Flow Injection Determination of Ethanol in Brazilian Brandy with a Compact, Low-Cost Refractive Index Detector

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Abstract

A simple, rapid and low cost method for the determination of ethanol concentration in aqueous solution by flow injection analysis (FI) has been developed. It was applied to determine the ethanol content in Brazilian brandy. The method uses a single line FI system with detection based on refractive index. A light-emitting diode and a photoresistor act respectively as light source and sensor. When distilled water is used as the carrier, the linear range is 0-15 % (w/w) of ethanol and the limit of detection is less than 0.01 % (w/w). Its linear working range can be further extended to 40 % when 30 % of ethanol solution is used as the carrier. The ethanol concentration of the carrier significantly affects both the relative signs and the relative heights of the positive (primary) and the negative (secondary) peaks. The height of the primary and of the secondary peaks or the sum of the two can be used as the analytical signal. The signals correlate linearly to the ethanol content only when the ethanol concentration of the sample is larger than that of the carrier.

Keywords: refractive index, ethanol, aqueous solution, photoresistor, LED, FI, brandy, spirits

Introduction

Flow-through photometers incorporating light-emitting diodes (LEDs) as sources of visible radiation and photodiodes or phototransistors as detectors provide a reliable, low-cost alternative to commercially available spectrophotometers [1]. LEDs are simple, small, inexpensive and long life. With a battery operated power source, the intensity noise is about 2×10^{-3} % of the total amplitude [2]. The first practical LED-based flow-through photodetector was described by Betteridge *et al* [3], where a light-emitting diode and a phototransistor act respectively as the light source and as the detector. It also functions as a differential refractometer due to the existence of two interfacial regions between the sample and the carrier. This is a general effect which is observed with many photometric detectors. It can be minimized or maximized [4]. Many efforts have been carried out to minimize or eliminate the refractive index effect in flow injection (spectro)photometric analysis [5-11]. Using analytically this effect, the detector functions as a refractometer [3, 4, 12-15].

The concentration gradient of the sample in the carrier, produced during injection, creates a refractive index gradient when the solute has a different refractive index to that of the carrier solvent [12]. The magnitude of this gradient can be measured by allowing a nonadsorbed light beam to pass through the detector volume. A change in refractive index within the flow cell causes reflection and refraction of the transmitted radiation, resulting in a change in the light intensity that reaches the photodiode detector [13]. Many factors affect the shape, magnitude, and direction of this response. The signal obtained in a system based on the refractive index detection is dependent on the magnitude of the concentration change as well as on the distance over which the change occurs [14].

In any analytical procedures, the magnitude of the analytical signal and the magnitude of the noise significantly affect the detection limit, which must be maximized and minimized, respectively [5]. The aim of this work is to develop a simple and rapid method for the determination of ethanol in aqueous solutions to be applied in real samples. The method uses a single line FI system based on a refractometric detector in which a LED and a miniature light dependent resistor act respectively as light source and sensor. In this work the conditions for the determination of ethanol were optimized by studying

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the minimization of the noise and the effects of various parameters, such as the sample volume, the flow rate and the composition of the carrier. An air pulse damper was used to reduce the baseline noise.

Experimental

The designs of the flow-through refractive index detector and of the analytical system are schematically shown in Fig. 1. The analytical system consisted of a flow cell, a rotary injection valve and a peristaltic pump (ISM 726, Ismatec, Switzerland). The flow cell, based on the design of Betteridge *et al* [3], was made of black polytetrafluoroethylene (PTFE). The channel diameter of 2 mm and the length of 30 mm served as the optical path. A yellow-light ultra bright LED (TLMP 7413) and a miniature cadmium sulfide light dependent resistor (PR) were fixed at the two ends of the flow cell. However, they were mounted in the cell in a manner which allows them to be easily replaced. The wavelength of the incident radiation can be altered by changing the LED. The light dependent resistor housed in an epoxy coated open frame TO-18 package is equivalent to the NSL19-M51 and has the characteristics: peak spectral response of 550 nm and cell resistance at 10 Lux (min-max) of 20-100 kΩ, as well as dark resistance (min.) of 20 MΩ. The PR was protected against the solutions with a piece of optical glass. The current applied to LED was *ca.* 25 mA using a 4-battery of 1.5 volts power source and a resistance of 150 Ω. The incident light passes through the sample and reaches the photoresistor at the other end of the flow cell, which measures the intensity of the light. The PTFE rotary injection valve fitted with a 50 or a 200 μl of sample loop was employed for the introduction of the sample. The signal of the photoresistor was displayed on a chart recorder (Model LR92425, Linear Instruments, Dubuque, Iowa) by means of a Wheatstone bridge converter (Leeds & Northrup Co., Philadelphia, PA). An air pulse damper described previously [16] was fitted into the carrier line between the peristaltic pump and the injection valve to minimize the noise of the baseline. The outside surfaces of the LED, of the PR and 15 cm of the flow in/out tubes of the cell were lacquered black to avoid ambient light to reach the photodetector. Special care was taken to thermally equilibrate both sample and carrier solutions to room temperature. The pumping tubes used were Tygon® tubes (1.5 mm I.D.). Polyethylene tubes with internal diameter of 0.9 mm were used in the manifold. All experiments were done at room temperature.

