

A Low Cost and Simple Lab-on-a-Chip for Spectrophotometric Determination of Ethanol

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Abstract

A simple lab-on-a-chip system was developed for the rapid determination of ethanol in different sample matrices, including gasohol and various alcoholic beverages. The colorimetric detection of ethanol using a spectrophotometer was based on the reaction between ethanol with 0.12 M ceric ammonium nitrate in acidic medium to produce a red colored product which gave a maximum absorption at 470 nm. A non-lithographic method was used for creating lab-on-a-chip molds to reduce manufacturing cost and preparation steps. The lab-on-a-chip device was fabricated from polydimethylsiloxane which consisted of a simple Y-shaped working channel. Under optimum conditions, a linear calibration graph was obtained in the concentration range of 0.20 - 20 % (v/v) ($r^2 > 0.999$). The limit of detection (3 SD) and limit of quantification (10 SD) were 0.039 and 0.13 % (v/v), respectively. The precision reported in terms of relative standard deviation (RSD) values was less than 1.40 % (n = 15). To demonstrate the lab-on-a-chip's performance, the determination of ethanol in gasohol and various alcoholic beverages was applied. The results obtained from the developed method compared with a standard gas chromatographic method were well correlated using the paired t-test and linear regression test. The results indicate that the proposed method has shown potential to extend the use of this simple lab-on-a-chip analytical device, due to its simplicity, low cost, lower reagent and sample consumption and high analytical performance. Moreover, the method of fabrication would be an additive manufacturing technique featuring a low equipment cost with no need for clean rooms.

Keywords: Lab-on-a-chip, ethanol, alcoholic beverage, gasohol

Introduction

In Thailand, ethanol has been commonly used in alcoholic beverages production and gasoline blends (known as 'gasohol'). An alcoholic beverage is a drink that typically contains 3 - 40 % ethanol by volume. Alcohol beverages are generally divided into 3 categories: beers (5 - 6 %vol), wines (12 - 13 %vol) and distilled spirits (28 - 40 %vol). The determination of ethanol in alcoholic beverage is important due to its social and economical implications, especially in relation to the taxed imposed in different countries [1,2]. Gasohol has been commercially available in Thailand since 2001. This type of fuel refers not only to low ethanol blends (E10, E20), but also to higher percentages (E85). Fuel blends with an 'E' prefix denote gasoline blended with ethanol, while the following numbers denote the percentage of ethanol in that particular blend by volume. For example, E10 is fuel blended with 10 % (v/v) ethanol and 90 % (v/v) gasoline. Ethanol acts as an octane booster for gasoline and can be considered as an environmentally friendly fuel substitute. Ethanol-based gasohol with higher concentrations of ethanol can damage the rubber seals and diaphragms of gasoline engines. Therefore, the amount of ethanol added to gasoline needs to be monitored to keep the quality production aligned with

the standard [3,4]. Consequently, the ethanol content is a key quality control for both gasohol and alcoholic beverages.

Several analytical methods have been reported for the determination of ethanol content in beverages and gasohol fuel including gas chromatography (GC) [5,6], high performance liquid chromatography (HPLC) [7] and enzymatic methods with electrochemical detection [8-10]. Flow-based techniques have also been proposed for ethanol analysis with colorimetric detection using dichromate or ceric nitrate as reagents to increase the degree of automation [11-17]. However, most of these methods have some limitations such as requiring relative expensive instruments and personal experience, lack of portability, long analysis times and large amounts of reagents and sample. Recently, the lab-on-a-chip system (LOC) or microfluidic system was developed for a large number of chemical and biological analyses, because of its advantages in using less reagents and sample, high throughput analysis, compactness of the systems, cost-effective disposable devices and safer platform for chemicals [18-21]. These miniaturized devices can integrate one or more laboratory function onto a single device of only millimeters to a few square centimeters scale and can be designed for a specific application. Lei *et al.* reported a microfluidic device with spectrometric detection for determination of ethanol content in wines and distilled spirits [22]. The microfluidic device was fabricated using photolithography to prepare a mold in a clean-room, followed by soft lithography to fabricate the device. The detection principle was based on chemical oxidation of ethanol by dichromate in acidic solution followed by detection of the produced Cr(III). This chip based method has advantages in terms of high throughput and portability but preparation of the microfluidic mold is rather complicated and expensive. In addition, the dichromate used in spectrometric detection is carcinogenic.

