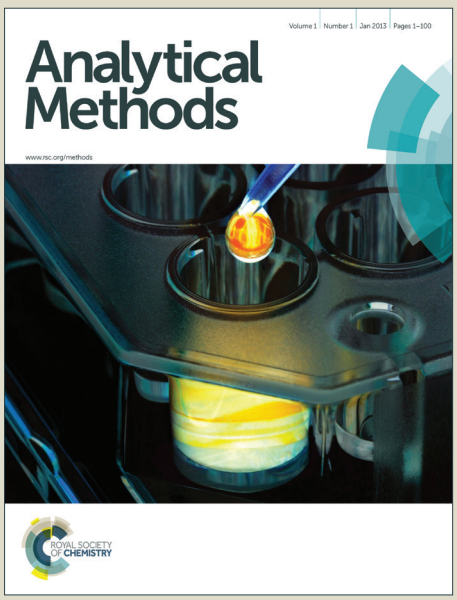
Analytical Methods

Accepted Manuscript



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Analytical Methods

TECHNICAL NOTE

Microemulsification-based method: analysis of ethanol in fermentation broth of sugar cane

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This article addresses important results for consolidation of microemulsification-based method (MEC), an approach recently proposed by these authors that represents a powerful output for deployment of point-of-use technologies. In MEC, the detection is conducted in solution with naked eyes. It relies on effect of analyte on formation of microemulsions (MEs). Minimum volume fraction of amphiphile needed to get ME (Φ_{ME}) is the analytical response whose measurement is based on a binary chemical information: the cloudy-to-transparent transition that occurs with microemulsification. Accordingly, this signal can be precisely detected with naked eye enabling precise determinations. Following experiments were accomplished: robustness investigation and direct determination of ethanol in fermentation broths of sugar cane. Dispersions were composed of water, oleic acid, and ethanol as hydrophilic (W), hydrophobic (O), and amphiphilic (AP) phases, respectively. Standards of analyte were added in W phase before the addition of AP in W-O mixture to attain the analytical curves. For application, the samples were directly used as W phase. Our approach was somewhat robust as regards to deviations in volumetric preparation of dispersions and changes in temperature and conductivity. Lastly, the reliability of MEC was evaluated in determination of ethanol in fermentation broths of sugar cane. The results were astoundingly accurate after direct analyses with naked-eye detection. Usually, the step of dilution and separation tools such as chromatography and electrophoresis are required for these samples. Limit of linearity, analytical sensitivity, and limit of detection were 70.00% v/v ethanol to water, -0.39, and 1.34% v/v, respectively. MEC stands out in relation to the other methods reported in literature for determination of ethanol in alcoholic beverage and fermentation broth when taking up parameters of wide linearity and low-cost. Indeed, our method is the unique that ensure precise determinations without instrumental detection, requiring only naked eye for detection. It represents a remarkable aspect for point-of-use measurements. Conversely, MEC is not applicable for trace chemical analyses because its poor limit of detection.

Introduction

Point-of-use platforms have emerged like an important area of the quantitative analytical sciences in the recent years. Such methods enable cheap, rapid, portable, and user-friendly assays bypassing the need for qualified operators.[1] Rapid testing methods contribute for accomplishment of in-situ experiments. This type of assay enhances the capacity to take fast decisions presenting high social and economic implications at industry, environment, and medicine.[2-9] Commonly employed point-of-use platforms rely on colorimetric reactions on paper. Herein, the detection is conducted by scanner, mobile phone, or smartphone whereas the assays are conducted through three assemblies, namely: dipstick, lateral flow, or microfluidics.[6,9] Meanwhile, such methods generate a somewhat poor precision regarding the sensitivity and specificity.[10] It is because different paper substrates are employed to construct the device, affecting the flow rates and interactions with analytes.

