Quantitative Determination of Ethanol in Local Thai Alcoholic Beverages by Raman Spectroscopy

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Abstract

This study focuses on various concentrations of ethanol content produced locally in Thailand. The alcoholic beverage with ethanol content of 35% and ethanol content of 40%, named sample A and sample B, were quantitatively analyzed using Raman spectroscopy. The characteristic vibration of CH- and OH band in the ethanol molecule (CH₃CH₂OH) were analyzed. To create the calibration curves (CH) and (OH), the band areas of stretching vibrations of the CH group within the wavenumber ranging from 2800–3000 cm⁻¹ and the OH group within the wavenumber ranging from 3100–3600 cm⁻¹ were plotted in comparison with the various ethanol concentrations in the ethanol-water binary standard solution (0–95%v/v with 5%v/v step). The calibration curve (CH) clearly demonstrated a linear relationship between the vibrational band area and the ethanol concentration with an R^2 value of 0.997. On the other hand, the calibration curve for OH exhibits poor linearity with a coefficient value ($R^2 = 0.947$). The calibration curve (CH) calculation yielded the exact acceptable values of ethanol concentration in samples A and B of about 33.88 ± 0.03 and 39.47 ± 0.02, respectively. The percentage difference between sample A and B (comparing with the Ebulliometer value) was about 2.69% and 4.22%, respectively. However, the calibration curve (OH) determined that the extreme percentage differences between samples A and B were 40.86% and 30.01%, respectively, due to strong vibration of H-bonding present in water which interrupts the OH stretching vibration in ethanol resulting in higher calculating error in the ethanol-based samples. Based on these findings, it can be stated that quantitative Raman measurement using a calibration curve (CH) performs well in estimating the ethanol content of locally produced alcoholic beverages in Thailand.

Keywords: Raman Spectroscopy, quantitative determination, ethanol concentration, calibration curve, vibrational mode

Article history: Received 06 October 2022, Revised 14 March 2023, Accepted 02 May 2023

1. Introduction

In everyday life, ethanol is utilized in products including drinks, solvents, protein hydration, and the self-assembly of biomolecular structures [1-4]. It is also often employed in biological, biochemical, and chemical processes in industrial settings. The precise concentration of ethanol in every solution must be considerably necessary. Because of the need to protect consumers from locally produced alcoholic beverages that are not qualified or contain excessive amounts of ethanol, quantitatively aqueous ethanol solution in distilled alcoholic drinks is very important. Additionally, the Thailand Excise Department stipulated tax regulations depending on the quantity or strength of alcohol. The quantity of ethanol in an ethanol-water binary solution should, therefore, be determined quickly, simply, and precisely. The electroanalytical method [5], gas chromatography (GC) [6], near infrared reflectance spectrometry (IR) [7], high performance liquid chromatography (HPLC) [8], amperometric biosensing [9], gas phase biosensing [10], and enzyme-base biosensing [11] are just a few of the methods used nowadays to measure and analyze the concentrations of ethanol. These methods are used the enzymatic, colorimetric, and titrimetric procedures. Traditional approaches, however, have severe drawbacks. For instance, it may be costly to do the analysis, difficult to prepare the samples, instrumental challenges, tough to remove the sample pretreatment, time-consuming process, and difficult to gather the results for in-situ detection. Faster, more affordable, and more accurate approaches have thus become crucial for development. Raman spectroscopy is prefer-

