip was placed flat (0°) to demonstrate that for some reactions, tilting the chip may not be necessary. The reaction occurred at the crossing point F, giving red color complex that migrated down vertically. Time was recorded when the red zone reached the detection point that was set at 2 cm down from the crossing point F. Migration of the reaction zone from the crossing point E toward valve V_A was not observed, even though the chip was placed flat. This is probably due to having more Fe^{3+} present in the lower section from F to V_B as compared to that in the upper section E to V_A.

The time-based calibration graph for Fe^{3+} was obtained as shown in Fig. 5. This calibration graph has positive slope, in contrast

Table 1
Comparison of the ascorbic acid contents in vitamin C tablet samples obtained from the proposed simple lab-on-chip and standard batch titration.

Vitamin C sample	Labeled amount (mg/tablet)	Standard titration method with DCIP (mg/tablet)	Lab-on-chip	
			With $KMnO_4$ (mg/tablet)	With NH_4VO_3 (mg/tablet)
Sample C1	500	478	481	513
Sample C2	1000	1084	1030	999
Sample C3	1000	957	1098	1063

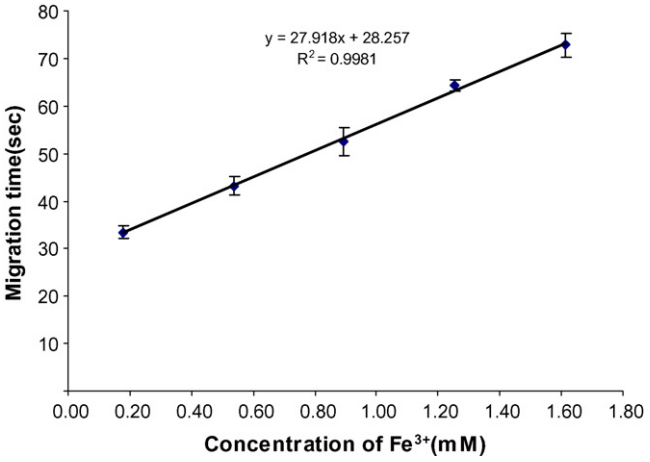

Fig. 5. Time-based calibration graph of Fe^{3+}.

to the negative slopes of the other calibration graphs observed in the previous examples. This difference can be explained by considering that in this system, sample solutions of various concentrations were injected vertically while the fixed concentration reagent was injected horizontally. Migration time, therefore, is in direct proportion (positive slope) to the concentration of sample in the vertical line. Linear working range was found over the chosen concentration range of 0.18–1.61 mM. The weight percent of Fe^{3+} in the nail sample was calculated to be 62%.

The same digested nail sample was also analyzed with batch molecular spectrometry and flame AAS. A calibration curve with linear working ranges 3.58×10^{-3} to 1.79×10^{-2} mM ($y = 8.2339x - 0.0004$, $R^2 = 0.9988$) was obtained with the batch molecular spectrometry. The average weight percent of Fe^{3+} in the nail obtained using the batch molecular spectrometry and AAS were 64 and 60%, respectively. They agreed well with the amount obtained from the simple lab-on-chip system.

4. Conclusion

A simple and economic lab-on-chip with time-based approach has been proposed and demonstrated with various reactions. The detection was based on migration of the colored reaction zone. Visual detection by naked eyes with a stop-watch can be incorporated. The techniques may be useful for on-site analysis by applying some coloration/decoloration reactions to the proposed simple systems. Further development by incorporating cartridge type pretreatment techniques to the proposed simple chip will be explored.

Acknowledgements

The authors thank the Commission on Higher Education (CHE) through Research Group (RG) grant for support. Additional supports from the Center for Innovation in Chemistry: Post Graduate Education and Research Program in Chemistry (PERCH-CIC), and The Thailand Research Fund are also acknowledged.

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