In this work, we developed a fast, inexpensive and simple method that used readily accessible materials to prepare lab-on-a-chip molds by laser cutting, followed by curing polydimethylsiloxane (PDMS) in the mold without any surface treatment to make a lab-on-a-chip device. The simple lab-on-a-chip system was demonstrated for determination of ethanol based on the reaction between ethanol and ceric nitrate in acidic medium to produce a red colored complex of Ce(IV)-ethanol [11,15]. The lab-on-a-chip system was successfully applied for analysis of ethanol content in different types of sample matrices and ethanol contents, including beers, Thai spirits and gasohol fuels. The proposed method also provided good analytical performance, high throughput analysis, was environment friendly and portable.

Materials and methods

Chemicals

All chemicals used in this work were analytical reagent grade and solutions were prepared in deionized water (Siemens, Thailand). Ceric ammonium nitrate (0.12 M $\text{Ce}(\text{NH}_4)_2(\text{NO}_3)_6$), used as a reagent solution, was daily prepared by dissolving 6.58 g of ceric ammonium nitrate (Kosdaq, Korea) in 100 ml of 0.3 M nitric acid (MacronFine Chemicals, USA). Standard ethanol solutions were prepared by diluting absolute 99.95 % (v/v) ethanol (QReC Chemicals, New Zealand) to the required concentrations.

Sample preparation

Commercially available alcoholic samples were obtained from local supermarkets. The alcoholic beverages consisted of 3 samples of beer and 2 samples of Thai spirits. These samples were diluted 5-fold in deionized water before direct analysis. Gasohol samples were purchased from petrol stations, Thailand. The samples consisted of a gasohol E85, 3 samples of gasohol E10 and 3 samples of gasohol E20. These samples were extracted with deionized water [14], followed by 1/5 dilution with deionized water before analysis.

Construction of lab-on-a-chip device

The process consisted of 2 fabrication steps: mold making and chip fabrication. Polymethyl methacrylate (PMMA) and a laser cutting system were used for creating the mold to reduce manufacturing costs and preparation steps. The channel pattern was firstly drawn using AutoCAD, a commercial computer-aided design and drawing software. The simple Y-shaped channel pattern, shown

in **Figure 1**, consisted of 2 upstream channels, one for the reagent solution and the other for the samples/standards, and a zigzag shaped mixer in the first part of the main channel. The width and depth of the Y-shaped channel were 1.0 mm and 0.8 mm, respectively. The design pattern was printed onto the PMMA sheet and then cut out using a laser cutting system (GoInterrich Co. Ltd, Thailand). This PMMA product was used as a mold for preparing the PDMS lab-on-a-chip device.

In the chip fabrication process, a polydimethylsiloxane precursor and a curing agent (Sylgard 184 Dow Corning, USA) were mixed in a 10:1.5 ratio by mass. To remove air bubbles from the PDMS that arise during mixing, the PDMS mixture was degassed for 20 min using a vacuum desiccator (BUCHI, Switzerland). The PDMS mixture was poured onto the PMMA mold, which was positioned in the bottom of a Petri dish. The PDMS-coated mold was cured at 60 °C for 30 min in a hot air oven (Memmert Germany). Then, the cured PDMS channel was peeled off from the mold and cut to size with a scalpel. Finally, the open PDMS device containing a Y-shaped channel was sealed with another flat PDMS (precursor:curing agent = 10:0.4) by thermal bonding at 80 °C for 40 min in a hot air oven without any surface treatment to form the PDMS chip. Holes for the inlets and outlet were punched into the PDMS chip with a needle, and small sections of disposable pipette tips were attached and fixed into place with 5 min epoxy. The dimensions of the PDMS chip were 3.0 mm depth × 40.0 mm width × 70.0 mm length.

Measurement system

The schematic diagram of the lab-on-a-chip system used in this work are shown in **Figure 1**. A pocket pump (Model HPN200, Yabegawa Elec.I. Japan) was used with 0.75 mm i.d. PTFE tubing (Cole Parmer, USA) for driving the reagent solution through the one upstream channel of the PDMS chip. A microliter syringe (Hamilton, Australia) was employed for injection of the standard or sample solutions in the other upstream channel of the PDMS chip. A standard/sample plug was mixed with the reagent within the zigzag shaped mixer of the PDMS chip. The reaction zone was propelled into main channel and passed through the end of main channel of a PDMS chip which was connected to the flow cell of a spectrophotometer (model UV-1000, Thermo separation). Finally, the reaction zone was pushed into the detection cell and the absorbance monitored at 470 nm.

