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A greener, fast, and cost-effective smartphone-based digital image procedure for quantification of ethanol in distilled beverages

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ABSTRACT

A novel procedure for the determination of ethanol in distilled beverages using smartphone-based digital images is proposed. It pioneering exploits the influence of ethanol concentration on the radiation absorption by phenolphthalein in alkaline medium. This effect is attributed to the low dielectric constant of ethanol in comparison with water, which affects the resonance structures of the phthaleins and the closure of the lactone ring. The color intensity was measured and converted to RGB values with a smartphone by using a free application (App). Aiming at portability, an innovative holder was built in a 3D printer; it integrated the smartphone and the measurement microtube to ensure the analytical measurements under proper focal distance and illumination provided by the smartphone camera. The procedure showed a linear response from 10.0 to 70.0% (v/v) ethanol ($r = 0.998$, $n = 7$), a coefficient of variation of 1.2% ($n = 8$) and a limit of detection (99.7% confidence level) estimated at 2.1% (v/v). Accuracy was assessed by comparison of the results achieved by the proposed and the AOAC reference procedures, which agreed at the 95% confidence level. The proposed procedure is practical, fast (< 1 min per determination), environmentally friendly (only 10.8 μg phenolphthalein is required per determination), and suitable for point-of-care determination of ethanol in distilled beverages.

1. Introduction

Ethanol in beverages originates from the fermentation of fruit and cereal sugars under strict physicochemical and biological control to meet the specificities of each beverage. During the production, transport, and storage processes, some quality parameters need to be monitored to assure stability, food safety, and product authenticity. The concentration of ethanol is the most important quality parameter in distilled beverages. The official method recommended by Association of Official Analytical Chemists (AOAC) is based on pycnometry [1], which provides accurate results, but requires a time-consuming distillation step, a precision balance, and temperature control that makes point-of-care measurements unfeasible. Although the sample throughput is improved by steam distillation [2], which takes ca. 5 min per sample, it requires a specific apparatus and the procedure is still laborious.

Alternative analytical procedures for the determination of ethanol in beverages exploit gas chromatography [3], near- [4] or mid- [5] infrared spectrometry with partial least squares regression, as well as fluorimetric [6], amperometric [7], or potentiometric [8] (bio)-sensors, or redox titration with potassium dichromate [9]. Some of these alternatives do not satisfy the requirements for point-of-care measurements, because they involve complex instrumentation assemblies, generate large waste amounts, and need controlled laboratory conditions to achieve reliable results. In order to circumvent some of these drawbacks, reagentless alternative procedures have been proposed for this application, such as those exploiting the Schlieren effect in flow analysis [10,11] or that based on thermal infrared enthalpimetry [12].

Measurements based on digital images have incorporated clear advances to analytical procedures, especially in relation to practicality, analytical productivity, simplicity, exploitation of low-cost instrumentation, and minimal waste generation. Procedures based on digital images have been applied for determinations of sulfite [13], methanol [14], furfural [15], and ethanol [16,17] in beverages. However, ethanol determination in beverages by this approach requires toxic reagents, e.g. chromium(VI) and cerium(IV), as well as highly acidic conditions and long reaction times, which are drawbacks for point-of-care measurements.

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The aim of this work was to propose a new approach for the determination of ethanol in beverages, by exploiting the effect of ethanol on the resonance structures of phenolphthalein in alkaline medium. The color intensity of the dye decreases proportionally to the ethanol concentration due to its effect on the dielectric constant of the medium, making feasible the analyte determination without any sample pretreatment. Aiming at point-of-care analytical measurements, the smartphone camera used as a photometric device was assembled to a specially designed 3D-printer platform to optimize the image capture without any other external device.

2. Experimental

2.1. Reagents and solutions

The solutions were freshly prepared with analytical-grade chemicals, deionized water (resistivity > 18 MΩ cm), and anhydrous ethanol (Merck).

A 1.0 mol L^{-1} sodium hydroxide stock solution was prepared by dissolution of the solid (Merck, > 99%) in water. A 1.0% (w/v) (31.4 mmol L^{-1}) phenolphthalein (3,3-bis(4-hydroxyphenyl)-1(3H)-isobenzofuranone, Sigma-Aldrich, > 99%) stock solution was prepared in 70% (v/v) ethanol. Further dilutions were performed with a 50% (v/v) ethanol solution. A 0.2 mol L^{-1} glycine buffer solution was prepared by dissolving the reagent and 2.34 g NaCl in water, and adjusting the pH to 11.5 with 1.0 mol L^{-1} NaOH solution [18].

Beverage samples (sugar cane spirit and vodka) were purchased at the local market (Piracicaba, SP, Brazil) and analyzed without any pretreatment.