The distilled water was further purified with a Milli-Q Plus unit (Millipore, Bedford, MA) and used throughout. Binary aqueous solutions of ethanol range from 0-50 % (w/w) were prepared with analytical grade ethanol (95 %). An ethanol solution of 5 % w/w was used to investigate the peak height variation as a function of experimental parameters. Deionized water or ethanol solution (10 % and 30 %) was respectively used as the carrier, and various concentrations of ethanol solutions were used as the samples.

The brandy samples were provided by the Laboratory of Drink Chemistry, Institute of Chemistry of São Carlos, University of São Paulo (São Carlos, Brazil). Standard addition experiments were performed by spiking the samples with ethanol solutions.
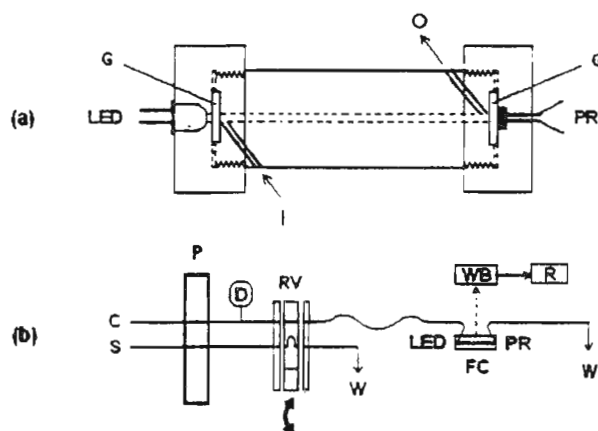

Fig. 1. (a) Sectional view of flow cell and (b) flow system for refractometric determinations. LED, light-emitting diode; PR, photoresistor; G, optical glass; I/O, flow in/out. C, carrier; S, sample; P, peristaltic pump; D, pulse damper; RV, rotary injection valve; FC, flow cell; WB, Wheatstone bridge; R, recorder; and W, waste.

Results and discussion

Reduction of the baseline noise

In a conventional FI manifold without the air pulse damper, significant noise was observed because of the pulse of the peristaltic pump (Fig. 2 a and 2c). The variation of the flow rate didn't reduce the noise (Fig. 2a). The insertion of the pulse damper between the peristaltic pump and the injection valve eliminates the effect of pulsation in the flow and

significantly reduces the amplitude of the baseline noise (Fig. 2b). Thus the air pulse damper was used throughout the present work.

