



# A reagentless flow injection system for the quantification of ethanol in beverages based on the schlieren effect measurement



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## ABSTRACT

A flow injection system comprising a gas-diffusion unit and schlieren effect detection was arranged in order to develop a simple methodology for the quantification of ethanol in different alcoholic beverages. It was possible to establish a linear range up to 25% (v/v), with a LOD and a LOQ of 0.6% and 2.0% (v/v), respectively; with good repeatability, RSD < 4.6%. Only 80  $\mu$ L of sample was needed for quantification with a determination rate of one determination per minute.

To evaluate the accuracy of the proposed method, a total of 43 alcoholic beverages, red and white table wine, port wine, beers, liquors, spirits and brandies were analysed. The results obtained by the developed method were in good agreement with the ones obtained by the reference method.

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## 1. Introduction

The determination of ethanol is a key parameter in terms of quality and stability for alcoholic beverages [1]. The official methods [2,3] for the determination of ethanol in wines and other alcoholic beverages are laborious and complex, usually involving the separation of the analyte from the sample matrix by distillation. Sample preparation is often the crucial step of the analytical processes, usually laborious and time consuming. Therefore, automation using flow-based systems turns to be an attractive solution.

Flow-based systems have gained increase importance on the automation of the steps required by the official methods for quantification of several analytes. These systems have started as automation tools for wet chemical analysis and further used in biochemical assays. The flow-based analytical methods rely on the combination of three principles: (i) reproducible sample injection, (ii) controlled dispersion of the sample zone and (iii) reproducible timing of its movement from the injection point to the detection [4].

In terms of ethanol quantification, flow-based systems have been used on the automation of methodologies using different approaches: overviews about reported methods applying enzymatic reactions [5] or colorimetric measurements [6] have already been published. In a previous work we have used a sequential injection analysis (SIA) system to develop an enzymatic methodology for the determination of ethanol [7]. The methodology was based on the spectrophotometric enzymatic

reaction catalysed by alcohol dehydrogenase in the presence of  $\text{NAD}^+$ . This method presented clear advantages when compared with the reference method; the sample consumption was largely reduced and the determination rate highly improved. With the aim of further downscale the sample and reagent consumption, the described methodology was adapted to a SIA system in a Lab-on-valve format [8]. It was possible to reduce nearly 85% the consumption of previously obtained. In spite of the above mentioned advantages, the above described methodologies involved the sample prior dilution before entering the system. To avoid this process, a gas diffusion unit (GDU) was coupled to a SIA system, where the analyte in its gaseous form was separated from the sample matrix prior to the enzymatic reaction. The introduction of the GDU provided extensive and reproducible dilution, as a result reducing the interference from the sample matrix on the spectrophotometric detection [5]. This is one of the most used separation techniques, due to its simplicity in the coupling to flow-based systems for continuous operation [9]. Furthermore the use of membranes for on-line separations is characterized by its simplicity, repeatability, reliability, and requires little or no sample pre-treatment [10].

By further exploring our previous experience, the aim of the present work was the development of a simple reagentless flow injection analysis (FIA) system for the quantification of ethanol in alcoholic beverages. To accomplish this objective, a FIA system comprising a GDU and a light diffraction measurement device was arranged. The detection system chosen for the proposed methodology is based on the so-called schlieren effect measurement. Actually, although this effect has been reported as a major problem in flow spectrophotometry [11–13], in this case we tried to turn it into our advantage to accomplish a simple

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detector with no need for derivatizing agents. Zagatto and co-authors [11,12] have been studying this effect in flow-based systems as it is normally associated to interferences on the spectrophotometric detection; at the same time it is a quite reproducible signal that can be used as a detection method. Any change in the refraction index between the moving carrier and injected sample or reagent causes a change in light transmission through the cuvette [14]. The schlieren effect is often associated with gradual variations in temperature and concentration, and consequently refractive index [13]. It can result by the combination of two factors; first the formation of stable liquid lenses under the laminar flow conditions of the system, and the other due to the poor mixing conditions of the flow-based systems [12].

The analytical potential of the schlieren effect has already been exploited; in fact, this type of detection by means of a light-emitting diode (LED) photocolimeter, has been coupled to a flow-based system and used for the quantification of ethanol [15] in distilled “cachaça”, rum, gin, vodka and white wine. Later, the same authors [16] tried to combine the schlieren effect measurement with the data generated by chemometric techniques to verify the adulteration of distilled alcoholic beverages. A different type of flow-based system, single interface flow analysis (SIFA) [17] was also used for the development of a system for the quantification of ethanol in spirits and white wine also based on the schlieren effect measurement. In the described work, spirit samples were subjected to a six fold dilution and white wine sample was degassed by ultrasound equipment. The authors refer that the quantification was not possible to establish in red wine samples due to its intrinsic strong absorption neither in alcoholic beverages with high sugar content. In this scenario, the aim of the present work was the development of a simple flow injection analysis system for the quantification of ethanol in alcoholic beverages with no sample or minor pre-treatment. Therefore a flow injection system coupled to a gas-diffusion unit and a charge-coupled device (CCD) detector was arranged. In the developed method, a well-defined volume of sample is injected in the carrier solution and propelled towards the donor channel of the gas-diffusion unit. The analyte is separated from the sample matrix by diffusion thorough the membrane to the acceptor channel, prior to analysis. The collected analyte is then transported towards detection. Consequently the system can be used not only in colourless samples, but also coloured samples as in red wine samples.

