

# Self-colorimetric determination of bio-ethanol using permanganate in fermentation samples

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## Research Article

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# Abstract

Without the aid of chromatographic techniques, quantification of bio-ethanol in fermentation-broth distillate becomes inconvenient. Potassium permanganate is preferable over potassium dichromate because of the latter well-known toxic properties, it is common used in ethyl alcohol determination either by visible determination of Cr(III) green optical density, a consumed Cr(VI) determination in strong acid medium by measuring band absorbance decrease at 267 nm or the unreacted Cr(VI) determination iodometrically after alcohol oxidation. Nevertheless, these titre methods arise difficulties experience analysts from multiple solutions preparation, standardization that should be carried out every day and to successful end point detection in the presence of Cr(III) green color which leads to a significant ethanol quantification error. Noteworthy permanganate-iodometry drawbacks as same as titre dichromate difficult practical procedures and multiple reagents employed.

In this laboratory a self colorimetric method was developed in neutral medium as alcohol-specific oxidizing agent precludes both of its undesirable high oxidizing properties and difficult titrimetric methodologies for bio-ethanol quantification.

It is based on unreacted permanganate optical density difference between a non ethanol-containing sample as a blank and ethanol-containing sample is directly proportional to the consumed permanganate amount in ethanol red-ox reaction and consequently directly proportional to ethanol content. This optical density difference versus ethanol concentration 1–6% v/v obeys Beer-Lambert law provides limit of detection, limit of quantification and correlation coefficient equal 0.17%, 0.56% and 0.999 respectively.

# Introduction

Regardless the chromatographic and enzymatic techniques for ethanol determination that may not be available in many of chemical laboratories, ethyl alcohol determination by titre acidic dichromate is everywhere in literature and applicable in most of winery and biofuel laboratories, noteworthy it comes back since the earlier pioneer work of Widmark [1] and Nicloux [2, 3], iodometry enables to determine the unreacted dichromate amount by titrating the amount of iodine liberated upon the addition of potassium iodide to the reaction mixture against a standard solution of sodium thiosulfate. Despite of the best repeatability and accuracy of this titre method but it experiences a lot of difficulties arise in end point color change successful detection especially in presence of the produced green color of Cr(III) repeatedly noticed especially with who not well-experienced in the art and this observation agreed to literature [4]. On the other hand, the bad repeatability of the direct spectrophotometric measurement of the resulting Cr(III) green optical density is not be recommended [5, 6] or by determining the consumed Cr(VI) by directly measuring the absorbance decrease of a band assigned at 267 nm [7].

Furthermore, the extensive study of Theodore and Rosalind using alkaline permanganate in ethanol determination after its oxidation into oxalic acid [8] as well as Evans and Day work regarding oxidation of ethyl alcohol by means of potassium permanganate reported the oxidized product in neutral medium is

acetic acid as an only product [9] inspired us in this laboratory to utilize neutral aqueous permanganate as a specific-alcohol oxidizing agent in ethyl alcohol determination spectrophotometrically precludes its undesirable high oxidation power and eliminates the difficulty involved in titre methods especially at high ethanol levels as closer as fermentation broth-ethanol [10, 11] content and to avoid dichromate well known high toxicity.

The present work depends on the oxidation of ethyl alcohol into acetic acid and reduction of permanganate (VII) into brown precipitate Mn(IV), the principle is based on the purple unreacted Mn(VII) optical density difference between a non ethanol-containing sample as a blank and ethanol-containing sample is directly proportional to the amount of consumed permanganate in redox reaction and consequently directly proportional to the amount of ethanol present according to the following equations:

$$C_B - C_S \propto C_{\text{Ethanol}}$$

Where  $C_B$  is permanganate concentration in the blank and  $C_S$  is the permanganate concentration after redox reaction and  $C_{\text{Ethanol}}$  is the ethanol concentration, from Beer-Lambert's law and at specific dilution.

$$\epsilon b A_B - \epsilon b A_S \propto C_{\text{Ethanol}}$$

Where  $A_B$  is absorbance assigned for non ethanol-containing sample and  $A_S$  assigned for absorbance of ethanol-containing sample after specific dilution,  $\epsilon$  is the permanganate relative absorbitivity and it is the same for both of blank and ethanol-containing sample and  $b$  is light path length, so

$$\epsilon b (A_B - A_S) \propto C_{\text{Ethanol}}$$

$\epsilon b$  is a constant value so absorbance difference between blank sample and ethanol-containing sample,  $\Delta A$ , and ethanol concentration plot, figure (1), gives a linear relationship obeys Beer-Lambert's law.