One powerful alternative to overcome the precision-related downsides is to make the experiments in solution. In addition, the solution-based detection relies on disposable systems and it allows the detection of diverse analytes according to review recently published by Paterson and de la Rica.[10] Such a wide application is stemming from the employment of modified nanomaterials. These structures facilitate the monitoring of colours after small changes on analyte content with the naked eyes as well. It bypasses the use of electronic readers improving the potentiality of method for in-situ analytical measurements.

In this paper, we specifically focus on further investigations into a rapid testing output that was recently proposed by these authors.[11] Their preliminary data were promising with respect to the deployment of point-of-use platforms by using solution-based-detection with naked-eyes. Conversely, our approach is not based on colorimetric chemical reactions[10] but in thermodynamics of colloids. Called the MEC (microemulsification-based method), it relies on effect of the analyte over the entropy of emulsions or Winsor systems (discussed below). It affects the formation of thermodynamically stable dispersions, the microemulsions (MEs). The analytical signal in MEC was the minimum volume fraction of amphiphile (AP) necessary to generate ME (Φ_{ME}) for a fixed ratio of water and oil. Herein, the production of nanodroplets in ME (transparent) ensures the visual detection of Φ_{ME} by monitoring the change of turbidity from heterogeneous dispersions (cloudy), emulsions or Winsor systems, as shown in **Fig. 1**.

Unlike the colorimetry wherein the response depends on increase/decrease in colouration or changes in tonality of a coloured medium, the measurements of Φ_{ME} are based on a binary chemical information: the cloudy-to-transparent transition that occurs with microemulsification. This conversion acts like a turning point in titration processes. Thus, the signal in MEC can be precisely detected with naked eye. This feature ensures not only screening analyses (positive/negative results) like the most of colorimetry rapid testing platforms,[10] but also precise quantitative experiments.[11] In colorimetry, there is a subjective uncertainty by personal and surrounding conditions in recording the colours with naked eyes.[12]

MEC meets the requirements for the development of powerful point-of-use tools. This method is simple, fast, cheap, and portable.

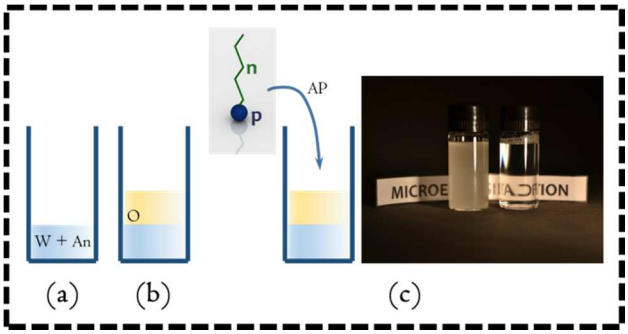

Fig. 1 MEC experimental routine when the solvent is polar. Preparation of solution of analyte (W + An) in polar solvent **(a)**, addition of oil (O) under a specific volume ratio **(b)**, and addition of amphiphile (AP) generating initially heterogeneous dispersions (photo on the right) and, then, microemulsions (photo on the left) by vigorously shaken the W-O mixtures **(c)**. Minimum volume fractions of AP needed to get microemulsion ($\mathbf{\Phi_{ME}}$) were used to plot the analytical curve since such a parameter depends on analyte contents. For application to samples, the routine is similar. Herein, meanwhile, the sample is applied directly as W phase, "W + An" in **(a)**. The symbols 'n' and 'p' are the non-polar and polar groups of amphiphile, respectively.

Besides, it allows precise determinations with a strong analytical performance taking up figures of merit such as precision, linearity, robustness, and accuracy. MEC still can operate with small sample volumes; values on the order of 20 µL for dispersion are enough to assure the visual detection of $\mathbf{\Phi_{ME}}$.[11]

The preliminary data obtained by MEC for analysis of water in ethanol fuels and monoethylene glycol in liquefied natural gas were precise, accurate, and robust regarding the deviations in dispersion preparing and temperature.[11] Further studies into the robustness of MEC are described herein. This parameter was also investigated as a function of changes in ionic strength for analysis of ethanol in water. In addition, this paper addresses a new application to study the reliability of method: the determination of ethanol in complex samples of sugar cane fermentation broths. Finally, we performed a comparative study between our method and some techniques for such an application from the literature.

