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Flow injection colorimetric method using acidic ceric nitrate as reagent for determination of ethanol

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ABSTRACT

Ceric ammonium nitrate has been used for qualitative analysis of ethanol. It forms an intensely colored unstable complex with alcohol. In this work, a simple flow injection (FI) colorimetric method was developed for the determination of ethanol, based on the reaction of ethanol with ceric ion in acidic medium to produce a red colored product having maximum absorption at 415 nm. Absorbance of this complex could be precisely measured in the FI system. A standard or sample solution was injected into a deionized water donor stream and flowed to a gas diffusion unit, where the ethanol diffused through a gas permeable membrane made of plumbing PTFE tape into an acceptor stream to react with ceric ammonium nitrate in nitric acid. Color intensity of the reddish product was monitored by a laboratory made LED based colorimeter and the signal was recorded on a computer as a peak. Peak height obtained was linearly proportional to the concentration of ethanol originally presented in the injected solution in the range of 0.1–10.0% (v/v) ($r^2 = 0.9993$), with detection limit of 0.03% (v/v). With the use of gas diffusion membrane, most of the interferences could be eliminated. The proposed method was successfully applied for determination of ethanol in some alcoholic beverages, validating by gas chromatographic method.

1. Introduction

Ethyl alcohol or ethanol is the most publicly known alcohol since it is contained in many products such as alcoholic beverages, antiseptic and fuel for vehicles. Determination of ethanol content is important for quality control of the products. Various methods have been used for quantification of ethanol such as densitometry, gas chromatography, high performance liquid chromatography, infrared spectroscopy, spectrophotometry and enzymatic biosensor. Although densitometry is recommended as a standard method for quality control of beverage products, it concerns the tedious and slow distillation procedure [1]. Flow injection (FI) pervaporation system with in-line densiotometric detection was developed to increase the degree of automation [2]. Gas chromatography is approved as a standard method for ethanol determination in beverages and gasohol fuel [3]. Chromatographic techniques lack portability and need long analysis time, although they provide simultaneous determination of several alcohols. Biosensor has advantages in terms of selectivity and portability but life-time of the sensor is not long and fabrication of the sensor is rather complicated and expensive [4]. Infrared spectroscopy is also limited in selectivity. FI with Near IR detection is proposed in order to increase degrees of automation of the method [5].

Spectrophotometric measurement is usually performed after separation of ethanol from other matrices by distillation. Chemical oxidation of ethanol by dichromate followed by spectrophotometric detection of the produced Cr(III) or the remaining Cr(VI) is the commonly used chemistry. This detection principle is also widely adapted to flow based techniques for determination of ethanol [6–11], which offer improved analytical performance such as being less tedious, having faster analysis, and having more automatic than batch wise method. However, dichromate is a carcinogenic substance, so it is of interest to reduce the amount needed, such as by using sequential injection (SI) [9], microfluidics [11] and microplate reader [12]. Enzymatic methods for determination of ethanol and glycerol in wines were introduced in FI [13] and SI [14] systems. These systems employed gas diffusion separation to increase selectivity. Electrochemical oxidation of ethanol on copper electrode under alkaline medium has been introduced in flow injection amperometric system [15]. However, this reaction is not very selective and the electrode is prone to fouling, which leads to low reproducibility. Since the reactions used either in spectrophotometric or electrochemical analysis are not selective, in-line separation of ethanol from sample matrices is needed, which could be done by gas diffusion [8,10,13,15] or pervaporation [6,7] technique, with the use of gas permeable

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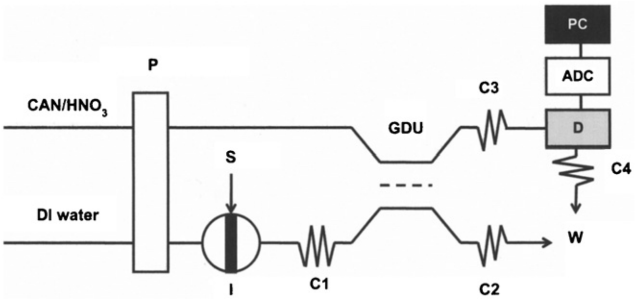

Fig. 1. FI manifold of flow injection system for determination of ethanol; P: peristaltic pump, I: injection valve, S: standard/sample, GDU: gas diffusion unit, W: waste, C1–C4: mixing coils, D: colorimeter, ADC: analog to digital converter unit and PC: personal computer.

membrane or recently with membraneless gas diffusion device [8,10].