## Results And Discussion

According to this assay, the ideal dilution giving the highest absorbance difference  $\Delta A$  between the blank and within ethanol concentrations interval, in addition to, gives blank sample absorbance closer to 2.5 before heating step and absorbance value closer to 2.1 after heating step due to water-permanganate oxidation was 0.375 ml (375  $\mu$ l):100 ml and the identified optimized reaction environment was incubation in water bath at 60°C for 30 min, it should be mentioned that no significant increase in absorbance difference between the blank and ethanol containing sample after this mentioned incubation time.

The equation describes oxidation reaction of ethanol in aqueous neutral permanganate is shown below.



The color decrease percentage absolute equation for ethanol determination as shown in the method details section derived by plotting both terms of absorbance difference and ethanol concentration.

Warming and adjusting the spectrophotometer at 525 nm then zeroing using distilled water.

A 5 ml of permanganate solution is mixed well with 1 ml of distilled water as a blank sample as well as six test tubes contain 5 ml of the same permanganate solution and 1 ml of ethanol concentration ranging 1–6% v/v (for ethanol dilutions, ethanol HPLC grade > 99.8% was considered as 100%) and incubate them in water bath altogether at 60°C for 30 min then cooling and centrifuged to down Mn(IV) precipitate or filtering.

A 0.375 ml (375 µl) of the clear filtrate is diluted to 100 ml for both of the blank and the other tubes set and measure the purple optical density at 525 nm.

Afterthen, calculating the difference in optical density between the blank absorbance and the other set absorbances followed by plotting ethanol concentrations on horizontal axis versus their absorbance difference on vertical axis.

Table (1) and figure (1) summarize the obtained data and the collected Beer-Lamberts equation.

Reference	Ethanol concentration v/v	Mixing, heating at at 60°C for 30 min, cooling and centrifuge or filtering to down Mn(IV) precipitate.	Absorbance 375µl:100 ml		Absorbance difference (A <sub>blank</sub> -A <sub>sample</sub> )	
			A	Ref.	ΔA	Ref.
Blank	0%	1 ml H <sub>2</sub> O + 5 ml of 5% permanganate	2.103	A <sub>B</sub>	-	-
S 1	1%	1 ml of 1% Alcohol + 5 ml of 5% permanganate	1.812	A <sub>S1</sub>	0.291	A <sub>B</sub> -A <sub>S1</sub>
S 2	2%	1 ml of 2% Alcohol + 5 ml of 5% permanganate	1.502	A <sub>S2</sub>	0.601	A <sub>B</sub> -A <sub>S2</sub>
S 3	3%	1 ml of 3% Alcohol + 5 ml of 5% permanganate	1.211	A <sub>S3</sub>	0.892	A <sub>B</sub> -A <sub>S3</sub>
S 4	4%	1 ml of 4% Alcohol + 5 ml of 5% permanganate	0.934	A <sub>S4</sub>	1.169	A <sub>B</sub> -A <sub>S4</sub>
S 5	5%	1 ml of 5% Alcohol + 5 ml of 5% permanganate	0.631	A <sub>S5</sub>	1.472	A <sub>B</sub> -A <sub>S5</sub>
S 6	6%	1 ml of 6% Alcohol + 5 ml of 5% permanganate	0.363	A <sub>S6</sub>	1.797	A <sub>B</sub> -A <sub>S6</sub>
Table (1)						

The equation shown in figure (1) represents Beer-Lambert's law of absorbance difference and ethanol concentration plot.

A comparison of this proposed neutral permanganate method and the reported titrimetric alkaline permanganate method is shown as below in table (2).