Ethanol is the most produced biofuel in world with a production of around 93 billion litters in 2014 from fermentation of renewable sources like sugarcane, beet, and corn.[13] Alcoholic fermentation is a key step in production of ethanol biofuel because diverse parameters may interfere on action of yeasts. These parameters are: change of temperature and pH, poor building up of nutrients, presence of toxic species, and ethanol in excess.[14] Thereby, the monitoring of ethanol in broths during the production of ethanol fuels is relevant to detect unconformities and provide a high efficiency of production.[15]

The fermentation broth and wine samples are complex presenting diverse species such as ions, sugars, and alcohols. These samples usually require indirect methods for accurate determination of ethanol due to chemical complexity. Herein, outputs applied for ensuring selective analyses include HPLC,[16] electrophoresis,[17] and gas diffusion separation[18] with, in general, electrochemical or optical detection. Astoundingly, we present in this paper the direct determination of ethanol in fermentation broth of sugar cane by employing just MEC with visual detection. It bypassed even the dilution of samples.

Experimental methods

Chemicals

Ethanol, NaCl, and Na_2SO_4, and $CaCl_2$ were supplied from Merck (Whitehouse Station, NJ) whereas oleic acid was obtained from Labsynth (São Paulo, Brazil). Particles of SiO_2 and $FeCl_3$ were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Deionized water (Milli-Q, Millipore Corp., Bedford, MA) was attained with resistivity no less than 18 MΩ cm.

Microemulsification

Experimental protocol of MEC depends on the nature of sample. According to its polarity, the analyte can be added in water (W), AP, or oil (O) phase for microemulsification process so MEC is applicable to polar, nonpolar, and amphiphile samples. The media tested herein are polar. In this case, W phase solutions are initially prepared changing the content of analyte standard is added in polar solvent. Such solutions are utilized to prepare W-O mixtures under a specific volume ratio. Succeeding, the generation of ME is conducted by transferring pure AP until cloudy-to-transparent transition. Detection of $\mathbf{\Phi_{ME}}$ was performed with naked eyes. Dispersions were attained in glass bottles or Eppendorf® tubes with the aid of micropipettes by vigorously shaken W-O mixtures after adding the AP. All of the measurements with MEC were made with $n = 4$ for each content of analyte. Value of $\mathbf{\Phi_{ME}}$ were obtained by gradually adding the amphiphile in a unique bottle containing the W-O mixture. The first attempt in finding $\mathbf{\Phi_{ME}}$ took approximately 4 min and it was intended only to get an approximate value of analytical signal. The other attempts, in turn, lasted less than 1 min. Continuing, the analytical curve is then constructed by relating the responses with the diverse analyte concentrations. For application, the microemulsification relies on employment of the sample directly as W phase. Finally, the analyte content is obtained by direct interpolation using the linear regression line equation fitted by least squares method. **Fig. 1** exhibits the MEC routine when the solvent of sample is polar, case reported in this paper.

Microemulsifications were performed at room temperature (23 °C) whereas the dispersions were composed of water (W), oleic acid (O), and ethanol hydrotrope (AP phase). The analyte was ethanol, added in W phase to get the analytical curves as described above. W-O mixtures had a total volume of 600 µL with 50.00% v/v oil to water ($\mathbf{\Phi_O}$). Such a faction does not take up the volume of AP. Lastly, the concentration of analyte was expressed as volume fractions of ethanol to water ($\mathbf{\Phi_E}$).