2.2. Apparatus

Digital images were acquired with the camera (13 megapixels) of a Samsung Galaxy J7 smartphone, equipped with Android 6.0, using the App Color Grab 3.6.1 (Loomatix®). The support for coupling the reaction microtube to the smartphone (Fig. 1) was built in acrylonitrile butadiene styrene (ABS) with a 3D printer (GTMAX-3D, model Corel H4), with the software simplify3D, version 4.0. With the dimensions indicated in Fig. 1, the support is also compatible with smartphones with centralized camera (e.g. Samsung J6, and Moto G6, LG X2). The adapter (Fig. 1, part A) can be designed to fit other kinds of devices.

2.3. Proposed procedure

Sample (500 μL) and reagent (200 μL) were transferred to transparent 1.5-mL polypropylene microtubes, which were immediately closed. After manual shaking, the microtube was placed in the holder at 10 cm far from the camera for photometric measurements (Fig. 2). The light emitted from the LED of the smartphone provided the required illumination for image acquisition. The reflected radiation, inversely proportional to the color intensity, was then quantified. G-channel readings provided the analytical responses and measurements were taken in triplicate. The values were manually transferred to Google Spreadsheets for data handling. The calibration curves obtained by least squares regression were used to determine the analyte concentrations.

The effect of the potential interfering species fructose, sucrose, and glucose (10, 30, and 60 mg L^{-1}), sodium sulfite (10, 20, and 50 mg L^{-1}), propanol and methanol (10, 20 and 30% v/v) was evaluated in relation to a 30% (v/v) ethanol standard solution.

For accuracy assessment, beverage samples were analyzed by the proposed procedure and by the reference method recommended by AOAC [1]. Briefly, 200 mL of alcoholic beverage was distilled about 35 min and the distillate was collected in a 200 mL volumetric flask; after reaching room temperature, the volume was completed with deionized water. The alcoholic concentration was then determined by pycnometry using tabulated density values for the water-ethanol mixtures.

3. Results and discussion

3.1. General aspects

Phenolphthalein (pK_a 9.3) is widely used as acid-base indicator due to its low cost and suitable color transition from colorless to pink (within pH 8.2 and 9.8). This indicator can be found in three forms in aqueous media: (i) a colorless monoanion (pH < 8.3); (ii) resonant structures involving the red-pink quinonoid and the colorless carbinol (at pH > 8.3); and (iii) colorless carbinol trianion in highly alkaline medium (pH > 12) [19,20] (Fig. 3). In polar protic solvents, the pink coloration characteristic of the quinonoid form predominates at pH above the transition range and below 12.0. On the other hand, in media of aprotic solvents such as $CHCl_3$, DMSO, and DMF, phenolphthalein is found in its colorless form. Partial discoloration is observed in aqueous medium after addition of solvents with lower dielectric constants, such as ethanol [19,21], which affects the resonance structures of the phthaleins by the closure of the lactone ring [19].

The analytical measurements were carried out by using a smartphone with an App for measurement of the RGB values. The spectral range covered by the G channel is strongly overlapped with the absorption spectra of phenolphthalein in alkaline medium. Thus, ca. 85% of the signal variation refers to the G channel, whose values vary proportionally to the phenolphthalein concentration.

Reliable smartphone-based photometric measurements require constant focal distance as well as stable illumination conditions. In order to achieve these requirements with a portable setup, a support for both the smartphone and the measurement microtube was designed with a 3D printer. The optimal distance from the camera to the microtube (sample probe) was set at 10 cm and constant illumination was provided by the LED of the smartphone.

3.2. Procedure optimization

Relevant experimental variables such as pH, buffer and phenolphthalein concentrations, sample volume, reaction time, and temperature were optimized by the univariate method.

As the procedure is based on the fading of the color of phenolphthalein, its concentration was the first parameter optimized in order to set a reference value without saturation of the G-channel. To this end, the volume of a 0.1% (w/v) phenolphthalein solution was optimized to maximize the effect of the ethanol concentration (Fig. 4A). Analytical signals obtained in the absence and presence of ethanol decreased inversely with the dye volume up to 20-μL phenolphthalein, and saturation was observed for higher volumes. The effect of ethanol concentration became more pronounced for higher concentrations of phenolphthalein. However, in order to prevent saturation of the G-channel, further studies were carried out with 15 μL of the reagent.

Variations in the volumes of the 0.1 mol L^{-1} NaOH solution within 5 and 25 μL, which provided final pH within 10.7 and 12.4, did not affect significantly neither the reference signal or the analytical response (Fig. 4B). As previously discussed, the color intensity of phenolphthalein is not affected in this pH range.

