

Microemulsification-Based Method: Coupling with Separation Technique

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Abstract

The outcomes described herein outline the potentiality of the microemulsification-based method (MEC) for development of rapid testing (point-of-use) technologies. MEC was recently proposed by these authors for analytical determinations wherein the detection is conducted in solution with naked eyes. It relies on effect of analyte over the colloid thermodynamics by changing the minimum volume fraction of amphiphile needed to generate microemulsions (MEs) (Φ_{ME}), which represents the analytical response of the method. We report in this paper the successfully coupling of MEC-based detection with gas diffusion separation. Such result extends the field of application of MEC in analytical sciences by improving its selectivity. One custom-designed module was constructed on PTFE for the separation measurements. It was utilized in combination with MEC for determining water in ethanol fuels using water/n-propanol/oleic acid MEs and water-rich compositions. In this situation, accurate direct determinations by MEC are not possible. In addition, further studies on analytical performance and robustness of MEC by using n-propanol amphiphile are described. The method was robust as regards to deviations in dispersion preparing and changes in temperature. Concerning the analytical performance, the analytical curves presented wide linear range with limits of linearity of up to 70.00% v/v ethanol to water (Φ_E). The limits of detection (S/N=3) were of 1.03%, 7.21%, and 0.68% v/v Φ_E for compositions with water- (region A) and oil-rich (region C) domains as well as equal volumes of water and oil phases (region B), respectively. With respect to the regions A and B, the analytical performance stressed herein exhibited best linearity and comparable sensitivities when compared to these levels reached with ethanol amphiphile (our first publication on MEC) rather than n-propanol.

Keywords: Point-of-care; Rapid test; Colloid; Interfacial tension; *In situ* analysis

Abbreviations: ME: Microemulsion; AP: Amphiphile; W: Hydrophilic phase of dispersions; O: Hydrophobic phase of dispersions; 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; Φ_W : Volume fraction of water to ethanol; $\Delta\Phi$: Absolute error determined for Φ_E

Point-of-use devices represent currently a key field in quantitative analytical sciences. These platforms are low-cost, fast, portable, and simple to use eliminating the necessity for qualified operators [1]. Rapid tests enable in-situ measurements presenting substantial social and economic implications at industry, environment, and medicine [2-9]. One potential output to perform point-of-use analyses is the accomplishment of the tests in solution with naked eye detection using disposable systems. It allows the determination of different analytes from the use of modified nanomaterial [10]. Naked eye methods bypasses the use of instrumental readers, an essential feature for in-situ technologies. Furthermore, the analyses in solution surpass precision-related downsides when making the tests on substrates such as paper [6,9]. In this case, the diverse paper substrates that are employed to fabricate the devices affect the flow rates and interactions with analytes [10].

This paper reports further investigations and application of the microemulsification-based method (MEC), a point-of-use platform that was recently proposed by these authors [11]. It relies on solution-based-detection with naked eyes. In contrast with colorimetric tools [10], MEC response depends on colloid thermodynamics by relying on effect of analyte on the entropy of emulsions or Winsor systems. It changes the formation of thermodynamically stable dispersions, the microemulsions (MEs). The minimum volume fraction of amphiphile

(AP) needed to get MEs (Φ_{ME}) for a fixed water-oil ratio expressed the analytical signal of the method. The generation of nanodroplets in MEs (transparent) allows the naked eye detection of Φ_{ME} by monitoring the change of turbidity from the emulsions or Winsor systems (cloudy) as shown in Figure 1a. This cloudy-to-transparent conversion acts like a turning point in titrations, ensuring the visual measurement of Φ_{ME} and, therefore, not only screening analyses (positive/negative data) as the most of naked eye colorimetry platforms [10] as well as precise quantitative analyses [11]. The response in colorimetry changes with the intensity of colour or tonality. Herein, subjective uncertainties by personal and surrounding conditions are observed [12].

MEC presents powerful aspects concerning the deployment of point-of-use tools. Such a method is straightforward, cheap, fast, portable, and provides precise analytical determinations with satisfactory precision, linearity, robustness, and accuracy. Lastly, volumes of approximately 20 μ L for dispersions assure the visual measurement of Φ_{ME} [11]. It contributes for a low sample consuming.

