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A green chemical analysis of ethanol using a smart phone

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ABSTRACT

This research presents a novel method for measuring ethanol concentrations using a smartphone. The method involves an oxidation reaction with potassium dichromate and concentrated sulfuric acid, resulting in a green-blue color formation. The color intensity, corresponding to ethanol concentrations ranging from 0 to 100%, was captured using a smartphone camera within a specialized photography box. The images were then analyzed using a specific application, converting the color signal into an absorbance value. The calibration curve demonstrated excellent linearity in the range of 0-0.55 v/v % and its detection limit is 0.01 v/v%, with a correlation coefficient exceeding 0.995. The method was successfully applied to measure ethanol in real samples, including ordinary rose water and a bitter wheat drink.

- The method is inexpensive.
- The method is rapid.
- The method is green.

Subject area:	Environmental Science
More specific subject area:	*Analysis*
Name of your method:	Green analysis
Name and reference of original method:	*EPA*
Resource availability:	*No*

Method details

Ethanol or ethyl alcohol or white alcohol or medical alcohol is prescribed as an antidote to reduce the effects of wood alcohol or methanol poisoning, it is used in 70% purity for disinfection, it is added to medicines to be absorbed faster and in some serums. In fruits, fruit sugars are produced by bacterial fermentation. Ethanol is quickly absorbed through the digestive mucosa, the toxic dose of 96% alcohol is one milliliter per kilogram, and if five milliliters per kilogram is consumed, it causes death. The toxic concentration of ethanol in blood plasma is 1 to 2 mg/ml (1000 to 2000 ppm) [1]. The liver is responsible for the metabolism of ethanol, with 90% of the metabolized substance being expelled via respiration and the remaining 10% being eliminated through urinary excretion. If consumed on an empty stomach, it causes nausea and vomiting, and in addition to abdominal pain, it may cause bleeding from the stomach lining. The amount of alcohol emitted from exhaled air is diagnostic. In addition, alcohol testing on blood and breath samples is also requested. Ethyl alcohol is converted into acetaldehyde in the cytosol of liver cells or digestive system cells by the enzyme

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Table 1
Relationship between wavelengths and color of solution.

Wavelength (nm) of absorption	Color of solution	Complementary color
400-435	Green-Yellow	Purple
435-480	Yellow	Blue
480-490	Orange	Blue-Green
490-500	Red	bluish green
500-560	Dark-red	Green
560-580	Purple	Green-Yellow
580-595	Blue	Yellow
595-610	Blue-Green	Orange
610-680	Green-Blue	Red
680-700	Green	Dark Red

alcohol dehydrogenase (ADH Class I). In the microsomal pathway, the cytochrome CYP2E1 enzyme present in the endoplasmic reticulum of liver cells converts ethanol first into acetaldehyde and then into acetate, and during this conversion, oxygen free radicals are produced, which are responsible for the toxicity caused by alcohol. Continuous use of alcohol leads to increased regulation of the CYP2E1 enzyme, which increases tolerance to alcohol. According to the World Health Organization, alcohol consumption is the cause of more than 60 types of diseases and tissue damage and leads to 2.5 million deaths annually. Several official and common methods of measuring ethanol are based on sample distillation and determining the specific gravity of the collected sample. While these techniques are precise, they are also labor-intensive and necessitate a substantial number of samples for distillation. Spectroscopy techniques offer greater sensitivity, but they are non-specific measurement methods and require distillation for sample preparation. Specific alcohol measurements can be achieved using methods such as gas chromatography or enzymatic assays, but these require specialized equipment that may not be readily available in many laboratories. A range of colorimetric methods have been employed for the quantification of ethanol in samples [2]. In general, the methods used to determine the amount of ethanol are spectrophotometry, colorimetry and gas chromatography (GC). However, the availability of tools is still limited due to their expensive prices. The spectrophotometer can be used in different ways. One of the ways to use it is to produce an absorption spectrum. If an object absorbs only a part of the visible wavelength, that object is seen in the complementary color of the absorbed light. For example, if an object is absorbed in the yellow area, that object is seen in blue color (which is the complement of yellow). Complementary color materials include three main colors: yellow, red, and blue, which are also sub-complementary and neutralize the effect. They do that their combination becomes black: Red-green, orange-blue, purple-yellow. In the visible region (400-700) nm, the absorption or transmission of the substance can be related to the color of the analyte. In this method, if a solution does not pass any visible wavelength, it is theoretically black in color. If it passes all visible wavelengths and does not absorb any light, the solution sample is white, and if we have a colored solution and we pass light through it that absorbs some of this light, then this absorbed light has a complementary color. For example, if the sample absorbs yellow color, it will appear blue at 580 nm, because blue is the complement of yellow. In fact, in visible spectrophotometry, one should choose a wavelength whose color is complementary to the color of the solution. Due to the fact that colored solutions have the maximum light absorption in their complementary color, based on this characteristic, the materials are examined in their respective wavelengths, and the intensity and weakness of this color depend on the amount of the substance in the solution. Therefore, in the visible spectrophotometry device, monochromatic light is created by analyzers such as filters, grids or prisms so that the concentration of a colored solution can be calculated. In this device, a filter is used as a monochromator. Therefore, a limited part of the wavelength is accessible (Table 1).