When the proposed procedure was applied to samples of distilled beverages, by using the optimized reaction conditions, i.e. 15 μL of 0.1% (w/v) phenolphthalein, 25 μL of 0.1 mol L^{-1} NaOH, and 1.0 mL of sample, for some samples the results disagreed with those obtained by the official reference method. This effect was probably caused by the presence of acidic species, which consumed part of the sodium hydroxide, thus affecting the equilibria involving phenolphthalein. Aiming at to overcome this drawback, buffer solutions were evaluated and, for practical purposes, these solutions were prepared with the selected phenolphthalein concentration (0.1% (w/v)). Best results were achieved with 0.2 mol L^{-1} glycine buffer solution at pH 11.5, in which phenolphthalein was stable for at least

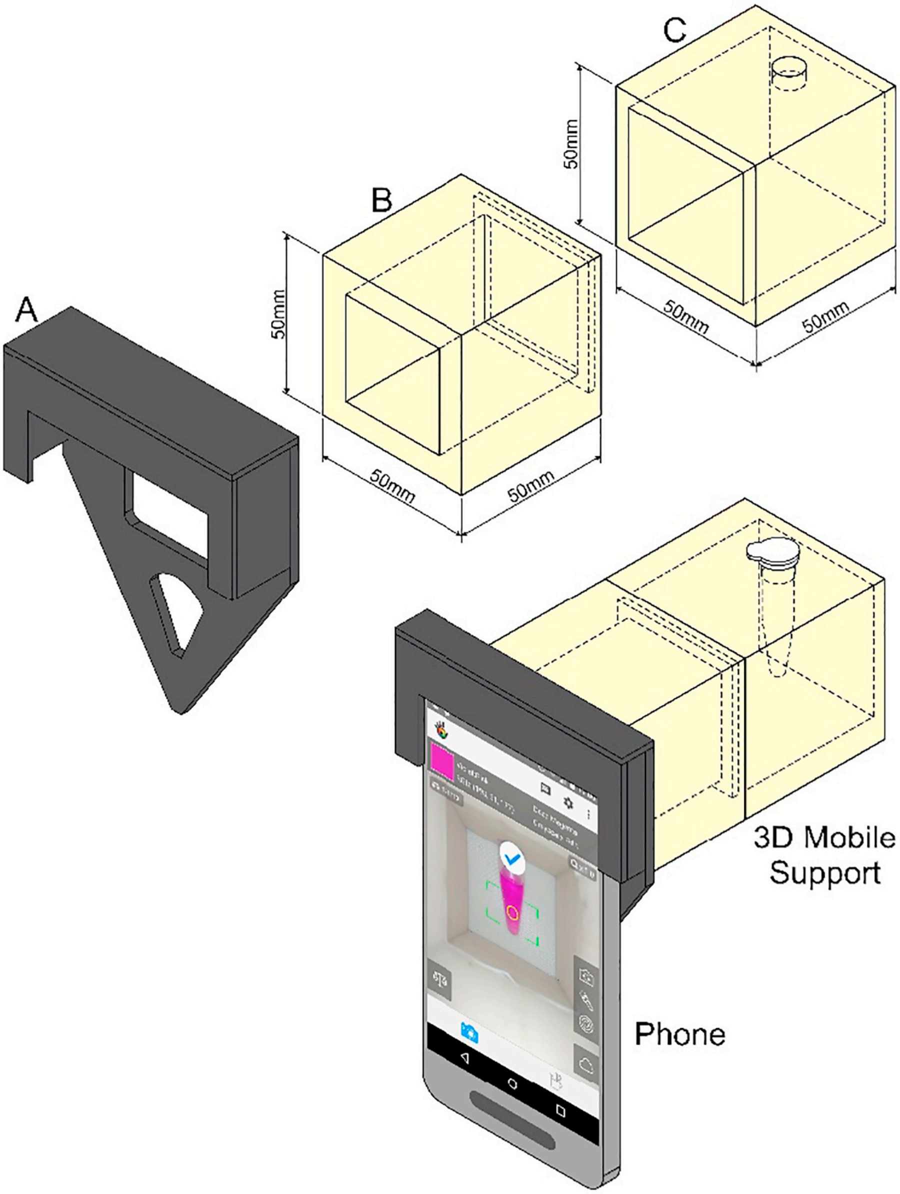

Fig. 1. Mobile phone holder built in ABS with a 3D printer. Mountable parts A, B, and C are interchangeable to be compatible with different smartphone formats (A) and solution vessels (C), i.e. cuvettes, glass tubes, or microtubes. Project available for download at www.tinkercad.com/things/0Fq3rSwDgtg.