Robustness test

The level of robustness was expressed by absolute errors calculated for $\mathbf{\Phi_E}$ ($\mathbf{\Delta\Phi}$, % v/v) in determination of ethanol in water. These errors were owing to deviations in volumetric preparation of dispersions and changes in temperature and ionic strength of W phase

To calculate $\mathbf{\Delta\Phi}$, analytical curves were initially achieved taking up (i) relative standard deviations (RSD) of 5.00% and 10.00% v/v in $\mathbf{\Phi_O}$ and several (ii) temperatures and (iii) ionic strengths. The $\mathbf{\Delta\Phi}$ values were related to 7.00%, 15.00%, and 25.00% v/v $\mathbf{\Phi_E}$ at 23 °C without deviations in $\mathbf{\Phi_O}$ and added salt (reference values). In all of the cases, the analytical signals associated to the reference values were used in linear regression equation of each curve to calculate $\mathbf{\Phi_E}$ for the MEs prepared by considering deviations in preparing of W-O mixtures and changes in temperature and ionic strength of W phase.

Temperature-function robustness was assessed by constructing analytical curves in the following temperatures: 18, 20, 23, 26, 29, 31, 33, and 35 °C. This range was selected by considering the usual

temperatures for in-situ tests, even in tropical countries like Brazil. For the robustness in terms of ionic strength (**I**), this parameter was mathematically expressed by conductivity (**κ**). **I** and **κ** are related to each other from equation:[19]

$$\kappa^2 = \frac{2e^2 N_A I}{\varepsilon_0 \varepsilon_r k_B T} \quad (1)$$

wherein **e**, N_A, ε_0, ε_r, k_B, and **T** are elementary charge, Avogadro constant, vacuum dielectric constant, medium dielectric constant, Boltzmann constant, and temperature, respectively. To attain the analytical curves in this case, the W phases were prepared by adding standards of ethanol in different aqueous media, namely: 10.0 and 500.0 mmol L^{-1} NaCl and 10.0 mmol L^{-1} Na_2SO_4, $FeCl_3$, $CaCl_2$, and $NiSO_4$

Application

Values of Φ_E in fermentation broths of sugar cane were determined by means of the analytical curve method. Samples were provided by Brazilian Bioethanol Science and Technology Laboratory (CTBE). Such samples were characterized as regards to presence of acetic, succinic, and lactic acids, glucose, fructose, sucrose, and glycerol. Their contents are shown in Supporting Information.

The conductivity of samples was measured by AJ Micronal AJX-522 (São Paulo, Brazil). Accuracy was tested by comparing the analyte concentrations determined by MEC and FTIR (Bruker Alpha, Billerica, MA). Student's t tests at 95% confidence level made statistical comparisons among the data recorded by the two methods.

Results and discussion

Analytical curve

Fig. 2 exhibits the analytical curve for standards of ethanol in W phase at 23 °C with 50.00% v/v Φ_O. We found the limit of detection (S/N = 3) was 1.34% v/v. The curve presented a wide linear range with limit of linearity of 70.00% v/v Φ_E and two regions with different analytical sensitivities: -0.39 and -0.88 for Φ_E greater than 40.00% v/v.

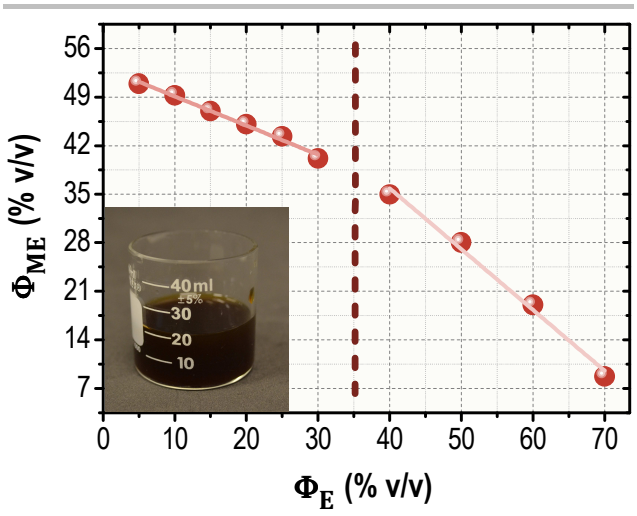

Fig. 2 Analytical curve for standards of ethanol in W phase at 23 °C (50.00% v/v Φ_O) with water-ethanol-oleic acid MEs. Inset: photo of one broth sample. R^2 were 0.9961 and 0.9907 (Φ_E higher than 40% v/v).