In this work, we developed a simple FI colorimetric system based on the reaction of ethanol and acidic ceric nitrate to produce a red colored complex of Ce(IV)–ethanol. Ce(IV) has been used as a colorimetric reagent for qualitative detection of alcohols for a long time [16]. The formation of 1:1 complex of Ce(IV)–alcohol was found, of which the change of color from yellow to red was observed [17]. Ce(IV) is present in the anionic form as a hexanitrato ceric species or other complexes with combination of H_2O, OH^- and $NO_3{}^-$ as ligands, with 12-fold coordination sphere of the Ce(IV). Alcohol replaces one of the Ce–O bonds of bidentate nitrate (or other monodentate ligands) leading to a complex composed of five bidentate nitrate groups, one monodentate nitrate group and one alcohol molecule [18]. This complex is not stable, and the alcohol will be oxidized to aldehyde and then to carboxylic acid while Ce(IV) is reduced to a colorless Ce(III) [18]. Apart from alcohol, Ce(IV) can form complexes with α-hydroxy acids such as lactic, malic and tartaric acids [19]. Despite this, the reagent is also not specific to ethanol but it is more selective than a strongly oxidizing agent, dichromate. Moreover, ceric ammonium nitrate is less hazardous than dichromate. In order to improve selectivity of the method, incorporation of a simple gas diffusion unit employing a PTFE plumbing tape as a membrane, the FI system was investigated for in-line separation of ethanol from the sample matrices. The proposed system provided good analytical performance and was successfully demonstrated for determination of ethanol in some alcoholic beverages.

2. Experimental

2.1. Chemicals and reagents

All chemicals used were of analytical reagent grade and deionized water (obtained from a system of Milli-Q, Millipore, Sweden) was used throughout. Reagent solution containing 0.04 M ceric ammonium nitrate and 0.3 M nitric acid was prepared by dissolving 2.20 g of ceric ammonium nitrate (BDH, England) in water and making up to a volume of 100 mL in a volumetric flask. Working standard solution of ethanol (0.1–10.0%, v/v) was freshly prepared by diluting the 99.5% (v/v) ethanol (Merck, Germany) with water.

2.2. FI colorimetric system

FI colorimetric system for determination of ethanol is shown in Fig. 1. It consisted of a peristaltic pump (Lachat, USA), a six port injection valve (Upchurch, USA), a planar gas diffusion unit [20], a lab-built colorimeter based on light emitting diode as a light source and light dependent resistor as a light sensor, equipped with a flow through cell of 10 mm path length (Hellma, Germany), a data acquisition unit [20] and a personal computer. Mixing coils and tubing for assembling the system were a PTFE tube of 0.5 mm diameter, except pump tubing for propelling the solution is a Tygon tube (Fisher Scientific, USA).

A home-made planar gas diffusion unit [20] was made of two acrylic plates (15 cm long, 4.8 cm wide and 1.0 cm thick), engraved for donor and acceptor channels (each 300 mm long, 1.5 mm wide, 0.75 mm deep). A PTFE membrane (teflon tape used for plumbing work) was placed in between the two plates to form channels for liquid to flow on each side.

2.3. Determination of ethanol by FI colorimetric method

Employing the FI set up as described above, a standard/sample solution (100 μL) was injected into a water carrier stream and flowed through mixing coil C1 to a gas diffusion unit, where the analyte diffused through a PTFE membrane into an acceptor stream of acidic ceric ammonium nitrate. Ethanol reacted with the reagent to produce a red Ce(IV)–ethanol complex which could be monitored for a color change by using a colorimeter with a blue LED as light source. A commercial spectrophotometer could be used by setting to measure absorbance at 415 nm. Output signal from the detector was recorded as FIA peak on a personal computer. Peak height was directly proportional to concentration of ethanol in the injected solution. A calibration graph was constructed by plotting peak height versus ethanol concentration and it was used for quantification of ethanol in sample.

2.4. Determination of ethanol by gas chromatographic method

Gas chromatograph (HP Aligent 6890 GC model, Hewlett Packard, Palo Alto, CA, USA). The following GC condition was used: carrier gas was helium, flow rate was 1.2 mL min^{-1}, injection volume was 1.00 μL, type of separation column was capillary column (Aligent 19091 N-113 HP – INNOWAX Polyethylene glycol), and detector was flame ionization. The method of internal standard calibration was utilized by adding n-Propanol as an internal standard to sample or standard solution of ethanol to obtain a concentration of 2% (v/v) n-Propanol. Ethanol standard solutions were prepared by dilution of the 99.5% (v/v) ethanol to obtain working standard solutions in the concentration range of 0.1–4.0% (v/v). All samples were diluted 20 times with DI water before analysis. Ethanol contents in samples were calculated from a calibration graph constructed from area ratio of ethanol to n-Propanol and concentration of the ethanol.

2.5. Sample preparation

Twenty-three samples of commercially available alcoholic beverages were purchased from local stores in Chiang Mai, Thailand. According to the label, ethanol contents in samples ranged from 5.0 to 40% (v/v). Samples were properly diluted with DI water (10 folds for distillated liquor, 20 folds for beer and 40 folds for wine) prior to injection into the FI system.