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able due to less time consumption and precise measurements for investigating such as metals [12], liquid solutions [13], polymers [14], and chemical plastics [15]. Due to various benefits of this technology in comparison with other techniques, Raman instrument is used to measure and analyze the ethanol concentrations. The methodology may circumvent the extremely flammable hydrogen gas that is often used for the frame in GC method by just correcting the back scattering of the photon from the sample to identify the characteristic of vibrational mode. Raman spectroscopy is not only the faster-corrected result but also the easier operation, nondestructive method and easyto-prepare samples for analysis. In other words, unlike other techniques such as GC, HPLC, and biosensing, Raman technique is less complicated. Furthermore, the technique uses light photon as a source to analyze the vibrations observed in the samples. Also, pretreatment is avoided for sample preparation. However, Raman spectroscopy lacks to measure the low concentration due to less sensitivity, but lower concentration samples can be analyzed using Surface Enhanced Raman Scattering (SERS) approach [16]. It is generally known that the inelastic energy of a photon measured by Raman spectroscopy matches the absorption energy of a vibrational molecular bond as observed in Infrared spectroscopy (IR). Not only Raman spectroscopy, but also Infrared reflectance spectroscopy (IR) can be used to determine the ethanol content. However, in the present work the researchers focus on Raman technique for measuring ethanol concentration. Raman spectroscopy has already been affirmed to be a tool for qualitative and quantitative analysis, and it allows structural fingerprinting with its narrow and highly determined band [17]. Identification of the inelastic light scattering from the optical phonon can be referred specifically to the characteristic of a molecules (bond strength and atomic mass) of each functional group which it can identify typically of substances. Generally, the acquisition of quantitative information can be gained by a Raman spectra line of the induced molecular vibration which is directly proportional to the amount of the molecule in samples [18]. It is widely known that the ethanol molecule (CH_3CH_2OH) consists of the ethyl (C_2H_5-) and the hydroxyl (-OH) group. Due to the specific characteristics of ethanol, much research has been conducted to determine the quantitative analysis of ethanol in ethanol-water binary solution using the CH or CC band as a standard curve, observing at 884 cm⁻¹ corresponding to stretching vibrations of C-C [18] and stretching vibrations of C-H ranging from 2800-3000 cm⁻¹ 19. However, OH vibrations show dominant feature if water molecules are present in the sample [20], which is observed in the range of $3100-3600 \text{ cm}^{-1}$ in a sample with OH vibrations.

The OH vibrations are observed in both the water and ethanol molecules exactly in the same region. The intensity and the shift observed in this region would bring significance to understanding their molecular changes in the sample. It is found that the intensity of the OH vibrational mode is observed to have various values when the concentration of ethanol changes from absolute ethanol to pure water. The present work provides novel analytical study the concentration of ethanol content in Thai alcoholic beverages observed from the vibrations of CH and OH modes using Raman instrumental technique. In this research, to establish a quantitative result using Raman technique, ethanol-water binary solutions were prepared for an ethanol standard solution. The stretching vibrations of the CH- and OH- bands were recorded, and the Raman integrated band area of the bonding groups (CH, OH) was analyzed in detail to generate a calibration curve, which was used to compute the quantitative concentration of ethanol in the samples. Finally, the linearity, accuracy, precision, and usability of the developed method in real samples (Thai alcoholic beverages) were examined and the results of this method were compared with that of result from the ebulliometer instrument.

2. Experiment

2.1 Examined samples

The locally available Thai alcoholic beverages (Red Bull Distillery (1988) Co., Ltd.) were used to determine the alcoholic concentration. Two samples of different alcoholic concentrations 35% (sample A) and 40% (Sample B) were used as topic of interest. Each sample was analyzed by placing 3 mL in quartz cuvette sealed with aluminum foil to avoid other noise during measurement. The ethanol concentration of the samples was examined with a procedure given in section 2.4 "Quantification of ethanol concentration using Raman spectroscopy".

2.2 Preparation ethanol-water binary solutions for an alcoholic standard solution

For the alcoholic standard solution, the twenty ethanol-water binary solution with different ethanol concentrations were prepared from the ethyl ethanol (95 %v/v AR1069-G2.5L; RCL Labscan Limited) and distilled water. The calculated amounts of this binary solutions were mixed into the volumetric flasks (10 ml). The ethanol concentration was expressed in term of the volume percentages, and it was varied from 0 %v/v (pure water) to 95 %v/v with 5 %v/v step. To avoid the possible error from vaporization of ethanol in the solution. Raman spectra was immediately collected after the solution was prepared. All solutions were prepared about 3 mL and taken in the quartz cuvette which were also stored in well-sealed vessels with the aluminum foil while measuring. Finally, both samples and standard solution were applied for Raman measurements in the same conditions.