60 days. In order to verify the buffering effect in the samples, additional experiments were performed by using sample volumes ranging from 400 to 700 µL plus 100, 200 or 300 µL of the buffer solution (Fig. 4C). All volumes of buffer solutions sufficed to keep the pH constant for up to 700 µL of sample (checked with a pH-meter). The difference in the signals related to the increase of the buffer volumes reflects the increase of phenolphthalein concentration in the reaction medium. As a compromise between the phenolphthalein concentration and the buffer capacity, further experiments were performed using 200 µL of buffer solution and 500 µL of sample. Under this condition, the previously optimized phenolphthalein concentration (ca. 46 µmol L^{-1}) is maintained.

The temperature did not significantly affect the results within the 20–40 °C range (variations lower than 3.5%), which is an attractive characteristic for a procedure devoted to point-of-care measurements. For higher temperatures, the reaction approached the steady state more quickly. Slopes of analytical curves within 10 to 60% (v/v) ethanol showed variations lower than 3% for a time interval higher than 100 h, which indicates the ruggedness of the method.

3.3. *Effect of potential interfering species*

The effect of constituents usually found in distilled beverages such as fructose, glucose, sucrose, sulfite, as well as methanol and propanol was evaluated in the absence and presence of a 30% (v/v) ethanol solution. The results (Fig. 4D) refer to the highest concentrations of the species evaluated. Sugars and sulfite did not affect the analytical response in a pronounced manner (signal variations lower than 10%). However, as expected, methanol and propanol in concentrations beyond 10% (v/v) affected the analytical response. This result reflects the structural similarity and chemical properties of these alcohols, but it does not hinder the proposed application because these substances must be absent from distilled beverages [16]. Moreover, the effect of absorption of CO_2 from the atmosphere was negligible during the assays.

3.4. *Analytical features and application*

Under the optimized conditions, the proposed procedure shows a linear response within 10 to 70% (v/v) ethanol (Fig. 5), described by

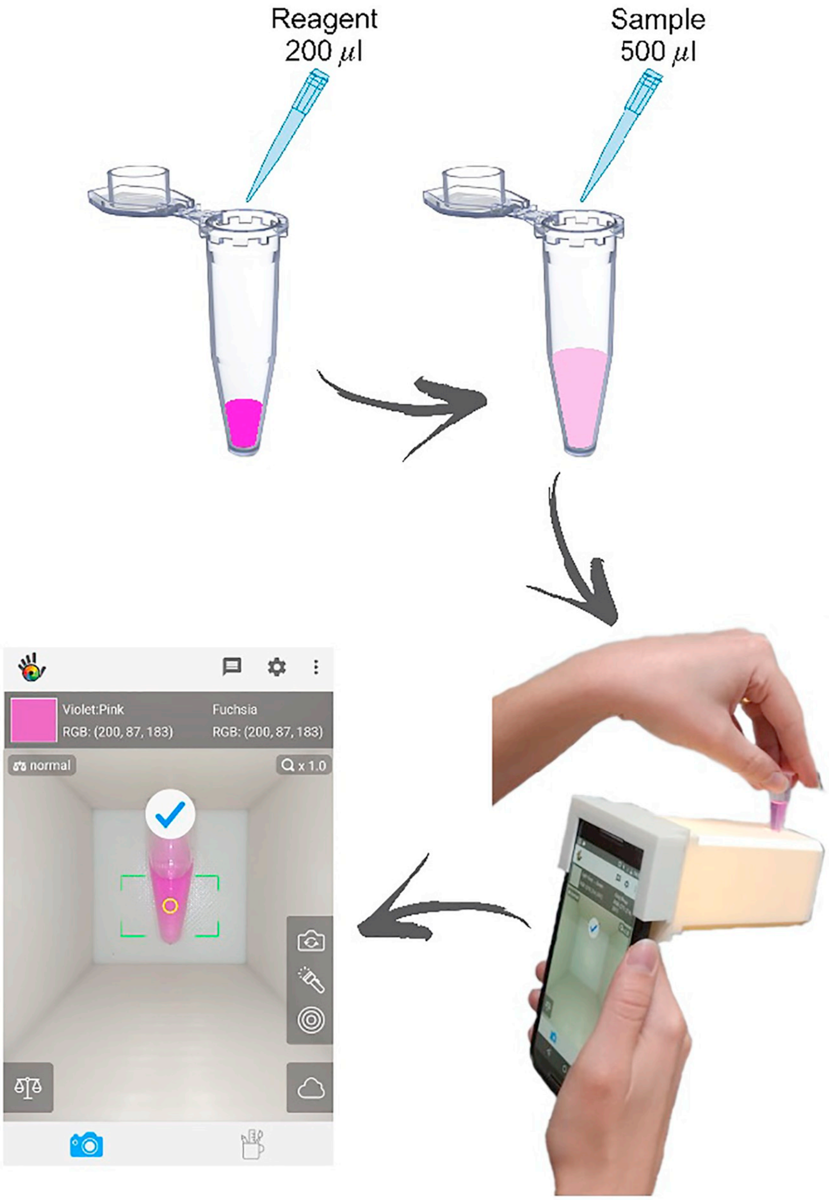

Fig. 2. Schematic representation of the procedure.