The first outcomes achieved by MEC were promising with respect to its analytical performance [11]. Direct analyses based on analytical

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Received July 07, 2015; Accepted July 21, 2015; Published July 28, 2015

Citation: Giordano GF, Higa KM, Santinom A, Gobbi AL, Kubota LT, et al. (2015) Microemulsification-Based Method: Coupling with Separation Technique. J Anal Bioanal Tech 6: 261 doi:10.4172/2155-9872.1000261

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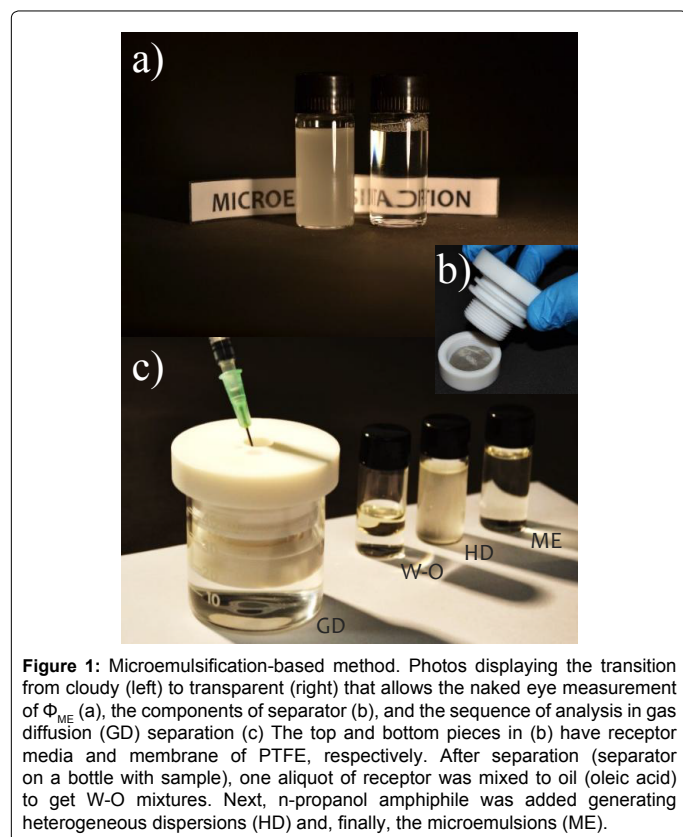


Figure 1: Microemulsification-based method. Photos displaying the transition from cloudy (left) to transparent (right) that allows the naked eye measurement of Φ_{ME} (a), the components of separator (b), and the sequence of analysis in gas diffusion (GD) separation (c). The top and bottom pieces in (b) have receptor media and membrane of PTFE, respectively. After separation (separator on a bottle with sample), one aliquot of receptor was mixed to oil (oleic acid) to get W-O mixtures. Next, n-propanol amphiphile was added generating heterogeneous dispersions (HD) and, finally, the microemulsions (ME).

curves were conducted for determining monoethylene glycol in samples associated to the processing of liquefied natural gas and water in ethanol fuels. For this latter case, accurate direct determinations were obtained by employing oil-rich MEs and dispersions containing similar volumes of the water (W) and oil (O) phases. Nonetheless, accurate direct determinations were not verified when using water-rich MEs. Considering the excess of water in this composition, we supposed these results were because the ionic strength of the samples of ethanol fuel. Studies addressed in literature revealed the presence of NO_3^- , K^+ , Ca^{2+} (0.5 to 3.5 mg L^{-1}) [13], Cu^{2+} , Zn^{2+} , Ni^{2+} , and Fe^{3+} (8 to 57 mg L^{-1}) in these samples [14].

We describe herein accurate determinations of water in ethanol fuels by MEC utilizing water-rich MEs. For this, the method was coupled with gas diffusion separation. It represents an important breakthrough by improving the reliability and selectivity of MEC, enlarging its range of application. In addition, further studies into analytical performance and robustness of MEC by using n-propanol AP are described. The robustness level was investigated as a function of deviations in dispersion preparing and changes in temperature.

Materials and Methods

Chemicals

Ethanol and n-propanol alcohols were supplied from Merck (Whitehouse Station, NJ) whereas oleic acid was obtained from Labsynth (São Paulo, Brazil). Deionized water (Milli-Q, Millipore Corp., Bedford, MA) was attained with resistivity no less than 18 $\text{M}\Omega \text{ cm}$.

Microemulsification

MEC routine relies on nature of sample. The analyte can be added in W, AP, or O phase for generation of MEs according to the sample polarity. As a consequence, MEC is applicable to polar, nonpolar, and amphiphile media. When the analyte is dipped into W phase, for instance, W phase solutions are initially obtained changing the concentration of analyte that is added in polar solvent. These solutions are used to attain W-O mixtures for a fixed volume ratio. Following, the microemulsification is made by adding pure AP until cloudy-to-transparent transition. The measured values of Φ_{ME} are, then, employed to construct the analytical curve. For application, the sample acts directly like W phase with the intent to stabilization of the dispersions. Finally, the analyte content is obtained through the linear regression line equation.