3. Results and discussion

3.1. Preliminary study of the reaction

Absorption spectra of 0.02 M ceric ammonium nitrate (CAN) and 0.02 M CAN with 10% (v/v) (0.017 M) ethanol in 0.15 M nitric acid solutions recording versus water, and of the Ce(IV)–ethanol complex versus CAN reagent blank were recorded in the range of 350–700 nm as depicted in Fig. 2. It was observed that the absorption of the complex occurred at a longer wavelength than that of

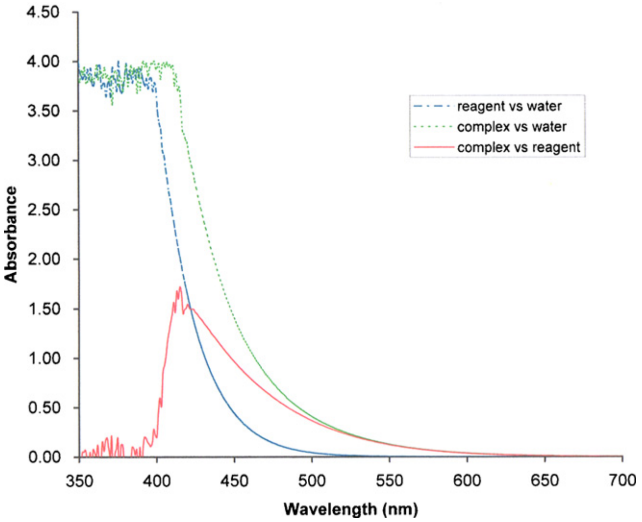

Fig. 2. Absorption spectra of (a) acidic ceric reagent (0.02 M CAN in 0.15 M HNO_3), (b) Ce(IV)–ethanol complex (0.02 M CAN and 1%, v/v (0.017 M) ethanol in 0.15 M HNO_3) with respect to water, and (c) solution of (b) with respect to solution of (a).

the reagent. The maximum absorption wavelength of the complex versus reagent blank was observed at 415 nm.

The reaction is fast and is the first order reaction with respect to ceric and alcohol [21]. The rate equation is as follows: rate = k[Ce(IV)][alcohol], with k of about 0.75 L mol^{-1} for ethanol. Although the complex had high intensity of color, the complex is not stable. Color of the complex faded rapidly which could be observed with naked eyes. In contrast, the reagent itself is stable. Stability of the complex was investigated by monitoring the absorbance of 0.02 M CAN-1% (v/v) ethanol solution at 415 nm versus reagent blank after the solutions were mixed together. It was found that the absorbance decreased dramatically and become zero within about 10 min (Fig. 3). The unstability of this complex might limit the use of this reagent for determination of ethanol in batch wise method but there was no problem in FIA because of a short and precisely controlled time interval between an injection of sample and the detection of the resulting product.

3.2. *Optimization of FI colorimetric method*

Employing the FI system as shown in Fig. 1, the optimum conditions for determination of ethanol were investigated. The following preliminary conditions were used: concentration of ceric ammonium nitrate of 0.05 M, flow rates of carrier and reagent solution of 1.0 mL min^{-1} each, sample volume of 100 μL, length of coils C1, C2, C3 and C4 of 50, 100, 50 and 50 cm, respectively and blue LED as a light source.

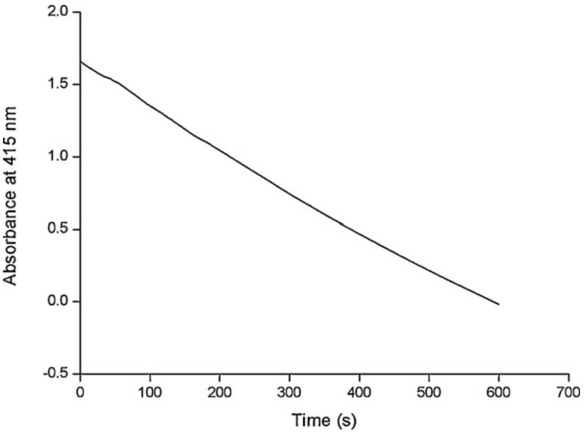

Fig. 3. Absorbance–time profile of 0.02 M CAN-1.0% (v/v) ethanol in 0.15 M HNO_3 solution. Absorbance at 415 nm was measured versus reagent blank.

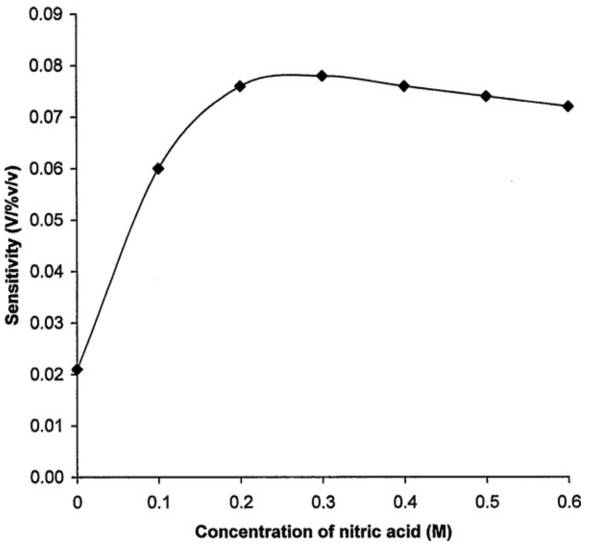

Fig. 4. Effect of nitric acid concentration on sensitivity of the method. (Condition: 0.04 M CAN, flow rate 1.0 mL min^{-1}, sample volume 100 μL, length of coils, C1 50 cm, C2 100 cm, C3 50 cm, C4 50 cm.)