This graph shows the amount of light absorbed as a function of wavelength. The image below shows the absorption spectrum of ethanol. The diagram shows a region of the spectrum with strong absorption of light at around 400 and 580 nm, and it is the green light, specifically light at 580 nm, that is strongly absorbed, this is the wavelength of light used to measure $Cr_2(SO4)_3$ concentration. [3].

However, measurement using this spectrophotometer and gas chromatography is also expensive and not all laboratories can afford it. Along with recent developments in digital imaging, digital image-based colorimetry has been used as an alternative in analysis methods [4]. In these methods, they use the red-green-blue (RGB) color space or its derivatives, such as hue and saturation. Digital image data can be obtained from a scanner, a webcam, a digital camera and a mobile phone [5]. The color and intensity of a digital image is usually 24-bit data (8-bit red, 8-bit green + 8-bit blue), an additive color space, in which red, green, and blue (RGB) lights are added together in various combinations. To create a wide range of color reproduction. Many colors can be created by combining different values of R, G and B. The intensity of each color has 256 levels (from 0 to 255). In this coloring, 16777216 colors (2563 or 224) are obtained. A value of R = 0, G = 0, B = 0 refers to pure black while R = 255, G = 255, B = 255 is pure white. With this system, the unique combination of R, G and B values provides these conditions to provide millions of different hues, saturations and hues [6-8]. This range of colors provides a very favorable database for quantitative measurements. Therefore, digital image-based methods can be used as an alternative to quantitative measurements in forensic toxicology. Decomposition terminology, RGB (red, green, blue), is one of the most common colors used to represent the color spectrum. Therefore, in this research, the method of determining ethanol content analysis using a simple colorimetry. This method uses digital images that can be obtained from smartphone cameras. The smartphone camera can be used as a sensor, reader and recording tool for complex color intensity. Each pixel is then processed with digital image techniques using an RGB application. The advantage of measurement using the digital imaging method is that the amount of material used for measurement is less and more efficient and does not require expensive and complex tools. The design of most laboratory tests goes in a direction that can easily be done on-site. The desired analyte, when present with the utilized solutions and reagents, results in a colored solution that can be observed without the aid of any optical instruments. The conventional method of reading the final color of the reaction is with a spectrophotometer, and for experiments that are performed using 96-well microplates, the use of an expensive and space-saving device is called a microplate reader. Using the image analysis taken with a mobile phone (to read the color of the test results) can be a suitable alternative to this type of expensive device. Green chemistry is the use of chemistry techniques and methods that reduce or eliminate the use or production of raw materials, products, by-products, solvents, reagents, etc. This is done in analytical chemistry in three different ways: 1) Reducing the amount of solvent needed in sample preparation. 2) reducing the amount and toxicity of solvents and reagents used in the measurement step, especially with automation and miniaturization. and, 3) development of alternative direct measurement methods without the need for solvents or reagents [9]. In this study, the reagent potassium dichromate is utilized for alcohol measurement. By employing a smartphone and a minimal volume of solution, the quantification of ethanol in various samples can be accomplished swiftly and sensitively. The novelty of this research lies in the use of a smartphone as a novel instrument for colorimetric analysis.

Experimental

Apparatus and software

A Vis-spectrophotometer from Unicam (USA), fitted with a one-centimeter glass cell (internal volume of 3 mL), and a Huawei P10 mobile phone equipped with a 12-megapixel color sensor were utilized in this study. The Spotxel® Reader 1.1 application was employed to process the digital images and extract data. Microsoft Excel was used for further processing of the digital image data.