The detection of Φ_{ME} was conducted with naked eyes. Dispersions were prepared in Eppendorf tubes with the aid of micropipette at room temperature (23°C). AP-added W-O mixtures were vigorously shaken for microemulsifications. The values of Φ_{ME} were detected by gradually transferring the amphiphile in a unique tube with the W-O mixture. The first attempt in finding Φ_{ME} was intended only to obtain an approximate analytical response. This step took approximately 5 min whereas the other attempts lasted less than 2 min.

Dispersions were composed of water (W), oleic acid (O), and n-propanol hydrotrope (AP phase). Ethanol analyte was added in W phase to get the analytical curves as the previously described experimental procedure. Such a parameter does not take into account the AP volume. The fraction of analyte was expressed by the volume fraction of ethanol to water (Φ_E).

Phase behavior

Φ_{ME} was obtained in diverse compositions of water/n-propanol/oleic acid MEs to attain a ternary phase diagram and, thus, to evaluate the phase behavior of dispersions. For this, the W-O mixtures were prepared using different volume fractions of oil to water (Φ_O). W-O mixtures had a volume of 600 μL in all of the measurements addressed in this paper.

Analytical performance and robustness level

Analytical curves were obtained to compare the n-propanol-observed results with those achieved by ethanol amphiphile (data in our first publication) [11].

The curves ensured the (i) calculation of merit figures (correlation factor, R^2 , analytical sensitivity, and limit of detection, LOD) in determination of water in ethanol fuels and the (ii) evaluation of robustness level as discussed below by establishing a relationship between Φ_{ME} signal and Φ_E content. Confidence intervals for each concentration level were calculated for $\alpha=0.05$ and $n=4$ in all of the cases. In both tests of analytical performance and robustness, analyses were conducted for three compositions of W-O mixture, namely: water- (region A) and oil-rich (region C) domains and dispersions based on equal volumes of W and O phases (region B). W-O mixtures were prepared with 5.00% (A), 50.00% (B), and 95.00% v/v Φ_O (C).

The robustness was mathematically expressed by absolute errors calculated for Φ_E ($\Delta\Phi$, % v/v) in determination of ethanol in water. These errors were because deviations in preparation of W-O mixtures and alterations in temperature.

Analytical curves were obtained to calculate $\Delta\Phi$ by taking up (i)

relative standard deviations (RSD) of 5.00% and 10.00% v/v in Φ_O and (ii) diverse temperatures, namely: 20, 23, 26, and 29°C. $\Delta\Phi$ values were related to 7.00%, 30.00%, and 60.00% v/v Φ_E at 23°C without alterations in Φ_O (reference values). Φ_{ME} signals related to the reference values were employed in the linear regression equations of all of the analytical curves in order to calculate Φ_E -considering deviations in dispersion preparing and changes in temperature for regions A, B, and C.

Application

MEC was coupled to gas diffusion separation seeking to improve its accuracy. This separation consists of separating volatile species from sample (donor media) to a receptor solution through gas permeable membranes [15]. Usually adopted membranes are hydrophobic and microporous. During the volatilization, a gas thin layer is stored in the micropores. Afterwards, the analyte diffuses through this layer that separates the donor and receptor solutions.

One custom-designed separation module was constructed on PTFE as approached in Figure 1b. It was utilized for determination of water in ethanol fuels using water-rich MEs (region A: 5.00% v/v Φ_O). The routine of analysis is generically shown in Figure 1c. To construct the analytical curve, deionized water (receptor) and PTFE membrane were placed inside the separation module. We inserted such a module manually on an external glass bottle containing ethanol standard (donor medium). The hydrophobic membranes were in contact with the donor and receptor solutions. The volumes of donor and receptor were 17 and 2 mL, respectively. Separations were based on diffusion of ethanol gas through membrane from donor to receptor [15]. The tests were made at room temperature (23°C) under two conditions of time: 5 and 20 min. To accelerate the homogenization of volatilized ethanol in receptor medium, a magnetic bar was inserted inside the extraction module. The stirrer was placed under the bottle with sample whereas the stirring was conducted with 500 rpm rotation speed. Subsequently, MEC was accomplished (protocol addressed above) using receptor water, oleic acid, and n-propanol as W, O, and AP phases of the dispersions, respectively, with 5.00% v/v Φ_O (570 μ L of receptor and 30 μ L of oleic acid to get W-O mixtures). Lastly, the microemulsification processes were conducted by adding n-propanol. Analytical curve was expressed in terms of Φ_E . This parameter allowed us to indirectly calculate the volume fractions of water to ethanol fuel (Φ_w). For application, the samples were dipped into the glass bottles (donor media) acting as W phase.

