Flow injection determination of ethanol in Brazilian brandies using a gas-permeable membrane and a gas flame ionization detector

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Abstract

A flow injection (FI) analysis system for the quantification of ethanol in brandies was developed. In the herein described FI system a gas-permeation membrane module is connected to a gas chromatography flame ionization detector (FID). In the membrane module one side of the membrane is in contact with the analyte-donor aqueous phase, whereas the other side is swept by a stripping flow of He which is the analyte-acceptor gaseous phase. The volatile component of the aqueous sample (ethanol) passes through the silicone membrane into the analyte-acceptor gaseous phase and is carried to the FID. The effects of the flow rate, sample volume, tubing length, temperature of the membrane module and the operational conditions of the gas chromatograph upon the signal were evaluated.

Keywords Flow injection analysis; Flame ionization detector; Ethanol; Brandies analysis; Gas-permeable membrane

1. Introduction

Ethanol is one of the most important analytes for food industry. Taxes regulation require exact determination of the ethanol content specially in spirits [1]. Official AOAC methods of analysis for distilled liquors include pycnometry, hydrometry, densitometry, refractometry and the William Field test [2], which needs sample distillation. Great efforts have been focused on the automation of ethanol determination by FI techniques which include the use of enzymatic [1,6-22] and non-enzymatic methods [3-6]. The non-enzymatic methods were unspecific whereas the enzymatic proved to be more specific. However, most of the samples had to be diluted prior to analysis owing to the kinetic saturation of the enzymes, a fact that causes an important additional source of error [1].

The technique of separation by a membrane coupled to a suitable detection device represents a powerful FI analytical tool that allows the analysis of a variety of analytes present in complex matrixes [23].

In this work a flame ionization detector (FID) was used for the detection of ethanol due to its high sensitivity and wide range of linearity towards organic compounds [24]. The aim of this work was to develop a fast and operationally simple procedure for the determination of ethanol in spirits using an FID coupled to a gas-permeation membrane module in an FI system. The method is based on the permeation of the ethanol molecules through a silicone membrane from the liquid matrix to the analyte-acceptor gas that carries the analyte to the FID.

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2. Experimental

2.1 Apparatus and Reagents

Fig. 1 depicts the apparatus developed for this work, which consists of a sample injector, a membrane module and an FID chromatographic detector. The sample injector is a self-constructed polytetrafluorethylene (PTFE) two way rotary injection valve [24]. The aqueous-ethanol solutions were pumped by a peristaltic pump (Ismatec ISM 726) using Tygon® pump tubes (Ismatec 1.52 I.D.). Deionized water was used to carry the sample to the membrane module where the analyte (ethanol) migrates to the analyte acceptor gaseous phase (Helium) and is transported to the FID. The membrane module was designed after modifications of an already described membrane system [25]. A silicone membrane was used in the module instead of the usual commercial PTFE microporous tape. This choice was based on the hydrophobic properties and on the low porosity of the silicone membrane, fact that avoids the permeation of water from the liquid donor stream to the gaseous acceptor stream, phenomenon observed when a PTFE membrane was used. The dimensions of the membrane were: thickness 0.10mm, length 110 mm, width 4 mm. The temperature of the membrane module was controlled with a Tempette TE-8D thermostat. A Hewlett-Packard 5890A gas chromatograph (GC) equipped with a flame ionization detector (FID) was used as the detector. The inlet pressure of the carrier gas was adjusted to 10 psi. The carrier gas line was connected to the inlet connection in one side of the membrane system. The outlet in the same side was directly connected to the GC through a 20 cm 1/16 inch stainless steel tube. A HP-5 capillary column (30 m × 0.32 mm × 0.25 μm film thickness, Hewlett-Packard), was used for the transport of the analyte to the FID. It is not unlikely that an empty fused silica capillary tube could be used for this transport, as the separation of the analyte is performed in the membrane module. The analytical signals, i.e., the ethanol peak areas, were processed by a Hewlett-Packard 3392A integrator.