Response signal

Fig. 3 shows a typical recording of the refractive index detector to the peak signal generated by injection of 1 % ethanol sample into the water carrier. A primary positive and a secondary negative peaks were observed as the sample passed through the flow cell. This phenomenon of positive/negative (primary/secondary) peaks observed in this work and also by other researchers [3, 12-14] usually is non-symmetric. The height difference between the first and the second peaks may be related to the time lag between the detection of the leading and of the trailing interfaces of the sample. This period allows extra broadening of the negative peak, which results in the concentration gradient loss [12]. The relative standard deviations for the primary, the secondary and the total (sum of the primary + secondary) peaks are respectively 0.8, 0.9 and 0.6 % (n=10). A detection limit can be evaluated from Fig. 3 where a signal to noise ratio of 2 gives 0.01 % of ethanol (w/w). This limit can be decreased further 2-3 times under the optimum conditions of sample volume (200 µl) and flow rate (2.2 ml min^{-1}).
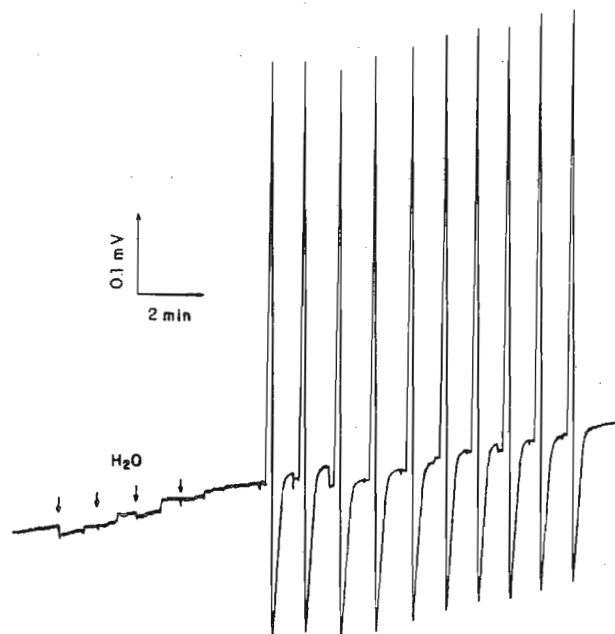

Fig. 3. Signal produced by the detector after injection of 50 µl of water and of 1 % ethanol solution into water carrier. Flow rate: 1.65 ml min^{-1}. Arrows represent the points of injection of water into carrier.

The shift of the baseline is responsive to room temperature changes as a consequence of the temperature dependence of the refractive index. Though the movement of the injection valve has some effects on the baseline, there is no response signal observed when the distilled water was injected into the water carrier, and it is easy to distinguish between the analytical signal and the fluctuations of the baseline caused by the injection of sample.

Optimization of the sample volume and of the flow rate

The influence of the injected sample volume on the primary/secondary signal peaks was studied over the range 30-400 µl, using a sample containing 5 % of ethanol at the flow rate of 1.65ml min^{-1}. The primary/secondary peak heights increase as the sample volume increases to about 150 µl, then is attained a "saturation level" for a sample volume larger than 150 µl. To perform the other experiments a sample volume of 200 µl was chosen.

The flow rate influencing the performance of the proposed system in the determination of ethanol was optimized by the sample containing 5 % of ethanol. The flow rate of the carrier was varied from
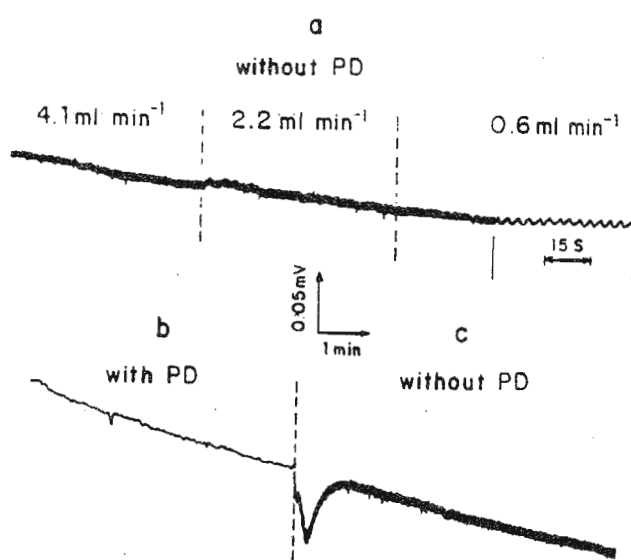

Fig. 2. Comparison of baselines obtained (a) and (c) without and (b) with the pulse damper for distilled water carrier (a) in different flow rates.