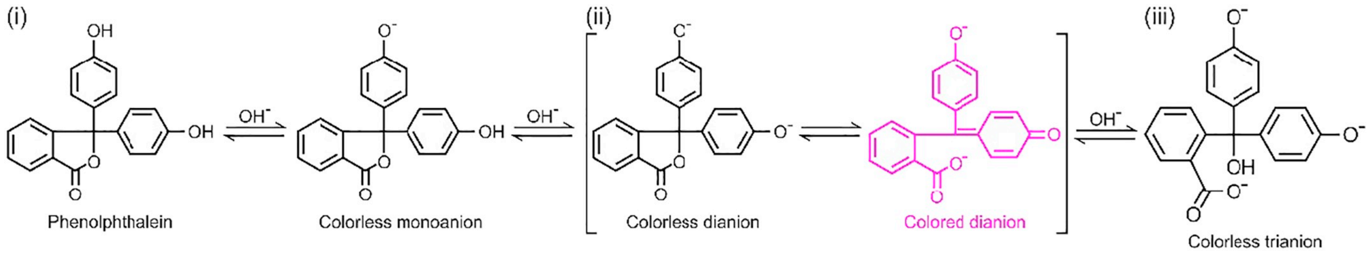

Fig. 3. Chemical forms of phenolphthalein in aqueous medium.

the equation G channel = (1.88 ± 0.06) [ethanol] % (v/v) + (27.21 ± 2.47) with $R^2 = 0.996$. Coefficient of variation (n = 8) and limit of detection (99.7% confidence level) were estimated as 1.2% and 2.1% (v/v), respectively.

For accuracy assessment, results obtained with the proposed procedure in the analysis of distilled beverages were compared with those achieved by the reference method. Results (Table 1) agreed at the 95% confidence level ($t_{calculated} = 0.148$; $t_{critical} = 2.571$), with relative errors < ± 10%, which is acceptable for the proposed application.

3.5. Performance comparison

Table 2 shows analytical features of some procedures for determination of ethanol in beverages based on digital images. Most of these procedures involve the reaction of ethanol with dichromate ions in a strongly acidic medium, generating Cr(III) [17,23]. In these procedures,