The water used in the experiments was firstly distillated in a glass distiller and then deionized using a Milli-Q Plus system (Millipore). Absolute ethanol (Nuclear – Casa da Química, Brazil) was used for the preparation of the standard solutions. The concentrations of the ethanol solutions were calibrated using an Abbé refractometer (Carl Zeiss Jena) at 20.00 ± 0.05 °C. Samples of Brazilian sugar cane brandies (cachaça) of various trade marks were analyzed with the proposed method without prior treatment.
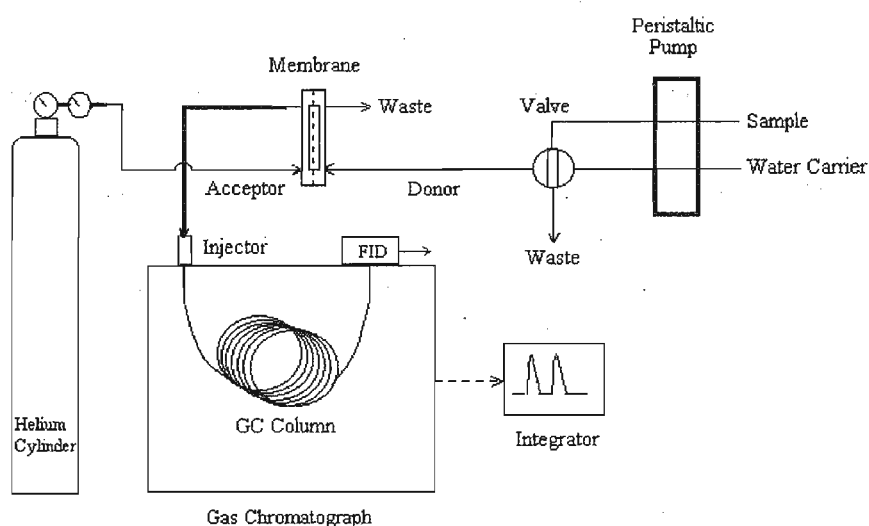

Fig.1 Scheme showing the major components of the FI - chromatographic system.

2.2 Procedure

The analyte-donor aqueous phase was pumped through the membrane module at a constant rate of 1.6 ml/min. A sample volume of 200 µl containing ethanol was injected into the water carrier. The analyte-acceptor gaseous stream (He, 10 psi) was introduced into the other side of the permeation cell. The GC temperature settings were: oven, 75 °C; injector, 250 °C; detector, 250 °C.

3. Results and discussion

The flame ionization detector is easy to use, exhibits a high sensitivity associated to a large linear response and low noise [24]. In addition, it is either insensitive or has a very low response toward gases such as H_2O, CO_2, SO_2 and NO_x.

The conditions for the determination of ethanol were optimized by studying the effects, upon the analytical signals, of the following parameters: flow rate of the analyte-donor aqueous phase, sample volume, tubing length (containing the aqueous phase) and the temperature of the cell membrane.

Fig. 2 shows the influence of the flow rate of the analyte-donor phase in the range of 0.45 to 4.2 ml min^{-1} upon the analytical signal. As can be seen the ethanol peak area decreases very rapidly as the flow rate increases and tends to a constant value as the flow reaches the value of 4.2 ml min^{-1}. This behavior of the analytical signal is attributed to the fact that at lower flow rates the time of contact of the sample with the membrane is larger leading to the permeation of more ethanol. The analyte-donor flow rate of 1.6 ml min^{-1} was used in this work as it results in an adequate compromise between the analytical frequency and the analytical sensitivity.

The effect of the sample volume upon the signal intensity was studied by varying the sample volume from 50 µl to 500 µl (Fig. 3). The analytical signal (peak area) augments in direct proportion to the sample volume (peak area = $5 \times 10^2 \times$ sample volume in ml).