0.6 to 7.0 ml min^{-1}. In Fig. 4, it can be observed that the primary and the secondary peak signals initially increase, decreasing above the flow rates of 2.2 ml min^{-1} (primary peak) and of 1.65 ml min^{-1} (secondary peak). Above 5.4 ml min^{-1} the height of the two kinds of peaks remain constant. Within 2.2 to 5.4 ml min^{-1} the signal of the primary peak initially decreases (2.6 ml min^{-1}) then increasing until 3.7 ml min^{-1} when it decreases again reaching the lowest value at 4.6 ml min^{-1}. From this flow the signal increases again until 5.4 ml min^{-1} remaining then constant up to 7.0 ml min^{-1}. The height of the secondary peak increases from the flow 0.6 to 1.65 ml min^{-1}. Then it decreases until 3.7 ml min^{-1}. From this flow the signal increases until 5.4 and 5.9 ml min^{-1} then slowly decreasing again. Fig. 4 clearly shows that the effects of the flow rate on the primary and on the secondary peaks are not identical. For example, the largest secondary peak appears at the flow rate of 1.65 ml min^{-1}, but the largest primary peak occurs at 2.2 ml min^{-1}. To perform the present work the carrier flow rate of 2.2 ml min^{-1} was chosen.

Flow rate has a large influence on the magnitude of the detector response and further on the analytical time and on the sampling frequency. The advantages of the application of higher flow rates is, by one hand, the facility to eliminate the interference of air bubbles in the tube lines and, by the other hand, a higher analytical frequency can be achieved. Depending on the used flow rate, an analytical frequency from 60 to 120 h^{-1} can be obtained.
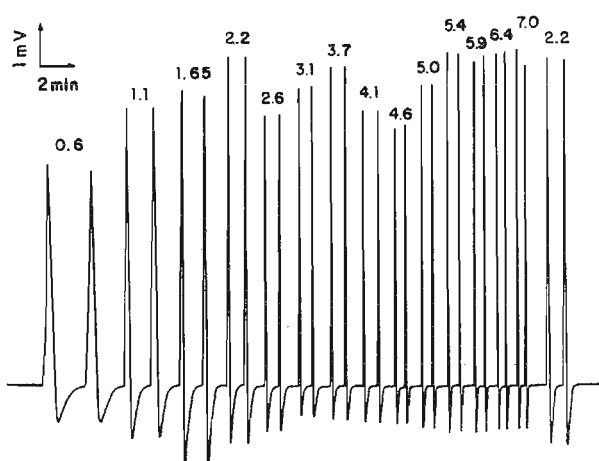

Fig. 4. Effect of flow rate on the response of refractive index detector. For 5 % of ethanol solution and 200 µl of sample volume using distilled water as carrier.

Effect of the composition of the carrier on the linear range and peak shape

The height of the primary and of the secondary peaks correspond to the relative magnitude of the refractive indices of the carrier and of the sample. Following the injection of ethanol solution into the water carrier, two peaks are observed: first a positive (primary peak) and then a second negative peak (secondary peak), relative to the baseline (Fig. 5a). In contrast, when an ethanol solution was used as the carrier and water or lower concentration of ethanol solution was the sample, the direction of the peaks signal was reversed (Fig. 5b and 5c).

Fig. 6 shows the correlation between the peak height of the primary and of the secondary peaks and the ethanol concentration. The results show that the peak heights can be employed as the analytical signal including the total peak height that is the sum of the primary and of the secondary. The total peak height is independent of the baseline level as it is measured from the maximum of the primary peak to the minimum of the secondary peak. Also as the total peak is the sum of the primary and of the secondary it is higher and therefore gives the best precision and the best limit of detection. When deionized water is used as the carrier, the linear range of the signal *versus* concentration is 0-15 % (w/w) of ethanol (curves A and B). It is possible to determine more concentrated solutions when the carrier is modified by adding ethanol. When 10 % (curves C and D) or 30 % (curves E and F) of ethanol solution are used as the carrier, the peak heights for the sample containing more ethanol than the carrier correlate linearly to ethanol concentration in the range from the carrier concentration to about 10 % of ethanol above that of the carrier. Thus the linear range is moved to 10-20 % and to 30-40 % (w/w) of ethanol, respectively. Usually the first peak caused by the leading interface of the sample is sharper in spite of not always higher, and the secondary peak is wider. It is interesting to note that when 30 % of ethanol solution was employed as the carrier, the secondary trailing peaks became higher than the primary leading peak for the case of the sample containing less than 30 % of ethanol, and the secondary peaks completely disappeared for the case of the sample containing more than 30 % of ethanol. As far as we know there is no report in the literature about this observation. This phenomenon indicates that the sample and the carrier are mixed more or less completely at the trailing interface when 30 % ethanol is the carrier and more concentrated samples are analyzed. The shape and the height of the peaks