Materials and methods

Ethanol 96 v/v% (Dr. Mojalli), concentrated sulfuric acid, and potassium dichromate (Merck) with laboratory grade purity were used.

Spectrophotometric measurement of ethanol

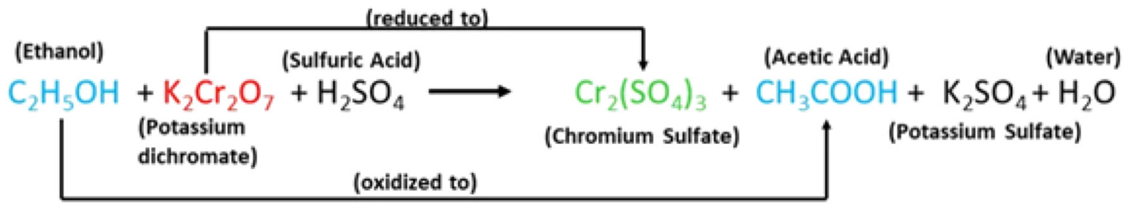
To carry out the distillation process, a flask with a small capacity and minimal rubber connections is desirable. An all-glass distillation apparatus consists of a 50 mL distilling flask attached to a vertical condenser by a glass connection, and the distilled solution is collected in a 5 mL rigid glass tube. In this technique, alcohol measurement is conducted using five milliliters of the distilled sample along with the derived solution. Initially, ethanol solutions of varying concentrations (0, 0.01, 0.025, 0.04, 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, 1, 5, 10, 25, 50, 100 v/v%) were prepared in water, each with a volume of ten milliliters. Following this, one milliliter of the standard solution was transferred to a test tube, to which five hundred microliters of potassium dichromate (2% by volume in dilute sulfuric acid) was added. This mixture was then vortexed for thirty seconds. Subsequently, the solutions were heated at 80 degrees Celsius for a duration of ten minutes. Finally, the absorbance of the colored solutions was measured using a spectrophotometer at a wavelength of 580 nm, and an absorbance calibration curve was plotted in relation to the ethanol concentration.

Mobile Phone-Based Measurement of Ethanol

Half a milliliter of each solution derived from the previous step was dispensed into the 96 wells of a round microplate. These wells were then photographed using a mobile phone. The color of the captured image was analyzed to obtain the signal corresponding to each concentration. In this technique, the logarithm of the signal from the reference solution exhibits a linear correlation with the signal from the standard solution, enabling the construction of a calibration curve.

Method validation

In this research, a simple colorimetric method based on an oxidation-reduction reaction of dichromate was used to determine ethanol. Ethanol is oxidized to ethanoic based on the following reaction with dichromate in an acidic environment:



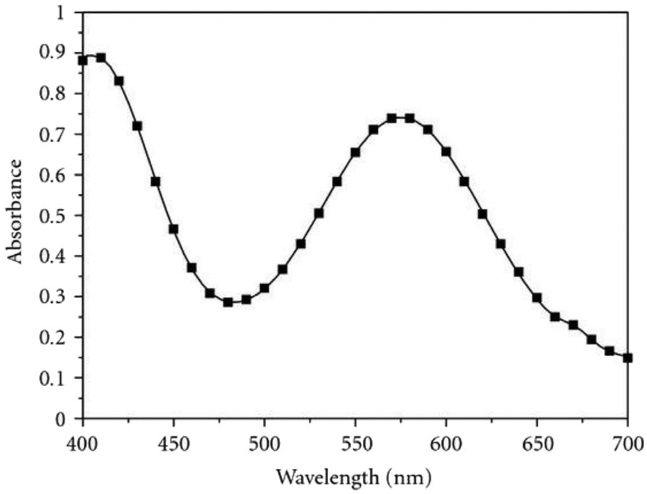


Fig. 1. The absorption spectrum of $Cr_2(SO4)_3$ (for ethanol determination).

Table 2
Figures of merit obtained for ethanol measurement with a photometer and smartphone.