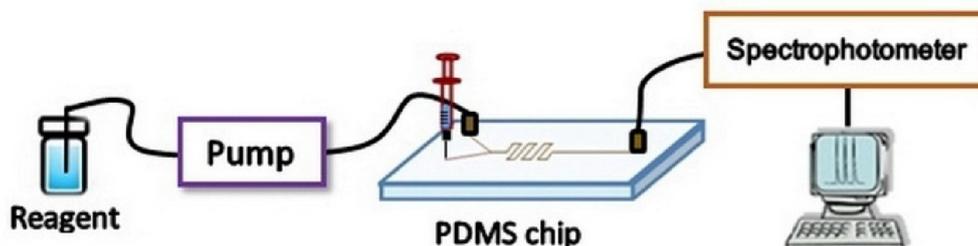


Figure 1 The schematic diagram of lab-on-a-chip system for the determination of ethanol.

Results and discussion

Optimization of the lab-on-a-chip device

Polydimethylsiloxane (PDMS) was chosen for fabrication of the lab-on-a-chip device due to its many advantages as a good elastomeric and inert material, ease of fabrication and economy. In the PDMS chip fabrication process, the effect of the mixing conditions (PDMS precursor:curing agent) and length of zigzag shaped mixer were investigated and optimized.

The effect of mixing ratios (PDMS precursor:curing agent) on the hardness of the PDMS chip were studied in the range of 10:1.0 to 10:2.0 for the PDMS channel and 10:0.2 to 10:0.8 for the flat PDMS.

The results indicated that increasing the curing agent ratio led to an increase in rigidity of the PDMS chip, causing leakage at the PDMS chip and flat PDMS interface. A smaller amount of curing agent resulted in lower cohesion of the polymer, which led to the PDMS chip breaking. Therefore, the mixing ratios between the PDMS precursor and curing agent at 10:1.5 and 10:0.4 were selected for the PDMS channel and flat PDMS, respectively.

The efficiency of mixing between the ethanol and reagent solution on the response signal of colored product measured by lab-on-a-chip system (**Figure 1**) was studied by varying the length of the zigzag shaped mixer within the PDMS chip over the range of 5 - 20 mm. It was found that the response signal increased with an increase on the length of the zigzag shaped mixer and leveled off at a length of 10 mm in all ethanol solutions (1 - 20 % v/v) as shown in **Figure 2**. The results indicated that more efficient mixing of the reagent and ethanol was achieved with increasing the length of zigzag shaped mixer as a result the signal of the colored product increased. However, zigzag shaped mixers longer than 10 mm decreased the sample throughput without a significant increase in the response signal. Thus, a zigzag shaped mixer 10 mm in length was selected for further experiments in order to keep size of the lab-on-a-chip device small.

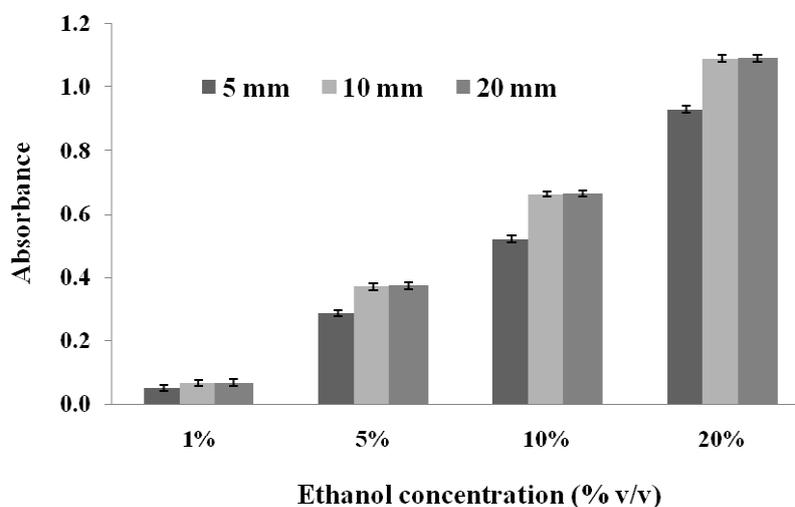


Figure 2 Effect of length of zigzag shaped mixer on the efficiency of mixing. (Conditions: 0.12 M ceric ammonium nitrate in 0.3 M nitric acid, flow rate of reagent 240 μLmin^{-1} , sample volume 4 μL).