3.2.1. *Effect of nitric acid*

Effect of nitric acid concentration in range of 0.0–0.6 M was studied by injecting a series of standard ethanol (0.1–10.0%, v/v) into the system while other parameters were kept constant as described above. Plotting peak height obtained versus concentration of ethanol yielded linear calibration graphs with r^2 higher than 0.999. It was found that sensitivity (slope of the calibration graph) increased with the increase of nitric acid concentration and leveled off at concentration higher than 0.2 M as shown in Fig. 4. The high cationic charge and smaller ionic size of the ceric ion may make ceric salts much more hydrolyzed in aqueous solution than other trivalent lanthanides, leading to the formation of hydroxide ceric complex ($\text{Ce}\,\text{L}_\text{m}\,(\text{OH}^-)^{(n-1)+}$) and the releasing of hydrogen ion [22]. In highly acidic solution the hydrolysis is suppressed and aquo complex ($\text{Ce}\ \text{L}_\text{m}\ (\text{H}_2\text{O})^{n+}$) should be predominant. Ethanol should replace H_2O ligand easier than the hydroxide ligand, hence the red colored Ce(IV)–ethanol complex was easily formed in acidic solution. Nitric acid concentration of 0.3 M was selected for further studies.

3.2.2. *Effect of ceric ammonium nitrate concentration*

Effect of concentration of ceric ammonium nitrate on sensitivity was investigated as similar to above. Calibration equations of $y = 0.067x + 0.001$, $y = 0.072x + 0.008$, $y = 0.073x + 0.009$ and $y = 0.071x + 0.022$ were obtained for ceric nitrate concentration of 0.02, 0.03, 0.04 and 0.05 M, respectively. In all case r^2 of the calibration graphs were higher than 0.998. As expected, too low of a concentration of ceric ammonium nitrate gave low sensitivity because of the slow reaction rate and the degradation of the complexed product. Ceric ammonium nitrate of 0.04 M was chosen as it provided high sensitivity and low background signal.

3.2.3. *Effect of mixing coil length*

Effect of length of mixing coil C1 was investigated over the range of 0–50 cm. It was found that sensitivity slightly decreased with the increase of mixing coil C1 length, due to the increased dispersion of the injected solution. However, with mixing coil length of 50 cm, a more reproducible peak profile was obtained than without mixing coil, so it was selected for further studies.

Effect of the length of mixing coils C2 and C3 was studied in range of 0–100 cm. The same length of coils C2 and C3 was considered in order to maintain the same pressure on both sides of the gas diffusion membrane, thus preventing deformation or breakage of the membrane. With increasing length of the coils, a slight increase in sensitivity was observed. A length of 75 cm each was chosen for coils C2 and C3 since having longer coils may cause breakage of the membrane. Coil C4 was incorporated in the system in order to increase pressure in the detection stream, preventing the evolution of bubbles in the line. According to the results, the length of this coil did not have affect on the sensitivity of the method. Coil C4 of 50 cm length was selected as it could maintain pressure in the line and provide reproducible peak profiles.

3.2.4. *Effect of sample volume*

Effect of sample volume was investigated by injecting a series of ethanol standard solutions in the concentration range of 0.0–10.0% (v/v), with different volumes of an injection loop. Sensitivity (slope of calibration graph) increased linearly with the injection volume and leveled off at about 150 μL volume. However, at higher volume a narrow linear range of calibration graphs were obtained. Thus sample volume of 100 μL, which gave a linear range up to 10% (v/v) ethanol was chosen.

3.2.5. *Reagent stability*

Stability of the reagent was examined by using the same reagent solution for construction of calibration graphs in the concentration range of 0.0–10.0% (v/v) ethanol at different times. Slopes of the calibration graphs of 0.078, 0.077, 0.075, 0.075, 0.075, 0.076 and 0.076 V/% v/v for the age of reagent of 0, 3, 6, 9, 12, 15 and 18 h, respectively were obtained, with $r^2 > 0.999$ for all the calibration graphs. This indicated that the reagent was stable within at least 18 h. Although the reagent might be stable up to 5 days based on observation of its color, it was daily prepared in all of our experiments.