are also different for the cases of ethanol solution injected into water carrier or *vice versa*. One of the reasons may be related to the changes of the physical properties of the solution besides refractive index due to the variation of the ethanol content. such as viscosity and density, which affect the dispersion behavior of the solute in the flow. In other words, the dispersion behavior of the leading interface for
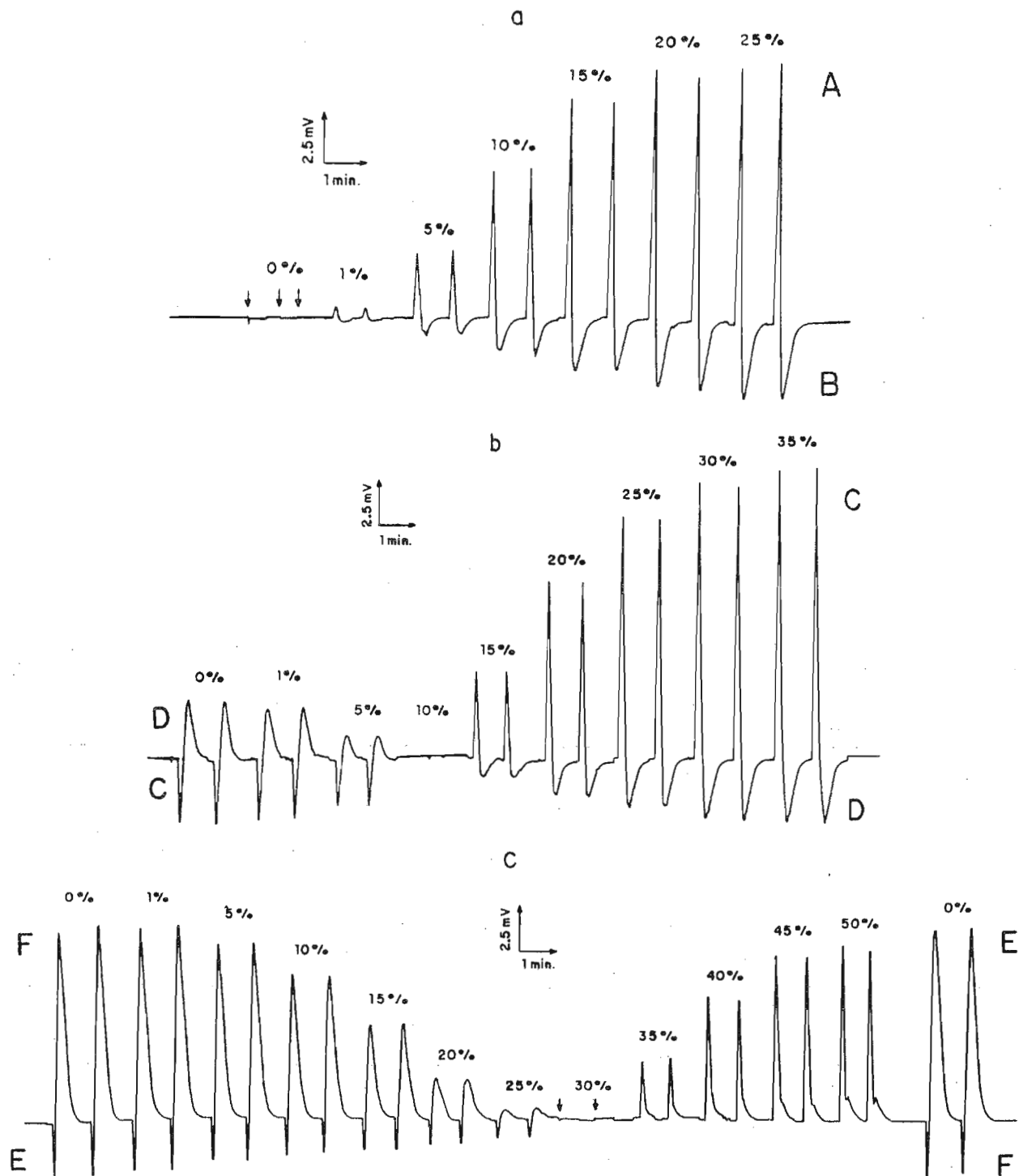