2.3 Raman measurement

The Raman spectra in backscattering configuration were obtained using an Ar-laser as a source with a wavelength of 532 nm (power around 0.95 Watt). An interference edge filter was used to prevent the elastic scattering signal (Rayleigh scattering) and allowed the wavenumber (Raman shift) to approach down to 200 cm⁻¹. The scattering phenomenon was collected in a monochromator grating of 1200 g/mm, and the distance of focus was about 140 mm (Micro HR, HORIBA Scientific). Spectra registration was performed by a CCD camera from HORIBA INSTRU-MENTS INC., EDISON, NJ, USA Model: 2048x70-UVS. The spectral resolution of the Raman spectroscopy was 2 cm⁻¹. All solution temperatures during measurement were maintained at 25°C. The Raman spectra of each solution were obtained from 250 to 3800 cm⁻¹. The measurements were made with the same parameters at a 15-second acquisition time, 2 times accumulation, and 1-second RTD time. Finally, the Raman spectra line was subtracted with a baseline using a Polynomial function for all measurements.

2.4 Quantification of ethanol concentration using Raman spectroscopy

To determine the quantification of ethanol, the stretching vibration of the CH_2 - and CH_3 -band (CH group) and the stretching vibration of the OH band (OH group) were obtained in the range of 2800–3000 cm⁻¹ and 3100–3600 cm⁻¹, respectively. The band area of the CH group and OH group were conducted to be the calibration curves for CH and OH, respectively. Two calibration curves were produced by plotting the band area from the sum of (i) the CH group (obtained by equation 1 [18]) and (ii) the OH group (obtained by equation 2) versus the ethanol concentration. Measurement of each band area was performed directly using Igor Pro program (WaveMetrics, Inc., Lake Oswego, USA).

Area_{OH} = Intensity<sub>$$\nu$$
=3229 cm⁻¹</sub> + Intensity _{ν =3422 cm⁻¹}(2)

To determine the ethanol concentration of samples (local Thai alcoholic beverages), band area from CH group and OH group of the samples were substituted in the calibration equation of calibration curve for CH and OH, respectively and the concentration of ethanol were calculated. The ethanol concentration results obtained from the calculation were compared with the alcohol concentration displayed on the label of the sample and correlated between the results and the value obtained from the alcohol solution in the sample (on label) were analyzed by statistics.

2.5 Measure by ebulliometer instrument

The ebulliometer instrument were obtained for measuring the ethanol concentration (degree) of Thai alcoholic beverages samples. The distilled water was added into the ebulliometer boiler for calibrating the zero point of the boiling point at room temperature (25° C). Later 100 ml of the samples were used for ebulliometer estimation. Sample A (35%) and Sample B (40%) were added to the ebulliometer boiler and recorded at the lowest constant boiling point. The zero of the scale was adjusted to the boiling point of the water and the percentage of alcohol which was examined from the scale.