3.3. *Optimization of gas diffusion system*

Five commercially available PTFE plumbing tapes with two different thicknesses, 0.75 (tape #1–#3) and 1.00 (tape #4–#5) mm, were tested to be employed as a gas diffusion membrane. Morphology of the membrane was examined by scanning electron microscope as illustrated in the SEM images of the membranes in Fig. 5. The thicker membrane has smaller pore size and the distribution of the pores on the membrane was more regular. Pore sizes of the membrane were in the range of about 0.3 × 1 to 5 × 25 μm. The ratio of area of pore per total area of the membrane was observed as follows: #4 Oishi < #5 Joint < #1 At Indy < #2 Protape < #3 Blue. As expected, PTFE tape of smaller thickness and larger pore size provided higher diffusion efficiency of ethanol, hence resulting in higher sensitivity as shown in Fig. 6. However, too thin and large pore membrane was easily broken, so the membrane #2 which provided medium sensitivity was selected.

Flow rates of donor and acceptor streams were identically maintained in order to avoid the building up of pressure difference between two sides of the membrane that may cause membrane deformation or breakage. Effect of flow rate in the range of 0.5–2.0 mL min^{-1} on sensitivity of the system with using various gas permeation membranes was investigated as shown in Fig. 6. As expected, an increase of flow rate resulted in a decrease in sensitivity because of the reduction in gas diffusion efficiency at high flow rate. As could be seen by the trend from the graph, one could select suitable flow rate to obtain sensitivity and sample throughput required for their application. Flow rate of 1.0 mL min^{-1} with membrane #2 was selected as it provided high sensitivity and adequate sample throughput for our application.

Geometry of gas diffusion unit also plays a role on diffusion efficiency [23]. Long, wide and shallow channel on the GDU provided high diffusion efficiency because it had high diffusion area per volume. However, too long of a channel caused high dispersion of the injected solution, thus reducing sensitivity and sample throughput. In this work, a gas diffusion unit 30.0 cm long, 1.5 mm wide and 0.75 mm deep was utilized and concurrent flow of the donor and acceptor streams was employed. Under the above selected conditions, the diffusion efficiency was found to be about 15%.

Temperature also affected the diffusion of ethanol through the membrane. According to the previous studies, high sensitivity was achieved with the increase of temperature [8,24–26]. However, using of relatively high temperature caused evolving of bubbles in the system and enough sensitivity was obtained with the operation at room temperature, so that the room temperature was recommended in literature [8]. According to our observation, with the experiment performed at room temperature of about 25 °C in an air conditioned room, the variation of less than 1% in concentration found for 5% (v/v) ethanol was observed. Slopes of the calibration graphs obtained during 18 h operation of the system were not significantly different as described in Section 3.2.5. A thermostat control unit might be needed if the system was operated at a place with high variation of temperature.

3.4. *Analytical characteristics*

Using the FI system as shown in Fig. 1 and a set of selected conditions: reagent solution consisting of 0.04 M ceric ammonium nitrate and 0.3 M nitric acid as an acceptor stream, water carrier as a donor stream, flow rate of each stream of 1.0 mL min^{-1}, sample volume of 100 μL, the length of coils C1, C2, C3 and C4 of 50, 75, 75 and 50 cm, respectively, and blue LED ($\lambda_{max} = 470$ nm) as a light source, analytical characteristics of the system were evaluated. A linear calibration graph ($y = 0.0873x - 0.0093$, $r^2 = 0.9993$) for the concentration range of 0.1–10.0% (v/v) ethanol was obtained as shown with the FIA peaks in Fig. 7. Detection limit calculated from three times standard deviation of blank/slope of the calibration graph [26] was 0.03% (v/v). Relative standard deviations for 11 replicate injections of 1.0, 5.0 and 10.0% (v/v) ethanol were 1.2, 0.5 and 0.3, respectively. Sample throughput of 20 h^{-1} was achieved and each injection consumed 3 mL each of reagent and carrier solutions. Sensitivity of the system using ceric ammonium nitrate as reagent was comparable to those using dichromate as reagent as summarized in Table 1. In addition, ceric ammonium nitrate is more environmentally friendly, since dichromate is a well known carcinogenic substance.

3.5. *Interference study*

It has been reported that the ceric ammonium nitrate reagent could react with other alcohols and α-hydroxy acids. However, by incorporating a gas diffusion unit (with hydrophobic membrane) to the FI system, the hydrophilic substances could be retained on the donor side of the membrane so they did not react with the reagent. Some substances which can affect the diffusion of ethanol through the membrane were also potential interferences. Interference was investigated by adding different concentrations of the potential interfering substances into 2% (v/v) ethanol standard solution. The solutions were injected into the FI systems with and without a gas diffusion unit. Table 2 shows the tolerance limit (maximum concentration of each substance that did not interfere or did not cause a change in peak height of 2%, v/v ethanol more than 5%) of some substances. It was found that methanol and propanol seriously interfere at low concentration (0.1%, v/v), either without or