Parameter	Neutral permanganate oxidation followed by photometry. (Proposed study)	Alkaline permanganate oxidation followed by iodometry. (Theodore and Rosalind 1936)
Specificity	Alcohol-specific oxidation	Non alcohol-specific oxidation
Reagents used	-Potassium permanganate	-Potassium permanganate -Sodium thiosulfate -Sulfuric acid -Sodium hydroxide -Potassium iodide -Starch -Sodium tungstate and mercuric sulfate (in case of protein and volatile substances rich samples)
Assay procedures testing standard ethanol sample 5% v/v.	5 ml of permanganate solution plus 1 ml of sample besides blank then heating at 60°C for 30 min and calculating $\Delta A$ followed by ethanol concentration equation from method details section.	(1 ml of 5% ethanol diluted to 25 ml) is mixed with 25 ml of 1 N permanganate and 10 ml of 5 N sodium hydroxide then heating for 20 min in boiling water then cooling and neutralizing with 10 N sulfuric and titration with 1 N sodium thiosulfate using starch indicator.
Technique used	Photometrical determination of absorbance difference between a blank and a sample.	Iodometrical determination of unreacted permanganate.
Correlation coefficient	0.999	Not applicable
Intercept	-0.005	Not applicable
Limit of detection	0.17%	Not applicable

Table (2), data from absorbance difference  $\Delta A$  and ethanol concentration plot

$M$  is the mean value calculated by the average of five runs

$SD$  is the standard deviation of five runs

$^1$  is calculated by (standard deviation/mean value) 100

$^2$  is calculated by mean value/true value

Parameter	Neutral permanganate oxidation followed by photometry. (Proposed study)	Alkaline permanganate oxidation followed by iodometry. (Theodore and Rosalind 1936)
Limit of quantification	0.56%	Not applicable
working range	1–6%	Not applicable
*Standard 5% v/v ethanol.	$M_{4.88} \pm SD_{0.091}$ v/v	$M_{3.91} \pm SD_{0.012}$ wt/v
Precision,, RSD%	<sup>1</sup> 1.86%	<sup>1</sup> 0.306%
Accuracy	<sup>2</sup> 97.6%	<sup>2</sup> 100.2%
Table (2), data from absorbance difference $\Delta A$ and ethanol concentration plot		
$M$ is the mean value calculated by the average of five runs		
$SD$ is the standard deviation of five runs		
<sup>1</sup> is calculated by (standard deviation/mean value) 100		
<sup>2</sup> is calculated by mean value/true value		

\* is chosen to predict high acceptable confidence level so far than limit of quantification

The limit of detection, LoD, is calculated using the following equation

$$\text{LoD} = 3 \text{ SD/slope of the linear equation}$$

Where SD is the standard deviation of the lowest analyte concentration that can be detected in comparison to the blank, and the slope value of the linear relationship equals 0.297

The Limit of quantification, LoQ, is calculated using the following equation

$$\text{LoQ} = 10 \text{ SD/slope of the linear equation.}$$

## Experimental Design

### Materials and Methods:

Original method details, Titrimetry, Theodore and Rosalind 1936.

## Alkaline permanganate oxidation followed by Iodometry

### Reagents and Equipments:

1-Potassium permanganate 3.3%

2-Sulfuric acid 10 N

3-Potassium iodide

4-Starch solution

5-Sodium thiosulfate

6-Sodium hydroxide 5 N

7-Water bath

8- Sodium tungstate and mercuric sulfate are added to ethanol-containing sample prior to distillation to prevent foams caused by presence of proteins and to remove volatile substances may be present and oxidizable other than ethanol under alkaline permanganate.

### Procedures:

1-A 25 ml of potassium permanganate solution (diluted if needed according to the expected ethanol percent under determination) and 10 ml of 5 N sodium hydroxide are added to both of 25 ml of non ethanol-containing sample as a blank experiment and 25 ml of ethanol-containing sample with mixing all the contents very well then placed in boiling water bath in an attached cooling closed condenser for 20 min

2-The solutions are cooled then neutralized by adding 10 ml of 10 N sulfuric acid followed by addition of 10 gm potassium iodide.