## 2. Materials and methods

### 2.1. Reagents and solutions

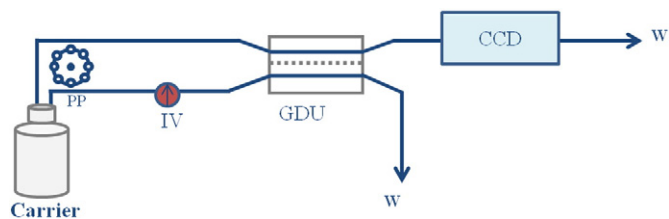
All chemicals were of analytical reagent grade, and ultrapure water (resistivity 18 M $\Omega$ /cm) was used throughout the work. Ethanol (C<sub>2</sub>H<sub>6</sub>O, Panreac, 221086) standards solutions were prepared daily by rigorous dilution of the stock solution.

Standard solutions of glucose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>, Merck, 108337), fructose (C<sub>6</sub>H<sub>12</sub>O<sub>6</sub>, Merck, 104007) and of bovine serum albumin (BSA, fraction V, 05484, Fluka) were prepared by rigorous dilution of stock solutions previously prepared by rigorous weighing.

Glycerol (Sigma G-6279), magnesium chloride hexahydrate (Merck, 5833), calcium chloride dehydrate (Merck, 2382); sodium chloride (Merck, 1.06404), L-(+)-tartaric acid (Merck, 1.00804) and sulphuric acid (Merck 1.00731) were used in the interference studies.

### 2.2. Apparatus

The flow manifold (Fig. 1) consisted of a Minipuls 3 peristaltic pump (Gilson, Villiers le Bel, France) and a four-way rotary valve (Rheodyne, Wertheim Mondfeld, Germany) connected by Tygon tubing (Gilson, Villiers le Bel, France) to a detection system; a CCD detector (Ocean Optics 2000, Winter Park, Florida, USA), two 200 mm fiber optic cables (FC UV-200) and a DH-2000 deuterium halogen light source (Top Sensor Systems, Eerbeek, The Netherlands). A 1 cm cuvette holder



**Fig. 1.** Configuration of the developed manifold for the quantification of ethanol in beverages. Carrier: Ultrapure water; PP: peristaltic pump-flow rate: 1.0 mL/min; IV: injection valve (80  $\mu$ L of sample); GDU: gas diffusion unit equipped with a Teflon membrane; flow cell of 1.0 cm with internal volume of 18  $\mu$ L.

(Ocean Optics, Winter Park, Florida, USA) and a flow-through cell with an internal volume of 18  $\mu$ L (Hellma 178.712QS, Müllheim, Germany) was used throughout. A gas diffusion unit with flow channels of 0.3 mm depth, equipped with Teflon membrane was used to perform the on-line dilution of the samples.

### 2.3. Reference methods

All wine and spirit samples were analysed as described by Wang [18]; the port wine samples were analysed after distillation as described in the Portuguese Standard [19]. The beer samples were analysed by GC with a FID detector [20].

### 2.4. Samples

All the samples tested are commercially available; one reference standard, with 15% of alcohol level (LGC5405, standard, nominal 15% (v/v)) was also analysed. The developed method was performed using the content of the same bottle used for the reference method. The port wine samples were analysed directly and after distillation. As beer samples are carbonated beverages, these had to be filtered and degassed before analysis [20]. Only a dilution step was required for spirit samples as they have alcohol content above 25% (v/v).

## 3. Results and discussion

### 3.1. Optimization of the manifold

As a first approach, the FIA system (Fig. 1) was set up to study the physical conditions, which could have some effect on the efficiency of the mass transfer on the GDU, like membrane surface, membrane path length and geometry, membrane porosity and thickness.

Initial conditions were established as 80  $\mu$ L of sample volume injected in a 0.6 mL/min flow stream, using ultrapure water as carrier. As preliminary studies, calibration curves with ethanol standards in a range from 5 to 20% (v/v) were performed at different wavelengths, from 450 to 600 nm. A linear response for all of the wavelengths used was observed. In terms of sensitivity, it was possible to observe a decrease with the augment of the wavelength. At the same time better repeatability was attained for the results obtained at 500 nm and higher wavelengths. As a compromise between sensitivity and repeatability, 500 nm was the chosen wavelength.

Hereupon, the configuration and length of the mass transfer unit used were studied. Three mass transfer units with different configurations

**Table 1**  
Configuration of the mass transfer units studied.

Mass transfer unit	Configuration	Surface area (mm <sup>2</sup> )	Channel depth (mm)
A	Linear	70	0.5
B	Linear	140	0.5
C	Zigzag	140	0.3

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