Fig. 5. Recorded peak profiles for flow injection determination of ethanol with carrier of: (a) deionized water: (b) 10 % (w/w) of ethanol solution: and (c) 30 % (w/w) of ethanol solution. The values above the peaks are the ethanol concentration (w/w) of the sample. Flow rate was 1.65 ml min^{-1} and the sample volume was 50 μl.
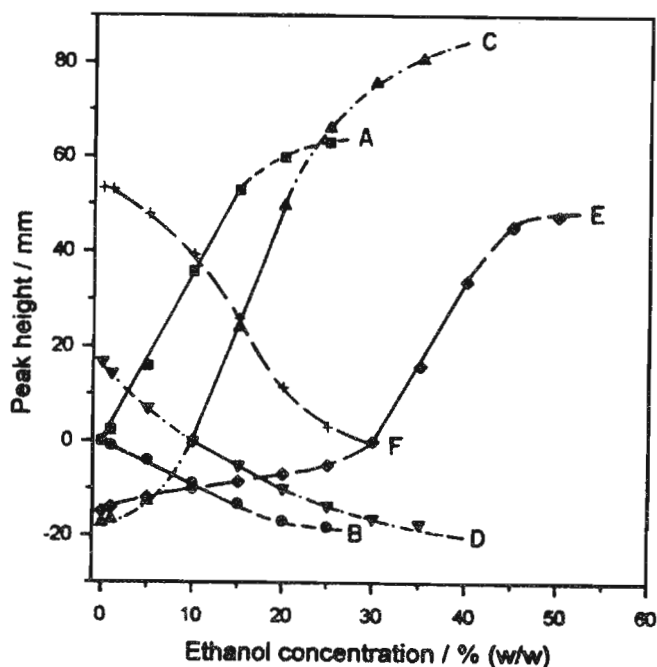
Fig. 6. Calibration curves for the FI determination of ethanol. A and B (related to the Fig 5a) - carrier deionized water; C and D (related to the Fig 5b) - carrier ethanol 10 % w/w; E and F (related to the Fig 5c) - carrier ethanol 30 % w/w. Flow rate was 1.65 ml min^{-1} and the sample volume was 50 µl.

Table 1. Determination of ethanol contents in drink samples [a]

Sample	Color	Cal. curve [b] / (w/w)	Stand. add. [b] / (w/w)
1	colorless	31.5±0.3	31.4±0.3
2	yellow	31.2±0.3	30.9±0.3
3	brown	34.0±0.3	34.3±0.3
4	light blue	35.6±0.3	37.6±0.3

[a] The carrier is 15 % of ethanol solution at the flow rate of 2.2 ml min^{-1} and 200 µl sample volume. [b] Mean of two measurements ± mean deviation.

ethanol solution injected into the water carrier is different from that of the trailing interface for the water injected into the carrier containing the same ethanol concentration. The ethanol concentration of the carrier affects the peak's shape and relative heights of first/second peaks. The asymmetry of the first and second peaks may be explained considering that, in the cell, the front and the rear of the sample form different concentration gradients [12].

Determination of ethanol in Brazilian brandy

Studies on the application of the refractive index detector in FI were carried out by methods for the determination of ethanol in drinks with different colors are given in Table 1. For the colorless, yellow and brow samples (LED is yellow) the results using calibration curve and standard addition methods give similar results. For the blue sample the difference between the results using the calibration curve and the standard addition methods is larger. This could be understood in terms of the absorption of the light by the colored solution. A suitable choice of the color of the LED or of a filter used from the wide range available will overcome this problem in most instances [3].

Conclusions

In summary, the method developed can be used to rapidly evaluate the ethanol content in distilled liquors. An ethanol concentration less than 0.01 % can readily be detected and the sampling rate up to 120 h^{-1} can be achieved. The relative signs of the first/second peaks relate to the relative magnitude of the ethanol concentrations (refractive indices) of the carrier and of the sample. The first peak is usually sharper but not always higher than the second. The ethanol concentration of the carrier has a significant effect on the relative height of the first/second peaks.

Acknowledgments

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