Selection of the reaction for ethanol

Ceric ammonium nitrate (CAN) has been reported as a reagent for qualitative analysis of ethanol in alcoholic beverages [11,15]. These methods demonstrate that the reaction between the ceric ion and ethanol is fast and occurs easily in an acidic medium. The red colored complex was stable within about 10 min [15], but there is no problem in lab-on-a-chip system because of the very short time interval between the zigzag shaped mixer and the end of a lab-on-a-chip device. Batch experiments were carried out to compare the performance of CAN with other strongly oxidizing agents such as dichromate and permanganate, which are usually employed as a reagent for ethanol analysis. The absorbance measured at a fixed time of 1 min, was used for the calibration plots. Calibration equations of $y = 0.0921x + 0.0036$, $y = 0.0382x + 0.0058$ and $y = 0.0192x + 0.0063$ were obtained for CAN, dichromate and permanganate reagents, respectively. All reagents gave linear calibration over the standard ethanol concentration range of 1 - 20 % v/v ($r^2 > 0.998$). The results showed that the CAN reagent is more sensitive than the other reagents. Ceric ammonium nitrate was therefore chosen to be used as a reagent for ethanol in this work

for better sensitivity and selectivity. Moreover, ceric ammonium nitrate is more environmental friendly than dichromate.

Optimization of the lab-on-a-chip system

Parameters that influence the sensitivity of the system were investigated as described below. A series of standard ethanol (0.50 - 20 % v/v) was used throughout the optimization studies employing the lab-on-a-chip system as shown in **Figure 1**. Each condition was quantified at least 3 times, and the results were shown as sensitivity obtained from the slope of a calibration graph plotting between absorbance measured and ethanol concentration. The criteria for the optimization are based on the balance of sensitivity, sample throughput and analytical performance.

Effect of ceric ammonium nitrate concentration

The effect of ceric ammonium nitrate concentration on sensitivity was studied in the range of 0.09 - 0.14 M. The results shown in **Figure 3** indicate that the sensitivity increased as the reagent concentration increased up to 0.12 M and remained stable afterwards, indicating the amount of reagent was enough for the reaction (at least at the tested ethanol concentration range). Consequently, a ceric ammonium nitrate of 0.12 M was chosen as the optimum concentration for further studies.

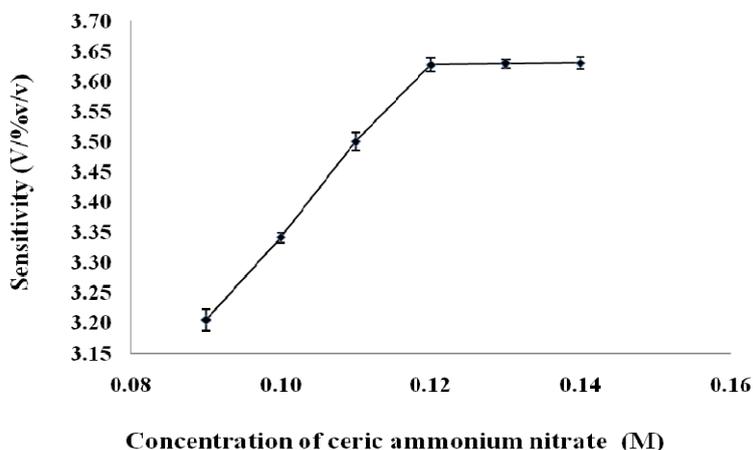


Figure 3 Effect of ceric ammonium nitrate concentration on sensitivity of system. Conditions: ceric ammonium nitrate prepared in 0.3 M nitric acid, flow rate of reagent $240 \mu\text{Lmin}^{-1}$, sample volume $2 \mu\text{L}$.