3-The iodine liberated is titrated against standard sodium thiosulfate as exactly the same strength as the permanganate solution.

### Calculation of ethanol content:

$[(\text{volume of sodium thiosulfate for the blank}) - (\text{volume of thiosulfate for the sample})] = \text{volume of thiosulfate equivalent to the permanganate used in the oxidation.}$

1 ml of 1 N of consumed permanganate is equivalent to 4.275 mg alcohol, (1 N permanganate, 3.3 gm permanganate in 100 ml H<sub>2</sub>O digested in hot water for 3-5 h).

### Proposed method details, the present study, spectrophotometry.

Neutral permanganate oxidation followed by photometry

## Reagents and Equipments

1-Potassium permanganate, analytical grade

2-Water bath

4-Spectrophotometer

## Procedures:

1-Weight accurately 5 gm potassium permanganate and dissolve in 100 ml distilled water stirring on cold using magnetic stirrer for 0.5 h till homogeneity.

2-Mix well 5 ml of permanganate solution to both of 1 ml distilled water as a blank and 1 ml of ethanol solution (maximum ethanol concentration 6% v/v) and incubate them in water bath at 60 °C for 30 min

3-Centerifuge or filter ethanol-containing sample using glass wool and dilute 0.375 ml from the clear filtrate (375 µl) to 100 ml distilled water for both of blank and ethanol sample and measure both absorbances at 525 nm after zeroing using distilled water and calculate absorbance difference  $\Delta A$  and color decrease percentage as follows.

$\Delta A = A_B - A_S$  where  $A_B$  absorbance assigned to Blank and  $A_S$  absorbance assigned to ethanol-containing sample after the previous mentioned 375µl:100ml dilution.

**Color decrease percentage (CDP) =  $(\Delta A/A_B)100$**

4-Plot absorbance difference and/or color decrease percentage versus ethanol concentrations 1-6% v/v.

## Conclusion

A facile colorimetric method for ethanol quantification 1–6% v/v has been outlined with acceptable accuracy and precision. Fermentation broth-distillate ethanol content is oxidized by alcohol-specific aqueous neutral permanganate into acetic acid by heating in water bath at 60°C for 30 min and the optical density decrease percent between a non ethanol-containing sample and ethanol-containing sample was used to determine ethyl alcohol content conveniently.

## Declarations

### Competing interests:

The author declares no competing interests.

## References

1. -Eric M. P. Widmark. *Biochem. Z.*, 131, 471,1922.
2. -M. Nicloux. *Bull. Soc. Chim. biol.* 13, 857, 1931.
3. -M. Nicloux, E. Le Preton, A. Dontcheff. *Bull. Soc. Chim. biol*, 16,1314,1934
4. -E. A. Crowell, C. S. Ough. A modified procedure for alcohol determination by dichromate oxidation. *Am. J. Enol. Vitic.*, 30, 61–63, 1979.
5. -M. B. Williams, H. D. Reese. Colorimetric determination of ethyl alcohol. *Anal. Chem.* 22(12), 1556–1561, 1950
6. -Pilone GJ. Determination of ethanol in wine by titrimetric and spectrophotometric dichromate methods: colaborative study. *J. Assoc. Off. Anal. Chem.*, 68(2), 188–190, 1985
7. -Andrea D. Magri, A. L. Magri, Fabrizio Balestrieri, Amalia Sacchini, D. Marini. Spectrophotometric micro-method for the determination of ethanol in commercial beverages. *Fresenius Journal of Analytical Chemistry.* 985–988, 1997
8. -Theodore E. Friedemann, Rosalind Klaas. The determination of ethyl alcohol. *J. Biol. Chem.*, 115, 47–61, 1936.
9. - W. L. Evans, J. E. Day. The oxidation of ethyl alcohol by means of potassium permanganate. *J. Am. Chem. Soc.*, 41,1267, 1919.
10. - Abdel-Naser Zohri, Mohamed Abdelwahab, Maysa Ali, Sara Ibrahim, Mohamed Abdelazim. In-situ sodium hydroxide-sodium bisulfate sugarcane bagasse pretreatment for biofuel production. *Egypt Patent Office.* 2017, 1349
11. - Abdel-Naser Zohri, Mohamed Abdelwahab, Maysa Ali, Sara Ibrahim, Mohamed Abdelazim. Data on morphological features change of pre-hydrolysis treatment sugarcane bagasse using in-situ sodium-hydroxide sodium bisulfate method. *MethodsX*, 2019, 24, 103971