Analyte	Equation	R2	LOD (v/v%)	LOQ (v/v%)	RSD%	DLR (v/v%)
Photometer	Abs= $0.6487C_{Et} + 0.0131$	0.9990	0.003	0.01	0.1-0.5	0.01-05
Brightness	Abs= $1.8509C_{Et} - 0.0004$	0.9944	0.0001	0.0003	0.1-0.5	0.01-0.075

Chromic acid first oxidizes the first-type alcohol to an aldehyde and then to a carboxylic acid, and converts the second-type alcohol to a ketone, but does not react with the third-type alcohols. Since the carbon atom is oxidized to alcohol of the first and second type, the orange hexavalent chromium ion turns into the blue-green trivalent chromium ion. The distinction between various ethanol concentrations is based on the emergence of a green color, which, when combined with the additional yellow hue of chromate ions, results in a variety of colors. This approach has received full approval for both qualitative and quantitative ethanol detection. The process of ethanol oxidation by dichromate is recognized as a non-selective method. Therefore, to reduce the interference of other reducing compounds in the sample, the dichromate consumed by the oxidation of ethanol to acetic acid is determined by the difference between the remaining dichromate concentration after the reaction. This can be calculated with the entire sample and the de-alcoholized sample. However, solvent distillation eliminates all volatile compounds, which can influence the control solution, particularly when volatile reducing agents, alcohols, or aldehydes are present. Generally, the interference of these compounds is negligible when their concentration is low. It's worth noting that the interference of the sample's volatile components affects the accuracy of methods based on the distillation process, as these compounds are collected with ethanol in the distillation solution. To optimize the conditions of the redox reaction, the concentration of dichromate was first examined. As depicted in Fig. 2, the linear range of the calibration curve extends from zero concentration to 0.15 v/v%, which is due to the insufficient amount of dichromate for higher ethanol concentrations. Other parameters such as temperature and time were examined, determining that the reaction needs to be performed at a temperature of 80 °C for ten minutes.

In this investigation, the absorbance of the solution obtained from the oxidation of ethanol with chromic acid at a wavelength of 580 nm was obtained as shown in Fig. 1. As can be seen, the linear range of the calibration curve is between 0-0.55 v/v% and its detection limit is 0.01 v/v%. (Fig. 3). In another method, using a smartphone and the Spotxel® Reader 1.1 application, the amount of signal was measured in microarray mode. By plotting the values obtained from alcohol color analysis by smartphone, the curves drawn in Fig. 3 were obtained.

The Beer-Lambert relation was used to convert these values into absorption numbers (the logarithm was taken from the obtained values) and then these values were used to draw the calibration curve. The results show that an acceptable calibration curve can be obtained only in the blue and violet modes and the Brightness mode (in the other two modes, the absorption is downward and does not create a linear relationship with the concentration, which indicates the absence of absorption in these two modes). The initial RGB color values were converted to log ratios following the Lambert-Beer law derivation formula as follow:

$$A = log\frac{S_0}{S} = abc$$

S_0, and S are the color values of blank and sample, and a, b, and c are absorptivity, optical path length, and concentration, respectively (Table 2).

In order to check the efficiency of the system used by the mobile phone, one sample of rose water and one sample of beer were evaluated using the mentioned method (Table 3).

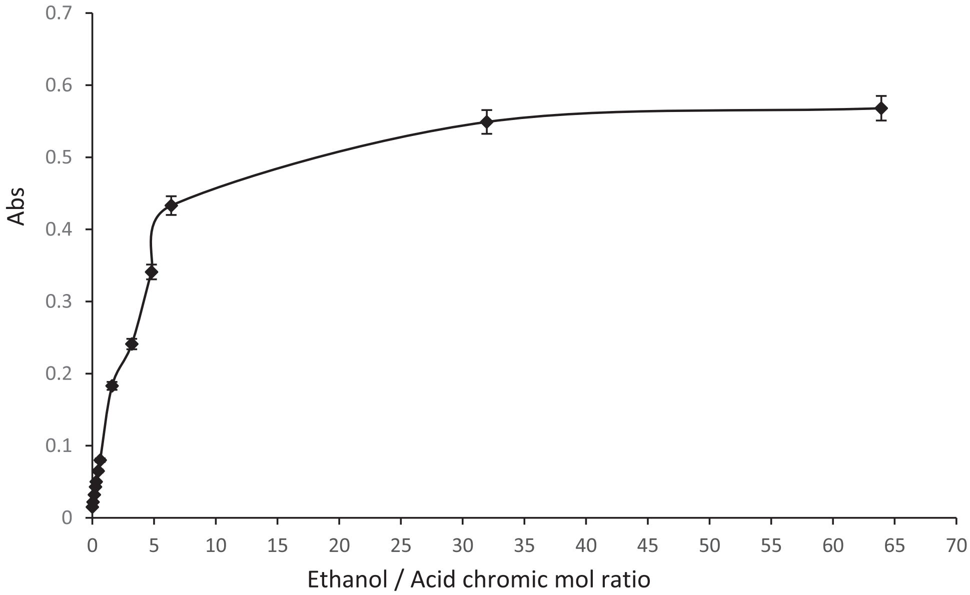


Fig. 2. The effect of the molar ratio of chromic acid and ethanol in the obtained solution (reaction conditions: sample volume one milliliter, potassium dichromate volume: 0.5 milliliter of 2% potassium dichromate in concentrated sulfuric acid).