Effect of nitric acid concentration

The influence of the nitric acid concentration on sensitivity was carried out by varying in the range of 0.0-1.2 M. It can be seen that the sensitivity increased with an increase of nitric acid concentration and dropped a little bit at concentrations higher than 0.3 M (**Figure 4**), which agrees with a previous report [15]. In acidic media, the hydrolysis reaction of the ceric ion to form hydroxide-ceric complex ($\text{CeL}_m(\text{OH})^{(n-1)+}$) is suppressed and the aqua complex ($\text{CeL}_m(\text{H}_2\text{O})^{n+}$) is dominant. Ethanol tends to replace H_2O ligand more easily than the hydroxide ligand, hence the formation of red colored Ce(IV)-ethanol complex was facilitated in acidic solutions [15]. Therefore, the concentration of 0.3 M nitric acid was adopted for the present study.

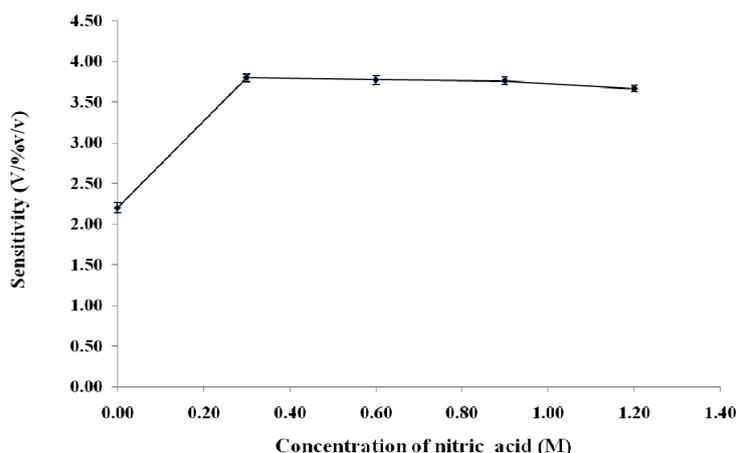


Figure 4 Effect of nitric acid concentration on the sensitivity of system. Conditions: 0.12 M ceric ammonium nitrate in nitric acid, flow rate of reagent $240 \mu\text{Lmin}^{-1}$, sample volume $2 \mu\text{L}$.

Effect of sample volume

The optimization of sample volume was investigated over the range of 1 - 5 μL . As illustrated in **Figure 5**, the sensitivity increased as a consequence of the increase of sample volume. However, volumes higher than 2 μL gave low reproducibility and the peak shape became broader. A narrow range of calibration curve was also achieved. Thus, a sample volume of 2 μL was chosen for all subsequent measurements to reduce sample consumption and obtain a linear range up to 20 % v/v ethanol.

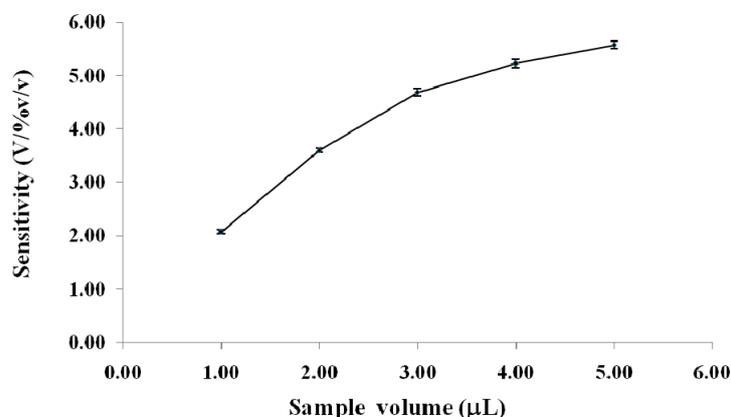


Figure 5 Effect of sample volume on the sensitivity of system. Conditions: 0.12 M ceric ammonium nitrate in 0.3 M nitric acid, flow rate of reagent $240 \mu\text{Lmin}^{-1}$.

Effect of flow rate

The effect of the flow rate of the reagent on the sensitivity was evaluated in the range of 60 - 240 μLmin^{-1} . The results indicate that the sensitivity steadily decreased as the reagent flow rate increased from 60 to 240 μLmin^{-1} . As expected, at higher flow rates the mixing efficiency was reduced which causes a decrease in the sensitivity as shown in **Figure 6**. Therefore, a flow rate of 120 μLmin^{-1} was selected by considering the choices of sensitivity, sample throughput and reagent consumption.