## Figures

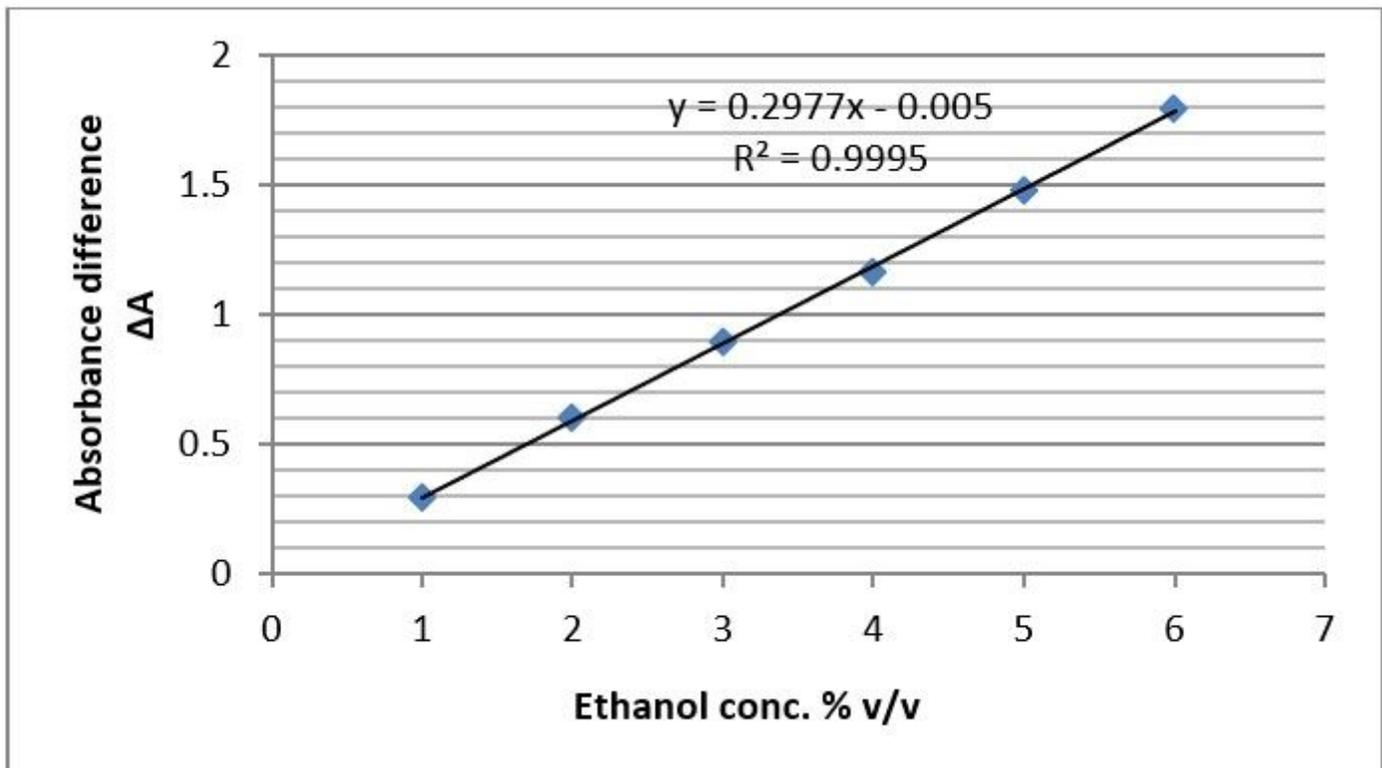


Figure 1

ΔA and ethanol concentration plot.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [graphicethanolpage0012.38.33AM.jpeg](#)