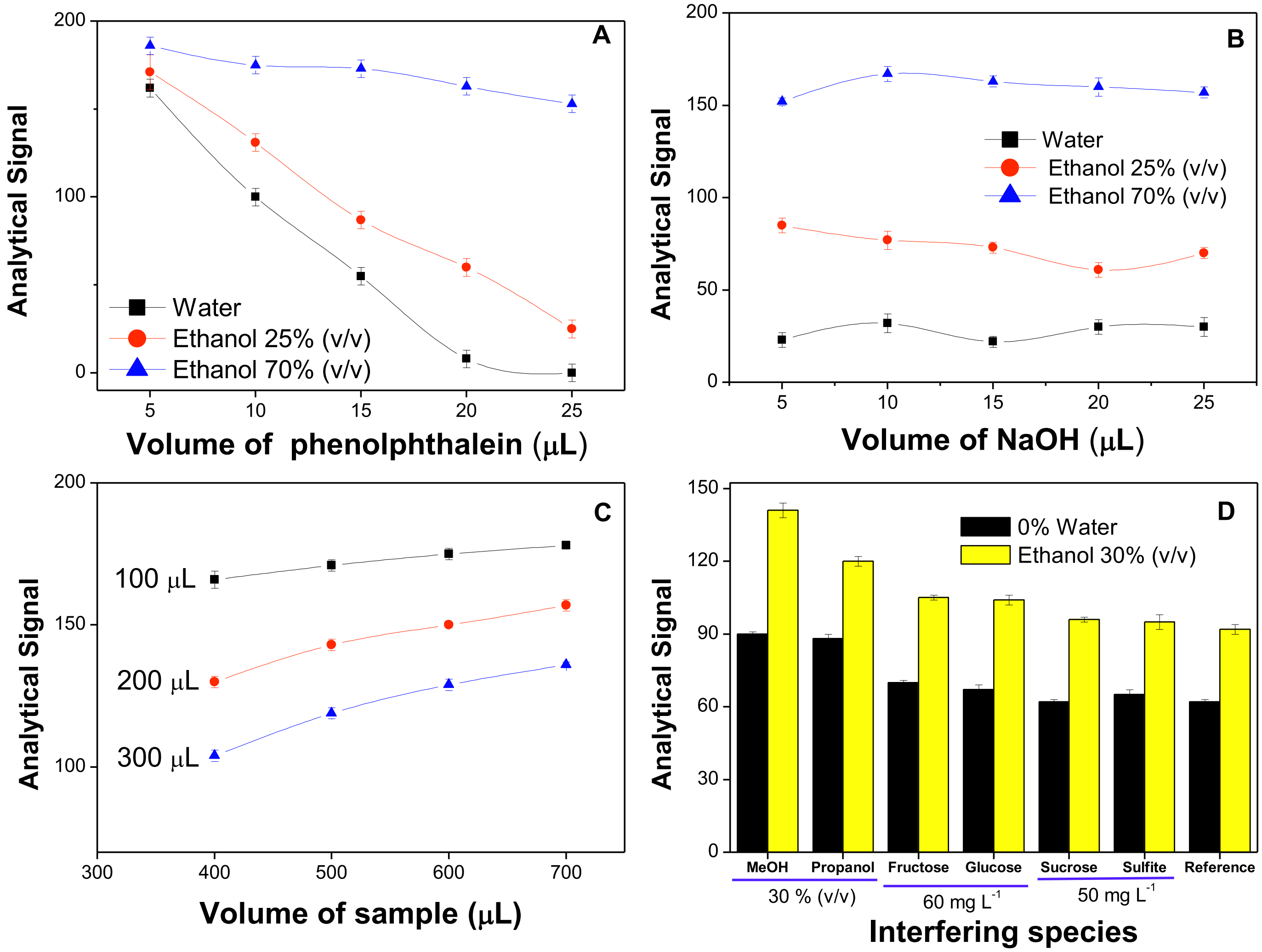

Fig. 4. Parameters evaluated in the presence and absence of the analyte. (A) volume of a 0.1% w/v phenolphthalein solution; (B) volume of a 0.1 mol L^{-1} NaOH solution; (C) sample volume for different buffer volumes; and (D) effect of potentially interfering species. Initial experimental conditions: 1.0 mL of sample, 15 µL of 0.1% (w/v) phenolphthalein and 20 µL of 0.1 mol L^{-1} NaOH. G-channel values were taken as the analytical signal.

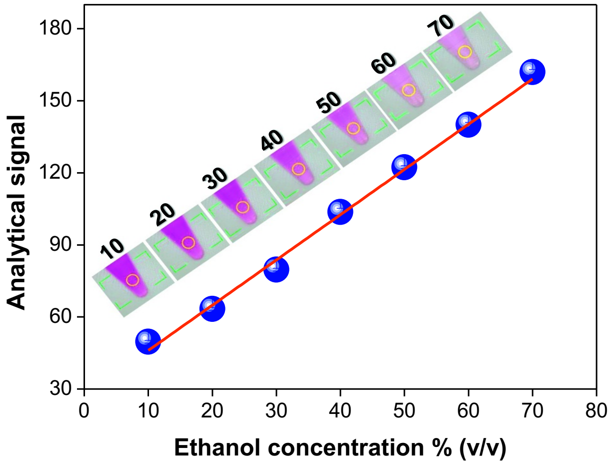

Fig. 5. Analytical curve for the determination of ethanol in distilled beverages. Images refer to the vials with ethanol standard solutions. G-channel values were taken as the analytical signal.

Table 1
Comparative results. Ethanol concentrations in distilled beverages % (v/v) as determined by the proposed and reference procedures.

Sample	Proposed method	Official method [1]	Relative error (%)
1	42.9 ± 0.3	39.4	8.9
2	32.6 ± 0.6	35.4	−9.4
3	38.0 ± 0.5	41.1	−7.5
4	40.1 ± 0.5	39.0	2.8
5	32.9 ± 0.6	32.8	0.3
6	45.2 ± 0.3	44.9	0.7

Mean value ± standard deviation (n = 3). Standard deviation of the official method was always lower than 0.1% (v/v).

digital images were captured with a digital camera under a controlled illumination environment and data treatment was carried out by ImageJ software (National Institutes of Health).