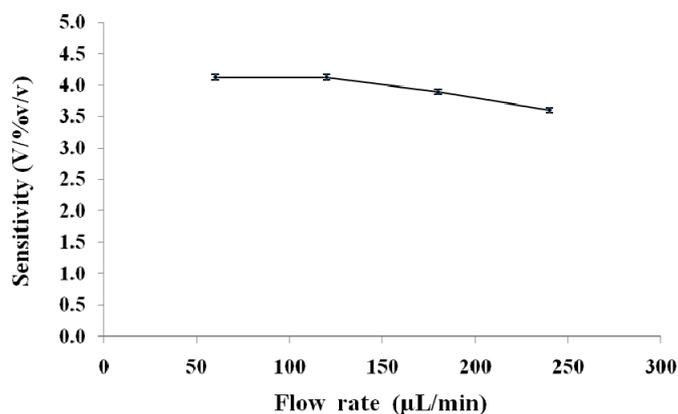


Figure 6 Effect of flow rate of reagent solution on the sensitivity of system. Conditions: 0.12 M ceric ammonium nitrate in 0.3 M nitric acid, sample volume 2 µL.

Analytical characteristics of the lab-on-a-chip system

Under the optimum conditions shown in **Table 1**, analytical parameters such as linear range, limit of detection (LOD), limit of quantification (LOQ) and precision were determined. The linearity was studied over a concentration range of 0.20 - 20 % (v/v) of ethanol with an excellent correlation coefficient being higher than 0.999. **Figure 7** shows the signals of ethanol standard solutions where each concentration has been injected 3 times to the lab-on-a-chip system. LOD and LOQ calculated from 3 and 10 times the standard deviation of blank/slope of the calibration curve were 0.039 and 0.13 % (v/v), respectively. The precisions were measured in terms of relative standard deviation (RSD) values at 3 different concentrations of standard ethanol (1, 10 and 20 % (v/v)). The RSDs of the method for intraday (n = 15) and interday (n = 9) were less than 1.34 and 1.40 %, respectively, which indicated a good repeatability of the method. The sample throughput was 45 h⁻¹, indicating that the proposed lab-on-a-chip method is fast and each injection consumed 0.32 mL of reagent showing that this method low chemical consumption. A comparison of the analytical characteristics of the present method with the previous published literatures for determination of ethanol in alcoholic beverages and gasohol fuels is summarized in **Table 2**.

Table 1 The optimum condition of lab-on-a-chip system for determination of ethanol.

Parameters	Studied range	Optimum value
Length of zigzag shaped mixer (mm)	5 - 20	10
Reagent concentration (M)	0.09 - 0.14	0.12
Nitric acid concentration (M)	0.0 - 1.2	0.3
Sample volume (µL)	1 - 5	2
Flow rate (µLmin ⁻¹)	60 - 240	120

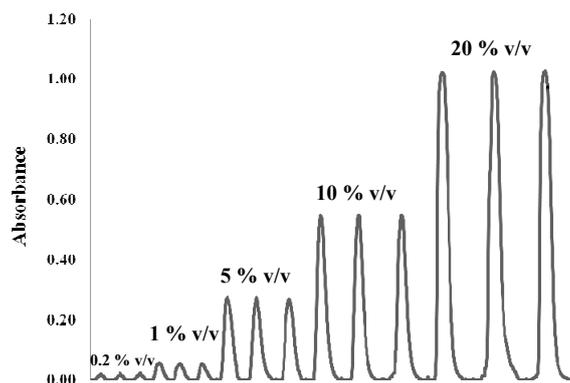


Figure 7 The signal profiles for a series of standard ethanol (0.20 - 20 % v/v) in triplicate measurements. Conditions: 0.12 M ceric ammonium nitrate in 0.3 M nitric acid, sample volume 2 μ L, flow rate of reagent 120 μ Lmin⁻¹.

Table 2 Comparison of analytical characteristics of the present method with other spectrophotometric/colorimetric methods for determination of ethanol.