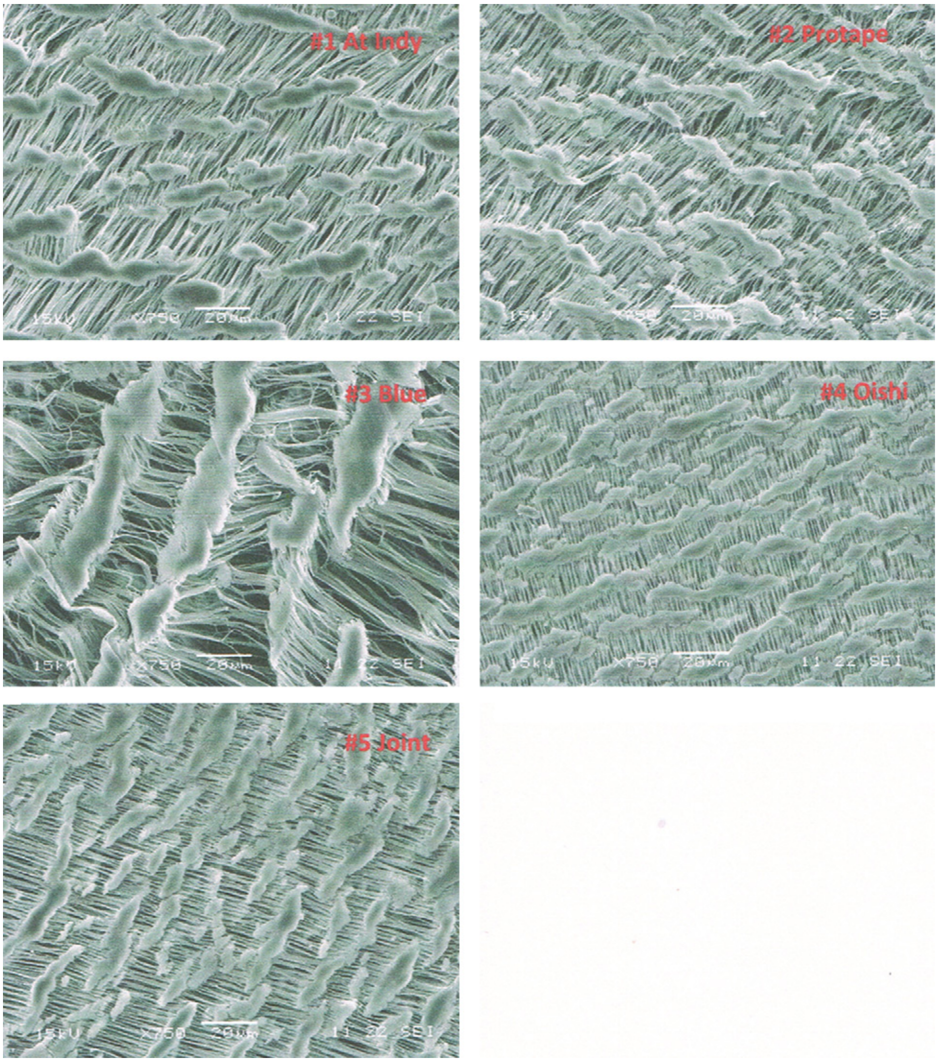

Fig. 5. SEM images of various PTFE membranes (plumbing tapes); (a) #1 At Indy, (b) #2 Protape, (c) #3 Blue, (d) #4 Oishi and (e) #5 Joint; Images were recorded by using scanning electron microscope (JSM-5910LV, JEOL, Japan), accelerating voltage: 15 kV, magnification: 750×, signal: secondary electron images.

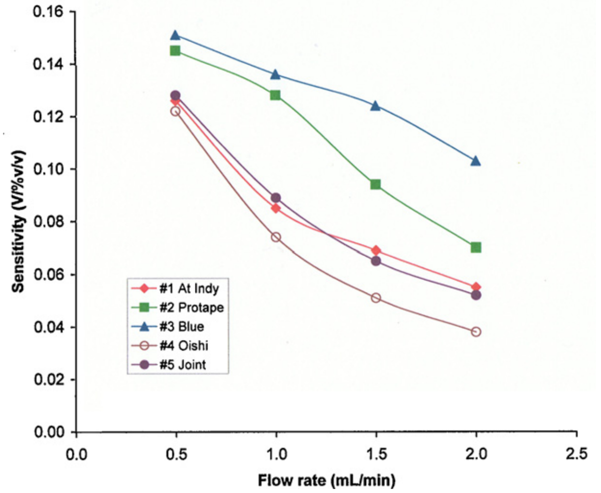

Fig. 6. Effect of flow rate on sensitivity of the method, using different membranes as described in Fig. 5. (Condition: 0.04 M CAN, 0.3 M HNO_3, sample volume 100 μL, length of coils, C1 50 cm, C2 75 cm, C3 75 cm, C4 50 cm.)

with the use of GDU. However, beverage samples contained very low concentration of these alcohols so they should not significantly affect the ethanol detection. Other long chain alcohols are not easily dissolved in water, so they should not interfere. Sugars showed negative interferences in both systems, except sucrose in the case

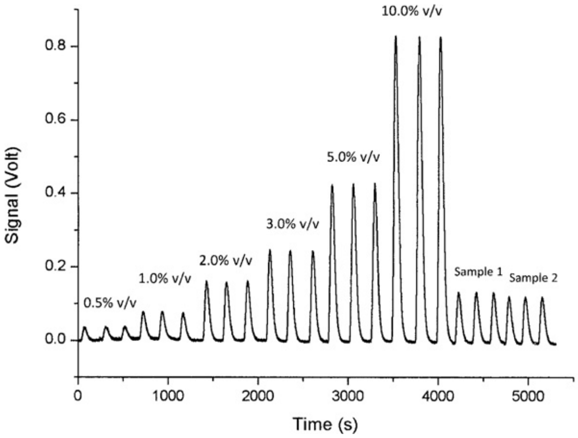

Fig. 7. FIAgram of ethanol standard solutions and some samples.