Negative deviations in Φ_{ME} are due to progressive addition of ethanol in W phase. It necessitates decreasingly volume fractions of ethanol for microemulsification. The reduce in Φ_{ME} by building up ethanol in W phase relates likely to the increase in surface activity phenomenon. It favours the thermodynamic stabilization of dispersions through the reduction in interfacial tension.[20,21]

Robustness

Assuming the theory of dispersions with non-ionic surfactants, the surface activity in MEs depends majorly on temperature rather than ionic strength. Herein, the temperature acts by modifying π_w and π_o.[20] Once the temperature increases, the π_w values are decreased due to reduction in solvation of polar groups of AP, diminishing the surface activity. Conversely, we observe an enhancement in π_o with temperature because the increase in the conformations of nonpolar groups. It raises the surface activity. Furthermore, this phenomenon changes with temperature and ionic strength because deviations in monomeric solubility of AP. It alters the surface activity by modifying the fraction of amphiphile adsorbed at W-O interfaces.

Considering the discussion above, Φ_{ME} do not show a simple and generic relationship with the parameters of temperature and ionic strength. In relation to deviation in W-O ratio, it affects the Φ_{ME} because the changes in π_w and π_o as well.

For the procedure of preparation of W-O mixtures, the $\Delta\Phi$ values were -1.23% (7.50% v/v), -1.54% (15.00% v/v), and -1.91% v/v (25.00% v/v Φ_E) considering 5.00% v/v RSD. Such errors for 10.00% v/v RSD, in turn, were -2.38% (7.50% v/v), -2.33% (15.00% v/v), and -2.28% v/v (25.00% v/v Φ_E). Analytical sensitivities, in turn, were -0.40 and -0.39 for 5.00% and 10.00% v/v RSD, respectively. Analytical curves obtained in this case are shown in Supplementary Information.

Resulting curves relative to temperature-function robustness as well as the obtained values of $\Delta\Phi$ are depicted in **Fig. 3**. Confidence intervals (n = 4) ranged from 0.11 to 0.39% v/v Φ_{ME}.

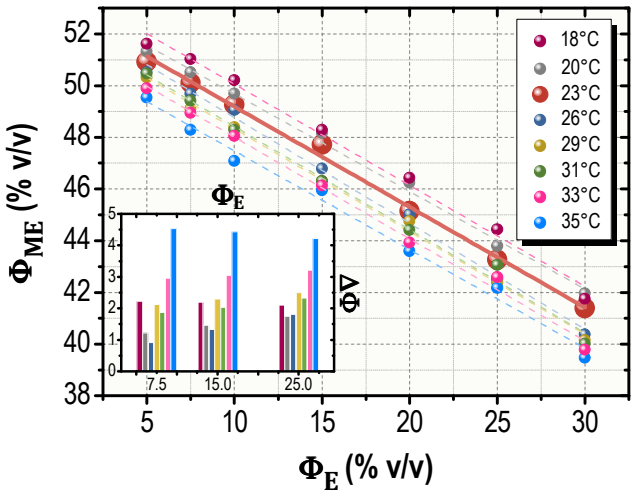

Fig. 3 Analytical curves for standards of ethanol in W phase at different temperatures to investigate the robustness using water-ethanol-oleic acid MEs. Inset: values of $\Delta\Phi$ as a function of Φ_E in three regions of analytical curves (7.50%, 15.00%, and 25.00% v/v Φ_E) for the changes of 23 to 18 °C, 23 to 20 °C, 23 to 26 °C, 23 to 29 °C, 23 to 31 °C, 23 to 33 °C, and 23 to 35 °C. All of the R^2 values were equal to or larger than 0.99. **S** values, in turn, were: -0.39 (18), -0.38 (20), -0.39 (23), -0.41 (26), -0.40 (29), -0.40 (31), -0.39 (33), and -0.38 (35 °C). $\Delta\Phi$ is in module; the values for temperatures greater than 23 °C were negative.