System	Reagent	Sample	Linear range (%v/v)	Detection limit (%v/v)	Precision (% RSD)	Sample throughput (h ⁻¹)	Ref.
FIA	0.03 M K ₂ Cr ₂ O ₇ in 1.5 M H ₂ SO ₄	Beer, wine and distilled spirits	0.5 - 30	0.27	0.5	16	[13]
FIA	0.2 M K ₂ Cr ₂ O ₇ in 4 M H ₂ SO ₄	Gasohol fuel	3 - 80	0.9	1 - 4.9	26	[14]
FIA	0.04 M CAN in 0.3 M HNO ₃	Beer, wine and distilled spirits	0.1 - 10	0.03	< 1.3	20	[15]
FIA	0.3 M K ₂ Cr ₂ O ₇ in 4 M H ₂ SO ₄	Fermentation brew	5 - 25	0.18	2.1	29	[16]
SIA	0.003 M K ₂ Cr ₂ O ₇ in 8 M H ₂ SO ₄	Wine	0 - 1.5	0.025	< 4	12	[17]
Microfluidic	0.15 M K ₂ Cr ₂ O ₇ in 6 M H ₂ SO ₄	Distilled spirits and wine	Up to 6	-	-	-	[22]
Lab-on-a-chip	0.12 M CAN in 0.3 M HNO ₃	Distilled spirits, beer and gasohol fuel	0.2 - 20	0.039	< 1.4	45	This work

Analytical application to real samples and validation

To demonstrate the analytical performance on real samples of the developed method, a lab-on-a-chip system was used to determine the concentration of ethanol in various types of sample matrices and ethanol contents, including alcoholic beverages (beer and Thai spirits) and gasohol fuels (gasohol E10, E20 and E85). In addition, results obtained from the developed method were also verified using reference gas chromatographic analysis [17] of the samples. Prior to analysis, alcoholic beverage samples were only diluted 5-fold in deionized water and gasohol samples were extracted with deionized water [14], followed by 1/5 dilution with deionized water. The determination results of the lab-on-a-chip method and gas chromatographic method are listed in **Table 3**. Ethanol contents obtained from both methods were in high correlation with no significant difference at 95 % confidence level ($t_{\text{stat}} = 0.986 < t_{\text{critical}} = 2.20$). The correlation coefficient between the proposed method and the GC method was 0.9999. Recovery studies were conducted by spiking standard ethanol into different samples to evaluate the degree of interference from the matrix. As shown in **Table 3**, the recoveries varied in the range of 98.8 - 107.7 %. These satisfactory results indicate that the developed lab-on-a-chip method provides accurate and precise results and it was not affected by the matrix.

Table 3 Ethanol contents in various alcoholic beverages and gasohol fuels determined by the proposed lab-on-a-chip method and the GC method with label values and recovery values in different samples (n = 3).

Sample	Ethanol content(%v/v)			Recovery (%)
	Label	GC method	Proposed method	
Alcoholic beverages				
- Beer	5.00	4.98± 0.02	4.97± 0.18	105.5
- Beer	5.00	5.02± 0.05	4.93± 0.22	102.7
- Beer	5.20	5.33± 0.03	5.32± 0.12	101.9
- Thai spirits	35.00	35.18± 0.08	35.01± 0.32	99.9
- Thai spirits	40.00	40.38± 0.04	40.46± 0.53	102.7
Gasohol				
- E10	-	9.58± 0.15	9.65± 0.15	102.4
- E10	-	9.39± 0.02	9.39± 0.10	101.1
- E10	-	9.55± 0.06	9.55± 0.10	100.6
- E20	-	19.92± 0.05	19.72± 0.15	107.7
- E20	-	19.88± 0.20	19.80± 0.28	101.8
- E20	-	19.82± 0.05	19.81± 0.20	99.8
- E85	-	79.40± 0.19	79.50± 0.39	98.8

Conclusions

A fast, simple and reliable lab-on-a-chip method has been developed for the determination of ethanol in different sample matrices and ethanol contents, including beer, Thai spirits and gasohol fuel. The sample throughput was 45 samples h⁻¹, with minimum sample and reagent volumes of only 2.0 µL and 0.32 mL, respectively. The ceric ammonium nitrate reagent had higher selectivity and negligible toxicity as compared to dichromate, which was commonly used as a reagent for ethanol analysis. According to the analytical characteristics study, the linearity, precision and sensitivity of the method are satisfactory. The results obtained from the developed method compared with a standard gas chromatographic method correlate well. The advantages of the proposed method show the potential to extend the use of a simple lab-on-a-chip analytical devices, due to their simplicity, low cost, lower reagent and sample consumption with tiny waste generation, portability and high analytical performance.

Moreover, the method of fabrication is simple, requiring lower equipment costs, less complicated steps and no need for clean rooms.

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