A paper-based sensor was recently developed for the detection of low concentrations of ethanol. Preparation of the device involved adsorption of alcohol oxidase onto a polyaniline film, followed of washing and drying, with storage at low temperature (ca. 4 °C). After the sample assay, the paper device was scanned and the image was processed with the ImageJ software [24]. Another procedure exploited formation of

Table 2
Procedures exploiting digital images for the determination of ethanol in beverages.

Analytical Procedure	Linear range % (v/v)	LD % (v/v)	CV %	R	Reference
Effect of ethanol on the resonance structures of phthaleins. Steps: Mixing glycine buffer + phenolphthalein with sample in a microtube. Detection: Smartphone with App Color Grab®	10–70	2.1	1.2	0.998	This work
Redox reaction of ethanol with dichromate in acidic medium generating Cr(III). Steps: Mixing sample and reagents in a porcelain plaque and measurements after 12 min. Detection: Digital camera and analysis using ImageJ.	1.0–20.0 25.0–50.0	0.25	–	0.999 0.980	17
Redox reaction of ethanol with dichromate in acidic medium generating Cr(III). Steps: Mixing sample and reagents in a porcelain plaque and measurements after 4 min. Detection: Digital camera and analysis using ImageJ.	10–70	1.72	< 2.8	0.995	23
Biosensor based on alcohol oxidase immobilized onto polyaniline. Steps: PANI film preparation; enzyme immobilization (> 30 min); biosensor construction; and color measurements. Detection: Image scanning and analysis using ImageJ.	0.01–0.8	0.01	1.6	0.996	24
Ethanol reaction with Ce(IV) in nitric acid medium to form a red complex. Steps: Mixing sample and reagents and measurements after 5 min. Detection: Multivariate PLS calibration through smartphone with the App PhotoMetrix Pro®.	0.62–5.0	0.19	< 0.5	0.998	16
Dynamic change of chemical waves in the Belousov-Zhabotinsky (BZ) reaction by ethanol. Steps: Gel formation in Petri dish (ca. 5 min), dilution of the sample and addition of the mixture in the petri dish. Detection: Chemical waves recorded using a smartphone and scale bar (ca. 5 min).	0.2–1.0	–	< 3	0.996	22

R: correlation coefficient; CV: coefficient of variation; LD: detection limit.

Cerium(IV)/ethanol complex, whose color intensity was monitored with a smartphone camera by the Photometrix® App [16]. Determination of ethanol in beverages was also performed using visible chemical waves in the Belousov-Zhabotinsky (BZ) reaction by perturbation with ethanol [22]. These waves were generated in a Petri dish and recorded using a smartphone camera to determine the velocity of the wave, which was inversely proportional to the ethanol concentration. These procedures aimed at most practical and cost-effective analysis, involving detection with widely accessible instruments. Despite these advantages, the use of toxic reagents (e.g. chromium(VI) and cerium(IV)) can be pointed out as drawbacks, in addition to the requirement of highly acidic conditions for the development of reactions.

Figures of merit of the proposed procedure were similar to those in Table 2, but the present proposal allows real-time monitoring of ethanol concentration, without any sample pretreatment or further data treatment for image processing. The use of single App for data acquisition and processing would make the procedure even more practical. Although this feature is provided by the Photometrix® free App, reliable results were not achieved in the present application because of the use of the smartphone LED as the only illumination source. The automatic adjust of focus and brightness during image acquisition hindered reading of the RGB values. Finally, the proposed procedure stands out by requiring only usual, inexpensive, and low toxicity reagents, in addition to portability provided by the specially designed 3D-built support.

4. Conclusions

The proposed procedure can be successfully applied to the determination of ethanol in distilled beverages, yielding reliable results in agreement with the AOAC reference method. Considering the high analytical demand, the present procedure stands out because of the fast chemical analysis, which requires < 1 min. The practicality, precision, widely available instrumentation, and low generation of an environmentally friendly waste, constitute themselves in advantages towards application of the procedure for point-of-care measurements, either in the production stages and/or quality control for distilled beverages.