$\Delta\Phi$ had values between 0.91 and 4.54% v/v. Errors calculated for temperatures greater than 23 °C were negative whereas the values for 18 and 20 °C were positive. The analytical sensitivity remained almost constant with the heating of MEs. Their values are shown in legend of **Fig. 3**.

The analytical curves achieved by adding salts in W phases as well as the values of $\Delta\Phi$ are shown in **Fig. 4**. Confidence intervals (n = 4) changed of 0.09 to 0.17% v/v Φ_{ME}. Herein, the absolute errors ranged from 0.01 to 2.90% v/v. Even though the biggest values of $\Delta\Phi$ were recorded for the more conductive solutions (containing 500 mmol L^{-1} NaCl), the conductivity of the W phases did not present a systematic effect over robustness. We did not observe, for example, the lowest absolute errors for data obtained with 10 mmol L^{-1} NaCl, less conductive media. Conductivities of W phase solutions are portrayed in Supplementary Information. Their values ranged from 0.5 to 38.9 mS cm^{-1}. The lowest errors were attained for 10 mmol L^{-1} Na_2SO_4, with values among 0.01 and 0.35% v/v. The W phases based on 10 mmol L^{-1} $FeCl_3$ had values of κ higher than those for solutions with 10 mmol L^{-1} Na_2SO_4 and $CaCl_2$. These phases exhibited similar conductivities. Herein, the MEs prepared in $FeCl_3$ did not show the biggest $\Delta\Phi$ that ranged from 0.71 to 0.86% v/v. Only the errors recorded for 10 mmol L^{-1} NaCl and $CaCl_2$ salts were negative. Lastly, analytical sensitivities remained almost invariable again as described in legend of **Fig. 4**. Indeed, the data of robustness of MEC did not present a systematic dependence on conductivity of dispersions.

Robustness is crucial for deployment of point-of-use analytical platforms. Such a parameter was somewhat acceptable for in-situ assays depending on conditions of temperature and conductivity as well as the needed levels of accuracy. High-accuracy experiments may require the employment of analytical curves based on specific either temperature or ionic strength to provide successful analyses. Once the analytical sensitivities remained practically constant for deviations in theses parameters, the correction of resulting data of concentration could be easily performed by taking only the linear coefficients of analytical curves.