Table 1
Analytical characteristics of some spectrophotometric/colorimetric methods for the determination of ethanol.

System	Separation	Reagent	Detection wavelength (nm)	Sample	Linear range (%, v/v)	Detection limit (%, v/v)	Precision (%R.S.D.)	Sample throughput (h^{-1})	Ref.
FI	In-line pervaporation	16 g/L $K_2Cr_2O_7$ in 8 M H_2SO_4	600	Wines	1–20	0.5	3	6	[6]
FI	In-line pervaporation	17 g/L $K_2Cr_2O_7$ in 6 M H_2SO_4	600	Molasses fermentation	1–10	–	1.5	20	[7]
SI	None	0.2 M $K_2Cr_2O_7$ in 4 M H_2SO_4	600	Distilled liquors	Up to 6	0.09	<1	19	[9]
FI	In-line membraneless gas diffusion	0.2 M $K_2Cr_2O_7$ in 4 M H_2SO_4	590	Gasohol fuel	3–80	0.9	1–4.9	26	[10]
Microfluidic	None	0.15 M $K_2Cr_2O_7$ in 6 M H_2SO_4; fiber optic detection	600	Distilled spirits and wines	Up to 6	–	–	–	[11]
Batch microplate reader	Off-line solvent extraction	100 g/L $K_2Cr_2O_7$ in 5 M H_2SO_4; detection performed in 96 well plate)	595	Yeast culture broth	Up to 8	~0.1	–	192	[12]
FI	Permeation through concentric silicon tubular membrane	0.15 M $K_2Cr_2O_7$ in 6 M H_2SO_4	600	Distilled spirits and wines	1–20	0.5	3.7	20	[24]
FI	In-line gas diffusion	Reduction of excess $K_2Cr_2O_7$ (0.1 M) in 8 M H_2SO_4 with ferrous ion and detection of ferric ion formed by potentiometry	–	Distilled spirits, beers and wines	5–40	–	0.8	25	[25]
FI	On-line gas diffusion membrane probe	0.3 M $K_2Cr_2O_7$ in 4 M H_2SO_4	600	Distilled spirits and red wines	Up to 50	–	<2	30	[26]
FI	In-line gas diffusion	0.04 M ceric ammonium nitrate in 0.3 M HNO_3	Blue LED (~470 nm)	Distilled spirits, beers and wines	0.1–10	0.03	<1.3	20	This work

of FI system without GDU. The presence of sugar at high concentration may lead to interference due to change of refractive index in the case of the system without GDU or decreasing of diffusion efficiency in the FI system with GDU because of the increase of boiling point of the solution (colligative property). It was clearly observed that the GDU helped reduce interferences from tartaric acid, glycerol and tannic acid, which gave positive interference in the system without GDU at low concentration. The membrane helped prevent diffusion of ionic species and large organic molecules to the acceptor side. Some substances did not interfere even at the maximum concentrations tried, which were the concentrations higher than those expected to be found in real samples.

However, on the application of the developed method to real samples of beer and wine, it was found that there were some substances in sample which can adsorb on the membrane, prevent the transfer of ethanol through the membrane and hence cause reduction in sensitivity. Fortunately, this problem could be solved by extensive dilution of the samples.

3.6. *Application to real samples*

The proposed FI system was applied for determination of ethanol in some beverages purchased from a local supermarket. The samples were prepared as described in Section 2.5 before injecting the solutions into the FI system. The same solutions were also analyzed by gas chromatographic method [3] as described in Section 2.4 for comparison. Contents of ethanol obtained from both of the methods were in good correlation ($\text{EtOH}_{\text{FIA}} = 1.029\text{EtOH}_{\text{GC}} - 0.509$, $r^2 = 0.9971$) as summarized in Table 3. According to *t*-test at 95% confidence level [27], both the results are not significantly different ($t_{\text{calculated}} = 0.57$, $t_{\text{table}} = 2.07$).

Table 2
Tolerance limits of some substances.