3. Results and discussions

3.1 Raman spectra of the samples and the ethanolwater standard solution

Figure 1a) and 1b) show the Raman spectra of 95% v/v ethanol, Thai alcoholic beverage samples and pure water ranging from 250 - 4000 cm⁻¹. The wavenumber position of these bands is estimated by fitting with Lorentzian equation and summarized in Table 1. The band positions (cm⁻¹) in each spectral line are found to be slightly shifted from their traditional values (blue shift or red shift). However, these positions of each band can be acceptable when compared with the reference values as shown in column 2 of Table 1. All wavenumbers of the bands performed the characteristic vibrational mode which corresponds to molecular formular of the ethanol (CH3CH2OH) as shown in column 3 of Table 1. Due to the transparency of the local Thai alcoholic beverage, the ethanol spectral line of the samples did not detect the fluorescence which is advantageous for undesirable spectra while correcting the vibrational band of ethanol. All ethanol band spectra have been obviously observed three strong bands of stretching vibrations of CH groups around 2883cm⁻¹, 2931cm⁻¹, and 2977cm⁻¹ which are assigned to the stretching symmetric vibrations of CH2, the stretching symmetric vibrations of CH3, and the stretching asymmetric vibrations of CH3, respectively. Furthermore, the researchers observe two strong overlapping bands of stretching vibrations of hydroxyl (OH) group from alcoholic solution and (OH) group from water molecule in the region of $3100 - 3600 \text{ cm}^{-1}$. The wide range is observed around 3422 cm⁻¹ and distinctly small shoulder around 3229 cm⁻¹. Due to dominating band of ethanol spectra, the ethyl (CH) and



Figure 1: Raman spectra of ethanol-water binary solutions, a) 95% v/v ethanol and b) Various Samples and pure water.

Table 1. The characteristic ethanol band of samples and ethanol standard in the wavenumber range $250-3,800 \text{ cm}^{-1}$ which correspond to the mode of vibration.

Raman shift (cm ⁻¹)	Reference (cm ⁻¹)	Vibrational mode		
444-445	440^{a}	Bending vibrations of C-C-O		
888-890	$884^b, 886^a, 888^c$	Stretching vibrations of C-C		
1054-1057	1053 ^b , 1054c, 1056 ^a	Stretching vibrations of C-O		
1096-1098	$1095^b, 1100^a, 1104^c$	Skeleton stretching vibrations of CH ₃		
1281-1283	$1279^b, 1280^a, 1287^c$	Deformation vibrations of CH ₂		
1457-1458	1455 ^b , 1456 ^a , 1462 ^c	Bending vibrations of CH ₃		
1630-1632	1630^{a}	Bending vibrations of water		
2726-2730	2730^{a}	Combinational frequencies		
2880-2883	$2880^b, 2884^a, 2887^c$	Stretching symmetric vibrations of CH ₂		
2928-2931	2929 ^b , 2932 ^a , 2934 ^c	Stretching symmetric vibrations of CH ₃		
2974-2977	2974 ^b , 2975 ^c , 2977 ^a	Stretching asymmetric vibrations of CH ₃		
3200-3500	3286–3615 ^b , 3330-3400 ^a	Stretching vibrations of OH group		
	3200-3420 ^d			
^a Reference [7]				
^b Reference [20]				

^cReference [4]

^dReference [21]



Figure 2: Raman spectra of ethanol-water binary solutions with various ethanol concentration (0 - 50 % v/v) with in region stretching vibration of CH and OH bands (2600-3800 cm⁻¹)



Figure 3: Fitting Raman spectra peaks of 50 %v/v ethanol-water solution in range $2600 - 3800 \text{ cm}^{-1}$ including with fitting information and residual peak fit located at the bottom and top of the graph, respectively.

the hydroxyl (OH) stretching vibrations bonding were considered as an advantageous band which gave us an opportunity to use these band as the calibration curves for quantification measurement of ethanol concentration in alcoholic solution.

3.2 Calibration curves by using the band of stretching vibrational of CH and OH groups in ethanolwater solution.

The Raman spectra of the ethanal-water standard solution whose ethanol concentration varied from 0-95 %v/v with 5 %v/v step were corrected. However, in this presentation, Figure 2 demonstrates the stretching vibration of CH and the OH band of ethanal-water standard solution with various ethanol concentration (0 - 50 % v/v, with 5 % v/v steps) in wavenumber ranging from 2600-3800 cm⁻¹. Despite changing the amount of ethanol concentration, the wavenumber position of the stretching vibration of CH and OH band remained practically unchanged. In contrast with the band position, the band intensity tended to vary related to the changing of ethanol concentration. The band intensity of the stretching vibration of CH groups was observed significantly rising with increasing of ethanol concentrations in contrast with the band intensity of the stretching vibration of OH groups which were also found significantly declining with increase in ethanol concentrations. These results powerfully confirmed that the linear relation between the band area of the stretching vibration of CH and OH groups with the ethanol concentration can be performed to produce the calibration curve as a standard for identifying the ethanol concentration in local Thai alcoholic beverages.