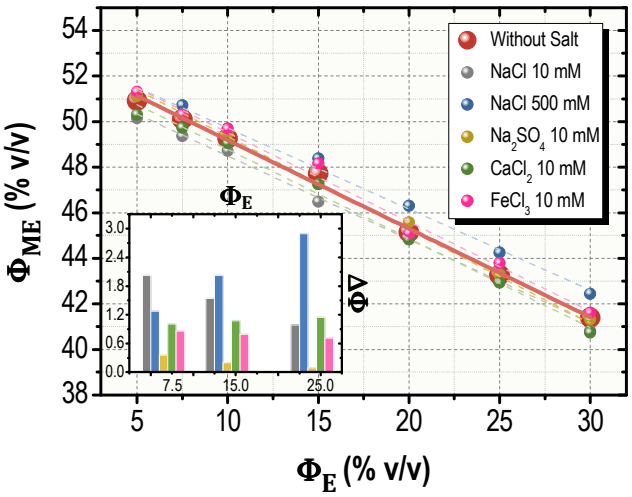

Fig. 4 Analytical curves for standards of ethanol in W phase at different ionic strengths to investigate the robustness using water-ethanol-oleic acid MEs. Inset: values of $\Delta\Phi$ as a function of Φ_E in three regions of analytical curves (7.50%, 15.00%, and 25.00% v/v Φ_E) changing the κ of W phase by adding ethanol in aqueous media, namely: 10.0 (gray) and 500.0 mmol L^{-1} (blue) NaCl and 10.0 mmol L^{-1} Na_2SO_4 (yellow), $CaCl_2$ (green), and $FeCl_3$ (pink). Other samples were visually similar to that shown herein. All of the values of R^2 were larger than 0.99. The values of analytical sensitivity, in turn, were: -0.39 (without salt, see **Fig. 2**), -0.37 (10.0 mmol L^{-1} NaCl), -0.36 (500.0 mmol L^{-1} NaCl), -0.40 (10.0 mmol L^{-1} Na_2SO_4), -0.39 (10.0 mmol L^{-1} $CaCl_2$) and -0.39 (10.0 mmol L^{-1} $FeCl_3$). $\Delta\Phi$ is in module; some values were negative as highlighted in main text.

Application

We used spectroscopy and chromatography to characterize the samples (photo exhibited in **Fig. 2**) as regards to the presence of sugars (glucose, fructose, and sucrose), acids (acetic, succinic, and lactic) and glycerol. Resulting data are stressed in Supplementary Information. Most abundant species were acid acetic and glycerol with concentrations on the order of 22 and 9 g L^{-1}, respectively.

Direct determinations by using the analytical curve (**Fig. 2**) were performed to the samples. The results are presented in **Table 1**. It shows also the conductivity of the samples. Wide linear range of MEC bypassed the step of dilution of the samples contributing for accuracy. Indeed, the fractions of Φ_E were in agreement with the data of FTIR. As described earlier, the samples herein investigated usually require indirect techniques and instrumental detection for accurate determination of ethanol. Thus, the data portrayed in **Table 1** are promising towards deployment of MEC-based analytical rapid tests.

Comparative study

Important features of different methods reported in literature for determination of ethanol in alcoholic beverage and fermentation broth are shown in **Table 2**. MEC stands out in relation to the other when taking up parameters of wide linearity and low cost. Further, this method is the unique that ensures precise experiments without instrumental detection, requiring only naked eye for detection. It is a remarkable aspect for point-of-use tests. Apart from this feature, the cost of MEC-based kits for chemical measurements would be significantly low because only the consumables would affect such a cost. Herein, we should to highlight volumes as reduced as 20 µL for final dispersions are enough for accomplishment of MEC.[11] Concerning analytical frequency, a powerful way to improve such a figure of merit concerns the employment of microfluidics (with optical detection to detect Φ_{ME}). Further advantages are resulting from conversion of bulk to microscale analyses, including decrease in chemical consumption (femto to nanoliter) and improvement in reproducibility.[23]

Conclusions

In summary, the findings reported herein represent a remarkable breakthrough in understanding and making the MEC a powerful platform for the development of point-of-use technologies. The method was somewhat robust as regards to changes in temperature and ionic strength and deviations in preparation of W-O mixtures

Table 1 Concentrations of ethanol (Φ_E, % v/v) in fermentation broths of sugar cane (B_1-B_4; a photo of one sample is exhibited in inset of **Figure 3**) determined by FTIR (n = 3) and MEC (n = 4)

Samples	FTIR (% v/v)	MEC (% v/v)	κ (mS cm^{-1})
B_1	15.5±0.1	15.9±0.3	5.3
B_2	15.5±0.2	15.8±0.5	5.2
B_3	15.6±0.2	16.1±0.4	5.3
B_4	15.7±0.1	15.9±0.3	5.3

Table 2 Comparison of some analytical figures of methods for ethanol determination in samples of alcoholic beverages and fermentation broths