The conductivity of samples was measured by AJ Micronal AJX-522 (Sao Paulo, Brazil). Statistical evaluation between the data attained by MEC and Karl Fischer titration (Metrohm, Titrando 890, Herisau, Switzerland) was made by Student's t-tests at 95% confidence level to assess the accuracy.

Results and Discussions

Phase behaviour

Phase diagram of dispersions composed of water/n-propanol/oleic acid at 23°C is exhibited in Figure 2. The regions above and below the binodal curve correspond to MEs and unstable dispersions, respectively. Average values of Φ_{ME} ($n=4$) were used to plot the diagram with confidence intervals of 0.18 up to 0.39% v/v.

Ethanol hydrotrope was employed as AP phase in our previous investigations [11]. Taking up the phase behavior for water/ethanol/oleic acid at 23°C, n-propanol AP generated an efficiency higher (it requires less Φ_{ME} for microemulsification) than that obtained with

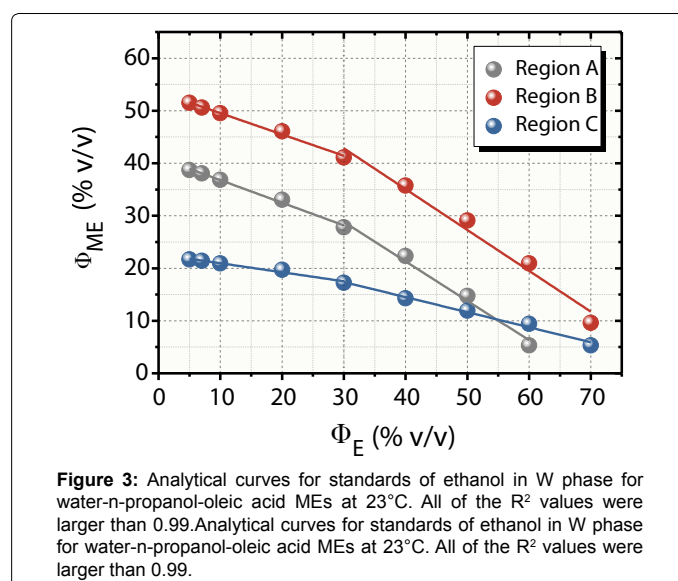
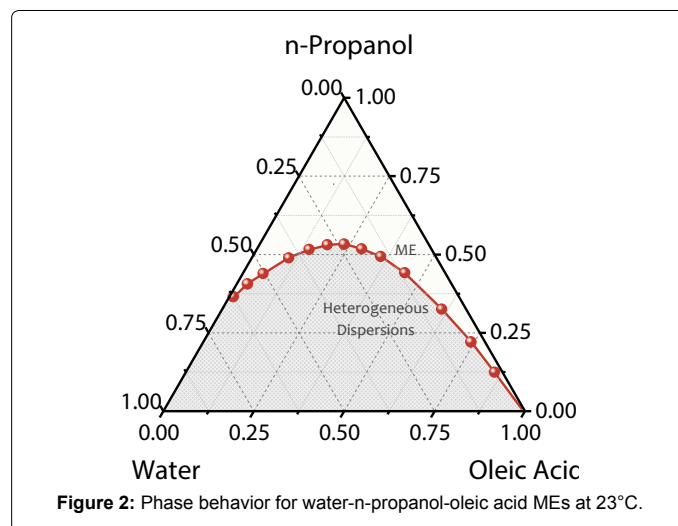
ethanol in the region of water-rich dispersions. In this region, the global average of Φ_{ME} for n-propanol was approximately 80% of the value attained with ethanol. It is in accordance with Traube's rule that defines an increase in surface activity with the size of AP hydrophobic chain, reducing the interfacial tension values [16]. Accordingly, the decrease observed in Φ_{ME} for n-propanol was expected. For MEs based on oil-excess and similar volumes of W and O phases, the efficiency was almost the same for ethanol and n-propanol amphiphiles.

The efficiency results achieved for water-excess dispersions outline an advantage as regards to the employment of n-propanol as AP phase rather than ethanol. In addition, the wide availability and low cost of this solvent in countries like United States represent an important feature regarding the successfully use of n-propanol in MEC.