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References

[1] AOAC Official Method 942.06 Alcohol by Volume in Distilled Liquors Pycnometer Method (1942).
[2] Modular distillation unit, KjelFlex K-360 (BUCHI): available in: https://bit.ly/2pZoSvh
[3] M.L. Wang, Y.M. Choong, N.W. Su, M.H. Lee, A rapid method for determination of ethanol in alcoholic beverages using capillary gas chromatography, J. Food Drug Anal. 11 (2003) 133–140.
[4] L.S. Mendes, F.C.C. Oliveira, P.A.Z. Suarez, J.C. Rubim, Determination of ethanol in fuel ethanol and beverages by Fourier transform (FT)-near infrared and FT-Raman spectrometries, Anal. Chim. Acta 493 (2003) 219–231.
[5] A. Debebe, M. Redi-Abshiro, B.S. Chandravanshi, Non-destructive determination of ethanol levels in fermented alcoholic beverages using Fourier transform mid-infrared spectroscopy, Chem. Cent. J. 11 (27) (2017) 1–8.
[6] S.S. Bozkurt, E. Merdivan, Y. Benibol, A fluorescent chemical sensor for ethanol determination in alcoholic beverages, Microchim. Acta 168 (2010) 141–145.
[7] R. Angeloni, M. Tomassetti, M. Castrucci, L. Campanella, Ethanol determination in alcoholic beverages using two different amperometric enzyme sensors, Curr. Anal. Chem. 11 (2015) 56–67.
[8] L. Rotariu, C. Bala, V. Magearu, New potentiometric microbial biosensor for etanol determination in alcoholic beverages, Anal. Chim. Acta 513 (2004) 119–123.
[9] G.J. Pilone, Determination of ethanol in wine by titrimetric and spectrophotometric dichromate methods: collaborative study, J. Assoc. Off. Anal. Chem. 68 (1985) 188–190.
[10] S.S.M.P. Vidigal, A.O.S.S. Rangel, A reagentless flow injection system for the quantification of ethanol in beverages based on the Schlieren effect measurement, Microchem. J. 121 (2015) 107–111.
[11] D.S. Silva, B.F. Reis, Evaluation of the Schlieren effect employing a LED-based photometer with a long-pathlength flow cell for reagentless photometric determination of ethanol in distilled ethanolic beverages, Microchem. J. 129 (2016) 325–331.
[12] A.S. Oliveira, C.A. Ballus, C.R. Menezes, R. Wagner, J.N.G. Paniz, B. Tischer, A.B. Costa, J.S. Barin, Green and fast determination of the alcoholic content of wines using thermal infrared enthalpimetry, Food Chem. 258 (2018) 59–62.
[13] L.P.S. Benedetti, V.B. Santos, T.A. Silva, E. Benedetti-Filho, V.L. Martins, O. Fatibello-Filho, A digital image analysis method for quantification of sulfite in beverages, Anal. Methods 7 (2015) 4138–4144.
[14] M.O.K. Franco, W.T. Suarez, M.V. Maia, V.B. Santos, Smartphone application for methanol determination in sugar cane spirits employing digital image-based method, Food Anal. Methods 10 (2017) 2102–2109.
[15] M.O.K. Franco, W.T. Suarez, V.B. Santos, Digital image method smartphone-based for furfural determination in sugarcane spirits, Food Anal. Methods 10 (2017) 508–515.
[16] F.C. Böck, G.A. Helfer, A.B. da Costa, M.B. Dessuy, M.F. Ferrão, Rapid determination of ethanol in sugarcane spirit using partial least squares regression embedded in smartphone, Food Anal. Methods 11 (2018) 1951–1957.
[17] L.P.S. Benedetti, V.B. dos Santos, T.A. Silva, E. Benedetti-Filho, V.L. Martins, O. Fatibello-Filho, A digital image-based method employing a spot-test for quantification of ethanol in drinks, Anal. Methods 7 (2015) 4138–4144.
[18] J. Lurie, Handbook of Analytical Chemistry, Mir Publishers, Moscow, 1975.
[19] K. Tsubaki, Colorimetric recognition using functional phenolphthalein derivatives, J. Incl. Phenom. Macrocycl. Chem. 61 (2008) 217–225.

[20] L. Nicholson, Kinetics of the fading of phenolphthalein in alkaline solution, J. Chem. Educ. 66 (1989) 725–726.

[21] M.W. Massey Jr., Z.A. Schelly, Dissociation field effect and temperature-jump kinetics of ethanolic and aqueous phenolphthalein, J. Phys. Chem. 78 (1974) 2450–2453.

[22] T. Somboon, S. Sansuk, An instrument-free method based on visible chemical waves for quantifying the ethanol content in alcoholic beverages, Food Chem. 253 (2018) 300–304.

[23] K.D. Pessoa, W.T. Suarez, M.O.K. Franco, M.F. dos Reis, R.P.L. Moreira, V.B. dos Santos, Development and validation of analytical methodology based on digital images of spot tests for determination of ethanol in cachaça, JCEC 3 (2017) 995–1010, https://doi.org/10.18540/jcecvl3iss7pp0995-1010.

[24] B. Kuswandi, T. Irmawati, M.A. Hidayat, M. Ahmad Jayus, A simple visual ethanol biosensor based on alcohol oxidase immobilized onto polyaniline film for halal verification of fermented beverage samples, Sensors 14 (2014) 2135–2149.