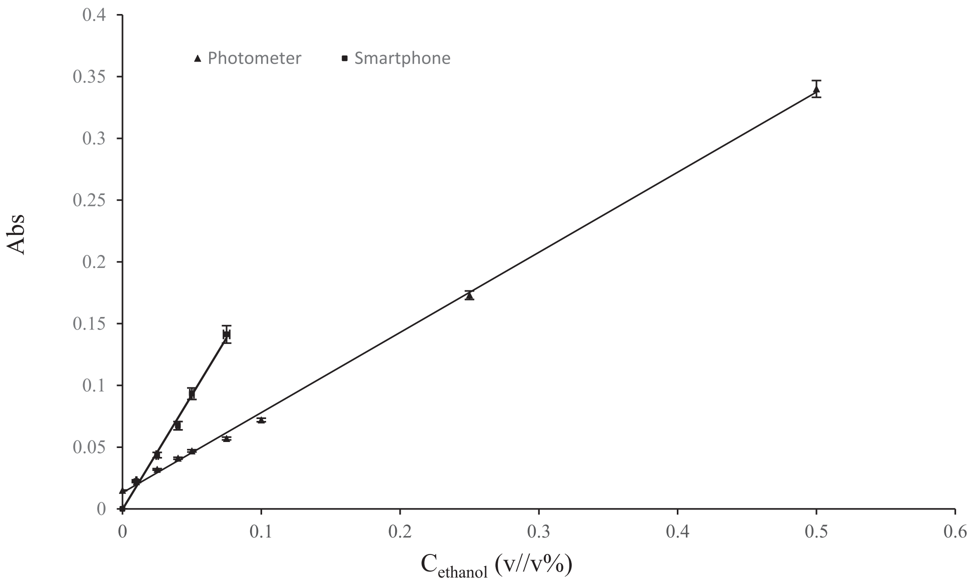


Fig. 3. Calibration curve obtained for ethanol using a photometer at 580 nm and smart phone.

Table 3
Analysis of real samples.

sample	$C_{ethanol}$ (v/v%)		Error%
	True	Obtained	
1	0.19	0.16	-15.7
2	0.38	0.37	-2.6

Table 4
Comparison of UV-VIS and RGB for determination of ethanol.

	UV-VIS	RGB	RGB benefits
Estimated time to process 96 samples (min)	100	5	20 ×faster
Solvent use for each sample (mL)	0.5	0.2	2.5 × less solvent
Glassware	Quartz or plastic cell	96 wells microplate.	-
Specialized equipment	Spectrophotometer	Mobile phone	40 × less costly
Cost for single measurement	800 $	1$	800 × less costly

In order to compares ethanol measurement with sspectrophotometer and mobile phone (RGB) and analysis time, equipment used, and ethanol measurement price is given in Table 4. It can be said that according to the analysis time for each sample and the price of the equipment used in each method, the cost of analysis with sspectrophotometer is much higher than mobile phone (800 times).

Conclusion

The method used is simple and problem-free, and the necessary reagents are easily prepared and stable for at least one year. The proposed method is easily performed using common reagents and equipment. It can be recommended for quick measurement or for the determination of ethanol in small or very low concentration samples. The study introduces a rapid and efficient method for ethanol measurement using a smartphone. The method leverages the oxidation reaction of ethanol, colorimetry, and image analysis. The technique has been successfully applied to real samples, demonstrating its practical applicability. Compared to traditional spectrophotometric absorbance measurements, this method offers a more limited calibration curve linearity but significantly higher measurement speed. It is necessary that the volatile and reducing compounds are present in a small amount in the sample. The reaction that you performed was a Brealyser test, with which the driver's consciousness can be checked with the changes that occur in the respiratory system, and specifically it can show the presence of alcohol in the exhaled air content (if the color of the compound If the tube filler changes from yellow to green, the result is positive).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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