Analytical performance

Figure 3 shows analytical curves for standards of ethanol in W phase at 23°C in the A, B, and C regions. The curves presented wide linear range with limits of linearity of 70.00% v/v Φ_E for B and C and 60.00% v/v Φ_E for A.

The regions A and B presented the best sensitivities and



detectabilities. All of the curves had an increase in analytical sensitivities upon 30.00% v/v Φ_E . Their values were: -0.43 and -0.75 for A, -0.41 and -0.78 for B, and -0.17 and -0.29 for region C. Lastly, the LODs ($S/N=3$) were of 1.03%, 0.68%, and 7.21% v/v Φ_E for A, B, and C regions, respectively. Confidence intervals ranged from 0.09 to 0.40% v/v.

It is important to highlight the differences between the tests for determining water in ethanol fuel using ethanol (our previous publication) [11] and n-propanol AP (this paper). In first case, the analyte in analytical curves was water that was added in AP instead W phase. The curves correlated Φ_{ME} and Φ_w . For application, the samples acted as AP. In addition, the O phase was chlorobenzene, a much more hazardous solvent than oleic acid as regards to safety and environment risks.

With respect to the regions A and B, the analytical performance stressed herein exhibited best linearity and comparable sensitivities when compared to these levels reached with ethanol AP [11]. In contrast, meanwhile, the data attained with ethanol were best for region C, presenting limit of linearity of 70.00% v/v Φ_w , analytical sensitivity of 2.04, and LOD equal to 0.32% v/v Φ_w .

The negative deviations in Φ_{ME} shown in Figure 3 are due to progressive addition of ethanol in W phase, requiring decreasingly AP volumes for microemulsifications. Such a decrease in Φ_{ME} by building up ethanol in W phase relates to the increase in surface activity phenomenon. It favours the thermodynamic stabilization of the dispersions by diminishing the interfacial tension [17].

Robustness

This parameter is essentially crucial for the development of point-of-use technologies by considering the accomplishment of in-situ assays wherein the effect of interferents on analytical response is critical. MEC was satisfactorily robust regarding deviations in preparing of W-O mixtures and changes in temperature.

Assuming the theory of dispersions with non-ionic surfactants, the surface activity depends mainly on temperature. Such a parameter reduces the polar group solvation and increases the number of nonpolar chain conformations of the amphiphile, diminishing and raising the surface activity, respectively [17]. Besides, this phenomenon changes with temperature owing to deviations in AP monomeric solubility. It alters the surface activity by modifying the fraction of amphiphile adsorbed at W-O interfaces. Therefore, Φ_{ME} do not show a simple and generic relationship with the temperature. In relation to deviation in W-O ratio, it affects the Φ_{ME} because the changes in surface pressures which are responsible for decreasing the surface activity and, thus, the interfacial tension.

For the procedure of W-O mixture preparation in which Φ_o was increased by 5.00% and 10.00% v/v, the values of $\Delta\Phi$ were positive for A and B and negative for C, with exception of the errors obtained in relation to 60.00% v/v Φ_E (reference value). The absolute error values changed among -0.31% and +5.39% v/v with a global average (in module) of $1.00 \pm 0.94\%$ v/v ($n=18$) and averages for each region ($n=6$) equal to $1.22 \pm 0.24\%$ (A), $2.85 \pm 1.28\%$ (B), and $1.39 \pm 0.76\%$ v/v (region C). Figure 4 illustrates the curves related to this robustness investigation as well as the calculated values of $\Delta\Phi$ for A, B, and C regions. The confidence intervals ranged from 0.08 to 0.42% v/v Φ_{ME} .

Resulting data showing the temperature-function robustness, in turn, are depicted in Figure 5. Confidence intervals were of 0.09 to 0.56% v/v Φ_{ME} whereas $\Delta\Phi$ ranged from +0.05% up to -9.46% v/v. Its global average (in module) was $1.69 \pm 0.84\%$ v/v ($n=27$) with averages