Substance	Unit of concentration	Tolerance limit	
		FI with GDU	FI without GDU
Methanol	%v/v	0.1, +	0.1, +
Propanol	%v/v	0.1, +	0.1, +
Glucose	$mg\,L^{-1}$	1000, –	1000, –
Fructose	$mg\,L^{-1}$	1000, –	100, –
Sucrose	$mg\,L^{-1}$	1700, –	1700, +
Acetic acid	$mg\,L^{-1}$	500[a]	500[a]
Citric acid	$mg\,L^{-1}$	200, –	100, –
Tartaric acid	$mg\,L^{-1}$	100, –	50, +
Tannic acid	$mg\,L^{-1}$	1000[a]	50, +
Glycerol	$mg\,L^{-1}$	4000, –	5, +
Acetaldehyde	$mg\,L^{-1}$	400[a]	400[a]
Ethyl acetate	$mg\,L^{-1}$	1000[a]	1000[a]

+: positive interference, –: negative interference.
[a] Maximum concentration tested.

Table 3
Ethanol contents in some alcoholic beverages, determined by the proposed FIA and gas chromatographic methods, and labeled values.

Sample	Ethanol content (%v/v)			%different[c]
	Label	GC[a]	FIA[b]	
1. Blended spirit 1	35	34.3 ± 0.1	34.3 ± 0.6	0.0
2. Blended spirit 2	35	34.7 ± 0.1	33.4 + 0.3	−3.8
3. Blended spirit 3	40	40.1 ± 0.1	39.9 ± 0.3	−0.6
4. Scotch whisky 1	40	38.4 ± 0.1	39.8 ± 0.6	3.4
5. Scotch whisky 2	40	36.0 ± 0.1	36.6 ± 0.2	1.7
6. White spirit	35	30.8 ± 0.1	31.2 ± 0.4	1.3
7. Gin 1	40	38.5 ± 0.1	39.2 ± 0.2	1.8
8. Gin 2	40	37.2 ± 0.1	38.9 ± 0.2	4.4
9. Vodka	40	38.3 ± 0.1	40.1 ± 0.8	4.5
10. Rum	40	38.0 ± 0.1	38.7 ± 0.2	1.9
11. Red wine 1	13.0	13.0 ± 0.1	13.0 ± 0.1	0.0
12. Red wine 2	12.5	11.1 ± 0.1	10.9 ± 0.2	−1.8
13. Red wine 3	12.5	12.3 ± 0.1	11.8 ± 0.2	−4.1
14. White wine 1	11.0	10.9 ± 0.1	10.2 ± 0.2	−6.4
15. White wine	11.5	11.9 ± 0.1	10.3 ± 0.2	−13.4
16. White wine	13.0	12.7 ± 0.1	12.0 ± 0.1	−5.5
17. Lynchee wine	12.5	11.9 ± 0.1	13.5 ± 0.3	13.4
18. Beer 1	5.0	4.7 ± 0.1	4.8 ± 0.1	2.1
19. Beer 2	5.0	4.6 ± 0.1	4.5 ± 0.1	−2.2
20. Beer 3	5.0	5.0 ± 0.1	4.6 ± 0.1	−8.0
21. Beer 4	6.4	5.7 ± 0.1	5.3 ± 0.1	−7.0
22. Beer 5	5.4	5.1 ± 0.1	4.7 ± 0.1	−7.8
23. Black beer	5.6	4.9 ± 0.1	4.9 ± 0.1	0.0

[a] Result from duplicate injections.
[b] Result from triplicate injections.
[c] % different = 100 × (FIA − GC)/GC.

By spiking standard ethanol into the samples #1 to #10, percentage recoveries were obtained in the range of 98.2–102.7%. It was found that the matrices of beer and wine samples affect a lot on the transfer of ethanol through the membrane. Standard addition method was firstly tried, by spiking ethanol standard solution into sample of different folds dilution (2, 5 and 10 folds), to obtain added concentration of ethanol of 0.5, 1.0 and 1.5% (v/v). However, this method cannot provide accurate and precise results. Moreover, sensitivity of the method decreased dramatically after several samples were injected. Fortunately, this problem could be solved by injecting the diluted samples (20-fold dilution for beer and 40-fold dilution for wine) and employing a lower range calibration graph (0.1–0.5%, v/v ethanol). The proposed method provided advantages such as low chemical consumption and fast analysis time.

4. Conclusions

The FI method for determination of ethanol was developed based on the reaction between ethanol and ceric ion to form a reddish colored product which has a maximum absorption at 415 nm. The detection by using blue LED as a light source was proposed. The reagent had higher selectivity and negligible toxicity as compared to dichromate, which was commonly used as a reagent for determination of ethanol. By incorporating a gas diffusion unit into the FI system, most of the interferences could be eliminated. This reaction also provides good sensitivity. Despite using a simple colorimeter as a detector, a linear calibration graph in the range of 0.1–10.0% (v/v) ethanol, with detection limit of 0.03% (v/v) was obtained. Sensitivity of the method could be increased by employing lower carrier flow rate, which provided higher mass transfer efficiency. On the other hand, if high sensitivity is not a priority, then high flow rate could be used to increase sample throughput of the method. The proposed method was successfully applied for determination of ethanol in some alcoholic beverages. Further development of the system to be used for the monitoring of ethanol in a fermentation tank is being carried out in our laboratory.

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