Figure 3 exhibits the fitting obtained from graph to calculate the integrated band area of the stretching vibrations of CH and OH groups from the Raman spectra for 50 %v/v ethanol standard solution in the range of $2600 - 3800 \text{ cm}^{-1}$. The fitting information shows the residual peak fit located at the bottom and top of the graph, respectively. The corrected Raman data and the fitting line are represented by the blue circle and red line, respectively. The Raman data was used to fit for multiple peaks using the Lorentzian equation. The fitting line covers overall the band of the stretching vibration of CH and OH groups (including the band of combinational frequencies at 2726 cm^{-1}). The data obtained from the results can be examined from the fitting information located at the bottom of the graph. The band positions (CH and OH group) obtained from the graph have already been reported in the previous section (Table 1) and the band area of the stretching vibration of CH and OH groups were corrected for producing the calibration curve. In the graph (Figure 3, 50 %v/v ethanol) the integrated band area of the stretching vibration of CH groups were obtained and show 274,090, 365,320, and 133,064 from the stretching symmetric vibrations of CH2, stretching

symmetric vibrations of CH3, and stretching asymmetric vibrations of CH3, respectively. Additionally, the integrated band area of the stretching vibration of OH groups were obtained which shows 278,380, and 773,130 from stretching vibrations of OH groups. As well as the other spectral line of each ethanol concentrations, the integrated band areas of CH and OH groups were added as shown in Equation 1 and Equation 2 for calculating the calibration curve for CH and OH group, respectively.

The calibration curve of ethanol was plotted with the integrated band area of CH and OH groups (0 - 95 %v/v with 5 %v/v step) versus the changing concentration of ethanol in ethanol-water based standard solution as shown in Figure 4 and 5, respectively. A linear response between integrated band areas of CH and OH groups and ethanol concentrations were observed. The results show that the relation between the ethanol concentration and the integrated band areas of CH and OH group are directly proportion and inversely proportional to each other as observed in Fig (4-5). Furthermore, the linear relationship between ethanol concentration and the integrated band area of CH group was obtained with high R2 value of 0.997. This result shows that the ethanol content in the alcoholic beverages samples could be determined with high accuracy by using the calibration curve (CH). However, the low linearity with coefficient value (R2 = 0.947) is found in calibration curve (OH). The linearity curve of ethanol concentrations was defined by following equation in which y was given the integrated band areas of CH and OH groups and x was the ethanol concentration, expressed in y=-180,350+19,175x and y=2,602,800 -24535x, respectively.

3.3 Ethanol content in local Thai alcoholic beverages

Table 2 summarizes the experimental concentration comparing with value from ebulliometer and the percentage difference (calculated and value from the ebulliometer) obtained from ethanol content in the local Thai alcoholic beverages. The concentrations were calculated in both calibration curve (CH) and calibration curve (OH). The reliability of the measurement was observed in terms of standard deviation of the mean (α). The results show that the ethanol concentration in Sample A and B calculated from the calibration curve (CH) were 33.88 ± 0.03 and 39.47 ± 0.02 , respectively. These values are acceptable precision which can be confirmed by comparing the experimental concentration with the ebulliometer value. The results of the technique show that Sample A (displayed in the label 35%) and Sample B (displayed in the label 40%) were $34.82 \pm 0.02\%$ and $41.21 \pm 0.01\%$, respectively. The percentage difference obtained from the experimental concentration using CH calibration curve and concentration from ebulliometer of Sample A and Sample B were found to be 2.69% and 4.22%,