Method	Sample	LOD (% v/v)	LOL (% v/v)	Analytical frequency	Sample dilution	Cost	Portability	Reference
FIA-GD-Amperometry on Copper oxide electrode	Alcoholic beverages	0.4	10.0	120 h^{-1}	1-fold	Medium	No	22
Microchip Electrophoresis	Beer/Wine	3.49x10^{-3}	0.029	18 h^{-}	50, 100-fold	Medium	Yes	17
Gas Chromatography	Alcoholic beverages	N.I.	63.3	5 h^{-1}	None	High	No	24
Amperometric Biosensor	Alcoholic Beverages	7.5x10^{-5}	8.7x10^{-3}	N.I.	~80-fold	High	Yes	25
FIA-GD-Spectrophotometry	Wine/Molasses	0.18	25.0	29 h^{-1}	5, 9-fold	Medium	No	26
SIS-GD-ADH-Spectrophotometry	Wine	0.05	25.0	21 h^{-1}	3.5-fold	High	No	27
GD-Voltammetry	Fermentation Broth	0.18	20.0	10 h^{-1}	1.5-fold	Medium	Yes	18
MEC	Fermentation Broth	1.34	70.0	12 h^{-1}	None	Low	Yes	This paper

N.I.: Not informed;
FIA: flow injection analysis;
GD: gas-diffusion separation;
SIS: sequential injection system;
ADH: alcohol dehydrogenase.

for determination of ethanol in water. Reliability of the method was satisfactory taking into account direct analyses of ethanol in complex samples of fermentation broths of sugar cane. Accurate measurements were possible without even step of dilution. For this application, dilution and separation methods like chromatography and electrophoresis are usually needful.

Taking up the conclusions, our data create new perspectives for point-of-use analysis by using the MEC, an approach based on colloid thermodynamics and solution detection. Deployed method ensures precise determinations bypassing subjective uncertainties by personal and surrounding conditions concerning the detection of analytical signal with naked eye. MEC has also a wide linear range and high robustness, essential parameters for in-situ assays. Furthermore, our method does not present drawbacks related to instability of chemicals like enzymes because MEC relies on use of solvents only beyond analytes. Conversely, it is worthwhile to highlight that such a platform is not applicable for trace chemical analyses because its poor limit of detection. Herein, tools based on spectroscopy or electrochemical, for instance, are recommended. In addition, developing methods to improve MEC selectivity is a key aspect that should be addressed in next investigations. Diverse species may change the media interfacial tension in other complex sample-base applications, thus generating interference on method. Thus, coupling of MEC with techniques like solid phase extraction should enlarge the employment range of MEC.

Acknowledgments

Financial support from *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP, Grant No. 2014/24126-6) is gratefully acknowledged. CTBE is recognized for providing and charactering the samples of fermentation broth.

Nomenclature

Φ_{ME} minimum volume fraction of amphiphile needed to get ME;
Φ_O volume fraction of oil to water;
Φ_E volume fraction of ethanol to water;
$\Delta\Phi$ absolute error determined for Φ_E;
κ conductivity;
I ionic strength;
γ_i interfacial tension.

Notes and references

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† Electronic Supplementary Information (ESI) available details about: *i)* analytical curves related to robustness test; *ii)* conductivity of W phases employed in robustness test, and *iii)* composition of samples.

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Microemulsification-based method:
analysis of ethanol in fermentation broth of sugar cane

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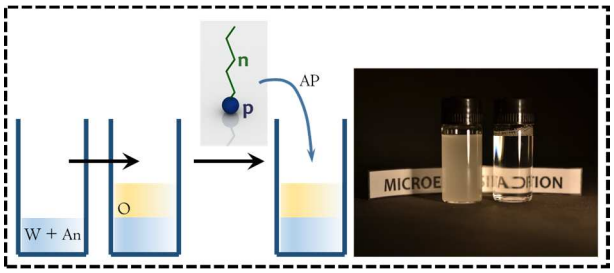

Nanodroplets in thermodynamically stable dispersions allows naked eye determinations. An: analyte; W, O, and AP; water, oil, and amphiphile, respectively.