After considering an adequate compromise between the intensity of the analytical signal and the analytical frequency, the volume of 200 µl was chosen to perform the herein described studies.
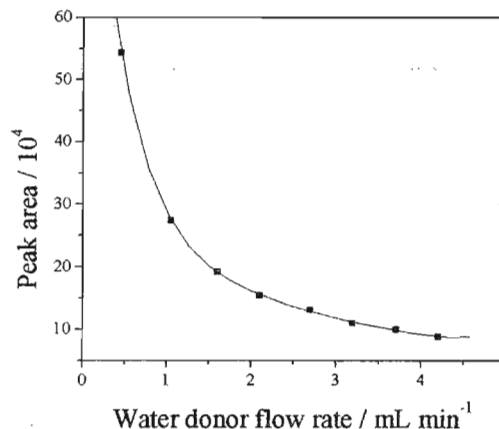

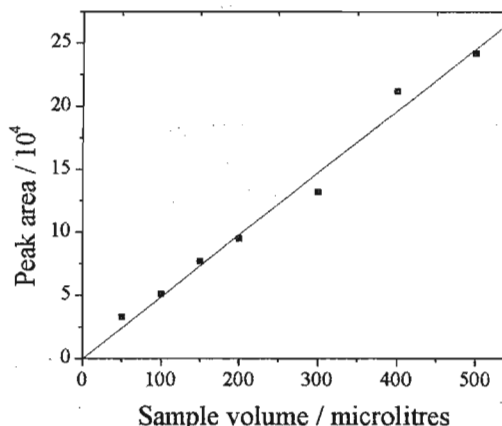Fig.2. Effect of the water flow rate on the analytical signal.

Fig. 3 Effect of the sample volume on the analytical signal. Analyte-donor flow rate: 1.6 ml min^{-1}.

The influence of the length of the tube that connects the injection valve to the membrane module was investigated for tube lengths of 6 cm to 39 cm (Fig. 4). It was observed that the analytical signal increases as the tube length is augmented from 6 cm to 25 cm, decreasing beyond this length. The length of 20 cm was used in this work. The format of the curve can be attributed to the relation between the dispersion of the ethanol in the tube and the burning efficiency of the

flame.

The effect of the temperature upon the permeation of the volatile compound through the membrane was also considered after varying the temperature of the membrane module (Fig. 5). An increase in the temperature of the membrane module increases the diffusion rate of the analyte through the membrane.
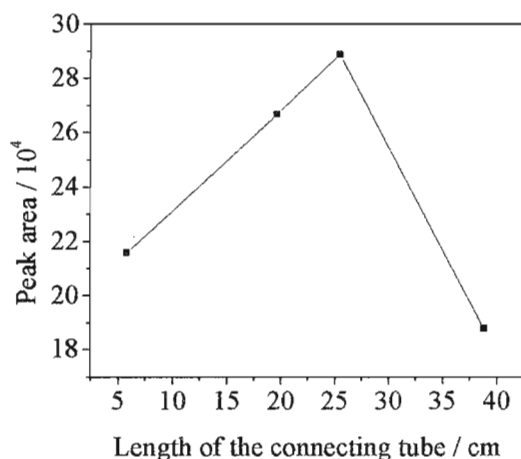

Fig. 4 Effect of the length of the tube placed between the sample injection valve (water donor flow rate) and the gas permeation FI-[membrane module].

Depending on the changes in distribution coefficients, this could result in either a positive or a negative change in the transition state and the transport efficiency [23].

In this study the temperature of the membrane module was varied from 20.0 to 40.0 °C in steps of 5.0 °C. When the set temperature was attained a time of 30 minutes was respected to guarantee the thermostatization of the device containing the membrane immerse in the bath. In the studied temperature range it was observed a linear relationship between the response signal and the temperature (peak area = -4 × 10^4 + 4.5 × 10^3 × t ; t = temperature (°C)).

Once the operational conditions of the FI-[membrane module]- FID system were established a calibration curve was made after the analysis of
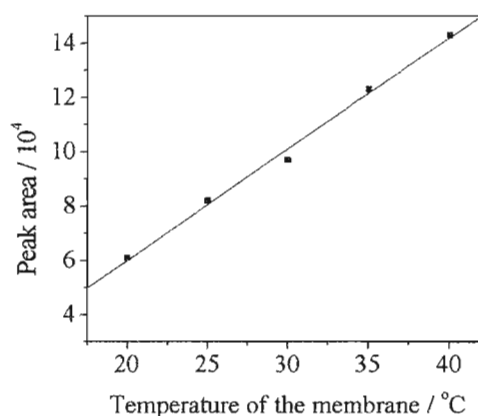

Fig. 5 Effect of the temperature on the silicon membrane of the FI-[membrane] device on the analytical signal.

aqueous ethanol standard solutions in the concentration range from 0 to 48.5 % w/w.