Figure 4: Calibration curve by using vibrational CH band of ethanol



Figure 5: Calibration curve by using vibrational OH band of ethanol

Table 2. Data	for Ethanol	Content of Loc	al Alcoholic Beverages

Local	% Alcohol (ethanol)				Difference (%) ^d	
alcoholic	Calibration curve (CH)		Calibration curve (OH)		Calibration	Calibration
beverages	Exptla $\pm \alpha^c$	Ebulliometer $\pm a^c$	Exptlb $\pm \alpha^c$	Ebulliometer $\pm \alpha^c$	Curve (CH)	Curve (OH)
Sample A	33.88 ± 0.03	34.82±0.02	49.05±0.09	34.82 ± 0.02	2.69	40.86
Sample B	39.47 ± 0.02	41.21±0.01	53.58 ± 0.03	41.21±0.01	4.22	30.01

 a Experimental calculation of ethanol concentration in alcoholic beverages samples with using the band area of CH group as a calibration curve

^bExperimental calculation of ethanol concentration in alcoholic beverages samples with using band area of OH group as a calibration curve

 $^{c}\alpha$ is a standard deviation of the mean.

^dCalculated as "Experimental - Ebulliometer" /Ebulliometer "×100", ignoring the sign

respectively. However, the unacceptable measurement of ethanol concentrations in the samples which were calculated by using calibration curve (OH) were observed. The extreme percentage differences between the experimental concentration and expected concentration of Sample A and Sample B were found to be 40.86%, 30.01, respectively. To use the stretching vibrations of OH band as a calibration curve could lead to inaccuracy in its results while analyzing because the interruption of the strongly vibrational H-bond of OH group network with the water molecules were performed [7]. However, the CH stretching band, which is the specific identity of ethyl molecules, exhibits high performance of the vibrational band contributing to the ethanol. Thereby the results, using the calibration curve (CH), present a high performance to developed quantitative Raman method for calculating the ethanol concentration in the samples. To our understanding, the calibration curve analysis of CH showed superior performance to analyze the ethanol content in various samples when compared with calibration curve OH analysis.

4. Conclusion

In this study, Raman spectroscopy was used as a tool to identify and determine the quantitative ethanol concentration in local Thai alcoholic beverages. The Raman spectra showed wavenumber ranging from 2600-3800 cm-1 for sample A (35 %v/v) and sample B (40 %v/v) corresponding to vibrational mode of CH and OH in the ethanol (CH3CH2OH) molecule. The baseline correction of ethanol spectra of various ethanol concentrations (0 - 95 %v/v) was used as standard. The integrated band area of stretching vibration of CH group with wavenumber ranging from 2800-3000 cm-1 and the integrated band area of stretching vibration of OH group with wavenumber ranging from 3100-3600 cm-1 were plotted and compared with the ethanol concentration to produce the calibration curve for CH and OH, respectively. The linear response between the integrated band area of CH and the ethanol concentration with a higher R2 value of 0.997 was observed. However, the low linearity with coefficient value (R2 =0.947) was found in calibration curve for OH. The ethanol concentration of Sample A and Sample B calculating from the calibration curve (CH) were found to be 33.88 ± 0.03 and 39.47 ± 0.02 . The percentage differences observed in its value were compared with the Ebulliometer and found to be 2.69% and 4.22%, respectively. The large percentage differences were observed for calibration curve (OH) for Sample A and Sample B and were found to be 40.86%, 30.01, respectively. From the error analysis, the value can be explained due to the strong vibrations of Hbonding available in water molecule which will interrupt the OH stretching vibrations of ethanol molecule. These results can be concluded that quantitative Raman measurement has successfully investigated and showed that using calibration curve for CH exhibits high performance to determine the ethanol concentration in local Thai alcoholic beverages.

Acknowledgment

This study was financially supported by the Research and Development Institute of Kamphaeng Phet Rajabhat University.

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