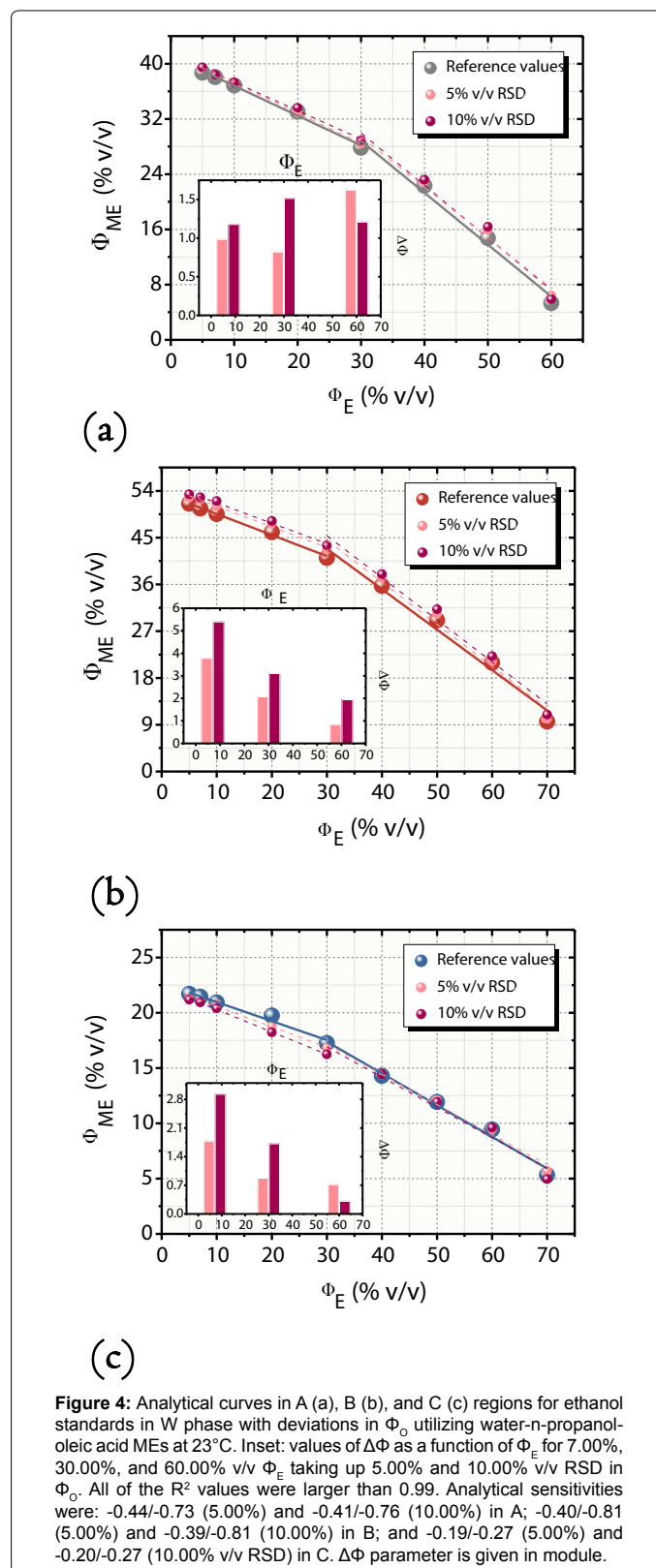
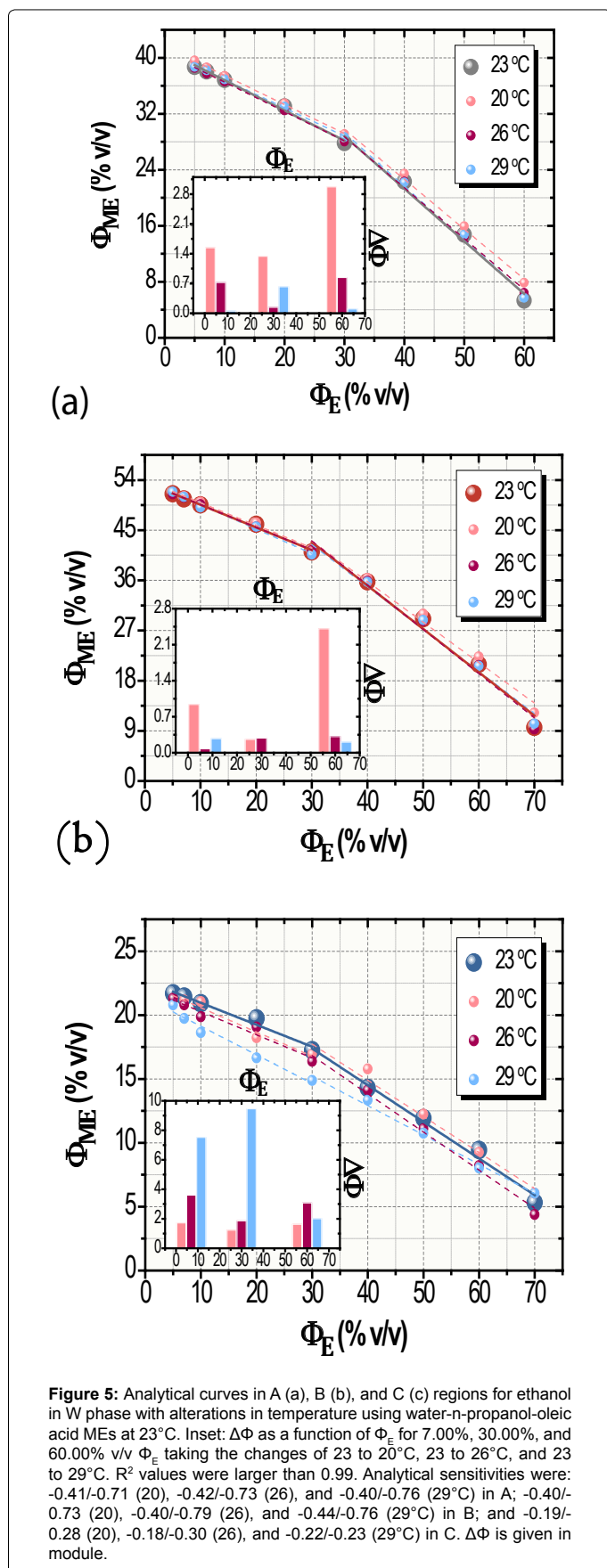