It was also established that the analytical circle, *i. e.*, the time necessary to perform one analysis is about 4 minutes and therefore the sampling rate is about 15 h^{-1}. A typical calibration fiagram is shown in Fig. 6.

The calibration curve is given by Eq. (1). The ethanol concentrations as percent by weight obtained for the cachaça samples after interpolation with Eq. (1) are listed in Table 1. For comparison purposes Table 1 also shows the ethanol concentration obtained after refraction index measurements.

$$\mathbf{C_{et} = 162.510 \ S_{et} \ ; r = 0.999} \qquad (1)$$

$\mathbf{C_{et}}$ is the ethanol concentration (% w/w) , $\mathbf{S_{et}}$ is the ethanol area (number of counts from the CG integrator) and **r** is the correlation coefficient. The data from duplicate determinations were used for the data regression that resulted in Eq. (1). The residues calculated for each known ethanol concentration ranged from -1.9 % to + 3.0 % and were uniformly distributed. Thus it may be concluded that data interpolation with Equation 1 was made at the maximum uncertainty of 3%.
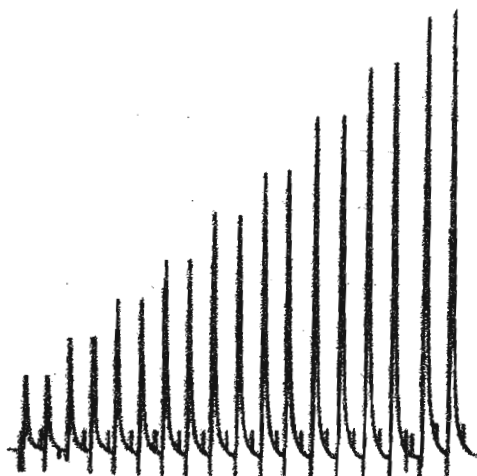
Fig.6 Typical calibration fiagram. Ethanol concentrations in % w/w are respectively from the left:9.7 ; 14.6 ; 19.4 ; 24.3 ; 29.1 ; 34.0 ; 38.8 ; 43.7 ; 48.5.

As can be concluded from the data of Table 1, the herein proposed method resulted in ethanol concentrations which are statistically identical to those obtained from refraction index measurements. This indicates that this FI-[membrane]-FID method is reliable. It should also be pointed that this method has the advantage of high sampling rate when compared to usual chromatographic procedure.

Table 1. Determination of ethanol concentration (% by weight) in Brazilian brandies (cachaça) using the proposed method in comparison to the refraction index method.

Sample	Proposed Method [a]	Refraction Index Method [b]
1	32.0	32.9
2	35.5	35.1
3	34.5	34.7
4	32.6	33.1
5	34.1	33.2
6	29.8	28.7
7	34.7	34.9
8	35.4	36.8

[a] - Maximum interpolation uncertainty of 3%.

[b] - Uncertainties 3%.

4. Conclusions

The proposed FI-GC system allowed fast quantification of ethanol in sugar cane spirits, with accuracy and precision comparable to those of traditional standard methods used for routine analysis. It also presents some advantages in relation to these conventional procedures. For instance, one of them is the capability of processing a large number of samples in short time periods. Also, the whole FI-GC process can be fully automated, which is specially suitable for its incorporation to routine control systems in sugar cane processing facilities, enabling real time control of the product. The hyphenation of the FI-[membrane] separator with a CG creates an analytical system with very interesting characteristics. The membrane separator module avoids the introduction of large amounts of water into the chromatographic column, improving the durability of this component. The fact that most of the stationary phases employed in GC are degraded by humidity is a serious drawback when direct injection of aqueous samples have to be made in routine procedures. Also, a large variety of stable, sensible and linear detectors – as the FID – are available as GC detectors, and coupling a FI module to a GC is a simple and direct way to extend their use to FI systems without need of more complex instrumental settings.

The chromatographic column was here solely used to transfer ethanol from the membrane separator to the GC. The extension of this system to quantification of several analytes per sample, after on-line membrane isolation and their chromatographic separation in the capillary column is presently in course.

5. Acknowledgments

6. References

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