Figure 4: Analytical curves in A (a), B (b), and C (c) regions for ethanol standards in W phase with deviations in Φ_o utilizing water-n-propanol-oleic acid MEs at 23°C. Inset: values of $\Delta\Phi$ as a function of Φ_E for 7.00%, 30.00%, and 60.00% v/v Φ_E taking up 5.00% and 10.00% v/v RSD in Φ_o . All of the R^2 values were larger than 0.99. Analytical sensitivities were: -0.44/-0.73 (5.00%) and -0.41/-0.76 (10.00%) in A; -0.40/-0.81 (5.00%) and -0.39/-0.81 (10.00%) in B; and -0.19/-0.27 (5.00%) and -0.20/-0.27 (10.00% v/v RSD) in C. $\Delta\Phi$ parameter is given in module.

for each investigated region ($n=9$) of $0.92 \pm 0.61\%$ (A), $0.59 \pm 0.47\%$ (B), and $3.56 \pm 1.91\%$ v/v (C). Herein, $\Delta\Phi$ had unsystematically diverse positive and negative values.

In both of the cases of robustness assessing, the analytical



sensitivities remained almost constant when compared to the curve at 23°C without deviations in Φ_O . Indeed, the robustness of MEC was outstanding. All of the values of analytical sensitivity are exhibited in legends of Figures 4 and 5.

Application

One potential factor for changing the signal of MEC is chemical interfering through modification of surface activity phenomenon. For example, a few samples associated to natural gas processing that presented diethylene and triethylene glycol in excess were not successful analyzed by MEC in our group to determine ethylene glycol. Hence, the development of alternatives for improving the MEC accuracy as regards to the presence of chemical interfering is necessary. In this case, a platform integrating gas diffusion separation and MEC detection was applied to test the adulteration of ethanol fuel by water utilizing analytical curve at 23°C and water-rich MEs. According to our preceding publication, direct determinations based on this composition did not generate accurate analyses as aforementioned [11].

The most usual adulteration of ethanol fuel is the excessive addition of water because the attained mixtures are colorless and do not present a distinctive smell. It produces loss of power and raise in fuel consumption rate [18]. Different methods were proposed for determination of water in ethanol fuels such as: i) near infrared spectrometry [19], ii) conductometry [20], iii) enthalpimetry [21], iv) cyclic voltammetry [22], v) photothermal detector [23], vi) ultrasonic propagation velocity [24], vii) evanescent field absorption spectroscopy [25], and viii) colorimetry [18].

The samples of ethanol fuel had $2.9 \pm 0.7 \mu\text{S cm}^{-1}$. Considering this low conductivity, we suppose a decrease in amount of diffused ethanol vapour due to tonometry was not observed.

High concentrations of ethanol in water (donor media) were used to construct the analytical curve by taking two aspects: the low analyte content that is volatilized in gas diffusion separation and the poor detectability of MEC. The resulting analytical curve at 23°C for a separation time of 5 min is portrayed in Figure 6. For Φ_E bigger than 40.00% v/v, the limit of linearity was of 70.00% v/v. Thus, the samples were previously diluted 50.00% v/v to water for application once ethanol is found in fuel samples on fractions of around 95.00% v/v. Negative deviations in Φ_{ME} are because the progressive increase in ethanol fraction as well as happens in Figure 3. Confidence intervals were of 0.08% to 0.21% v/v Φ_{ME} .

According to Figure 6, the analytical sensitivity was only -0.06. Ways to improve such a poor sensitivity were studied, namely, the increase in the time and temperature of separation. For the warming of donor solutions, the separator was immersed in thermostatted bath that was placed on the stirrer. Measurements at 40°C during 5 min produced non-linear data (not shown). Conversely, assays at 23°C for 20 min generated linear data with enhancement in analytical sensitivity, -0.12. The analytical curve for 20 min is shown in Figure 7. The values of Φ_{ME} reduced with the time of separation because the increase of volatilized ethanol in receptor media that is employed for the subsequent performing of MEC.

For application, gas diffusion separations at 23°C for 5 min generated accurate results at 95% confidence level despite the low sensitivity. This time was adopted by considering the gain in analytical frequency. The data recorded by Karl Fischer and MEC are demonstrated in Table 1 after correcting Φ_E through dilution factor and its conversion in Φ_w .

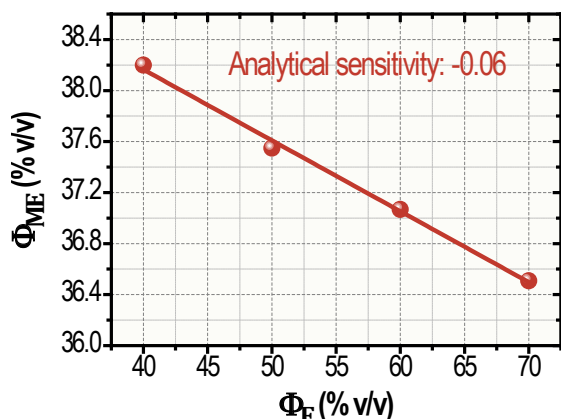


Figure 6: Analytical curve in region A for ethanol added in donor solutions considering separations at 23°C for 5 min and MEC using receptor water-n-propanol-oleic acid MEs. R^2 was 0.9950.

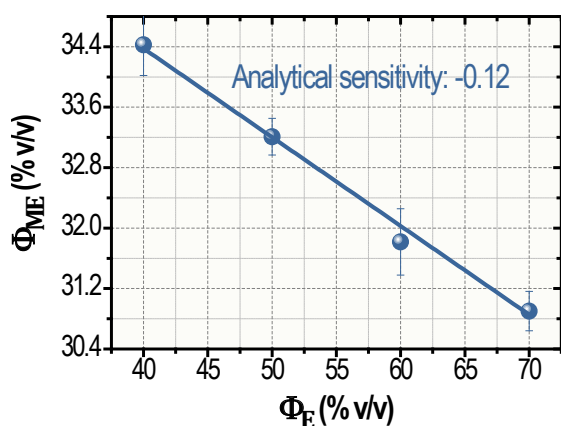


Figure 7: Analytical curve in region A for ethanol added in donor solutions taking up separations at 23°C for 20 min and MEC in receptor water-n-propanol-oleic acid MEs. R^2 was 0.9941.

Samples	Karl Fischer (% v/v, n = 3)	MEC (% v/v, n = 4)
F ₁	5.1 ± 0.1	5.1 ± 0.1
F ₂	5.1 ± 0.1	5.2 ± 0.1
F ₃	5.0 ± 0.1	5.0 ± 0.2

Table 1: Fractions of H₂O (Φ_w) in ethanol fuel (F₁-F₃) determined by Karl Fischer and MEC with gas diffusion separation.

Conclusion

In summary, the results stressed in this paper outline the potentiality of MEC for the deployment of point-of-use analytical technologies. The method was robust as regards to deviations in preparation of W-O mixtures and changes in temperature for the determination of ethanol in water. Furthermore, the successful coupling with gas diffusion separation extends the field of application of MEC in analytical sciences by improving its selectivity. Herein, other sample preparation methods could be used such as solid phase extraction. For the platforms integrating gas diffusion separation and MEC-based detection, the time showed to be a satisfactory parameter with the intent to increase the sensitivity. In this case, the microemulsification could be also made with greater volumes of dispersion. This fact helps for precision and

accuracy by enhancing the gap among the values of Φ_{ME} for diverse concentrations of analyte.

Acknowledgment

Centro Nacional de Pesquisa em Energia e Materiais is recognized for its facilities. Financial support from the *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP, Grant No. 2014/24126-6) is gratefully acknowledged.

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