AUTOMATIC ANALYTICAL MICROSYSTEM FOR THE SPECTROPHOTOMETRIC DETERMINATION OF TITRATABLE ACIDITY IN WINES

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INTRODUCTION Titratable acidity is one of the key parameters of wine due to its closed relationship with both its quality and character. Its control during wine production as well as in the final product is of great interest for wineries in order to ensure the desired characteristics. European and American official organizations, the *Organisation Internationale de la Vigne et du Vin* (OIV) and the Association of Official Analytical Chemists (AOAC International) respectively, propose similar methods for its determination. These methods consist in the titration of samples to a certain pH against a standard alkaline solution by potentiometric or visual titration. However, these methods are laborious and time consuming. Alternatively, other methods, including some flow-based systems, have already been proposed. Despite offering some advantages in comparison to the classical ones, these methods still present some drawbacks, including expensive instrumentation [1], or complex procedures [2], among others. Herein, we propose an automatic continuous flow spectrophotometric microanalyzer for monitoring titratable acidity in wine, displaying low reagents and sample consumption, simple instrumentation, high portability and high throughput.

METHODS The analytical determination is based on the decrease of the blue coloration of a buffered bromothymol blue (BTB) solution when it is mixed with the standard or the sample solution. This change in absorbance is monitored at 607 nm. The whole system consists of three programmable three-way microvalves, a peristaltic pump, a microfluidic platform, and a miniaturized optical detection system. The scheme of the fluidic set-up is depicted in Fig.1.A.

The programmable valves permit the on-line automatic preparation of the different standard solutions, as well as the sample dilution, by multicommutation. The microfluidic platform, which was entirely fabricated using cyclic olefin copolymer (COC) as the substrate material, was constructed by using a 3D-multilayer approach. The fabrication process regarding these type of platforms is described in detail elsewhere [3]. The platform consists of three different inlets, two micromixers and a detection chamber where the absorbance measurements take place (Fig.1.A). Inside the microfluidic platform, a buffered BTB solution is mixed with deionized water. This diluted BTB solution is, then, mixed with the carrier solution where the standard or the samples are injected. The microfluidic platform perfectly fits with the miniaturized optical detection system by using a lock-and-key concept [4]. The detection system consists of a LED emitting at 607 nm and a PIN photodetector mounted in a home-made polymeric structure. The LED and the photodetector are connected to a printed circuit board (PCB), which is connected to a data acquisition card (DAQ).

RESULTS The hydrodynamic conditions for the microsystem were evaluated and optimized, establishing a total flow rate of 1500 µL min^{-1} and a sample injection volume of 10 µL. The chemical composition of the different solutions was also optimized: deionized water was used as the carrier solution as well as for the on-line dilution of the buffered BTB solution (35 mM phosphate buffer solution adjusted to pH 7.8, with 500 ppm BTB). A 0.50 g L^{-1} tartaric acid solution was used as stock solution for the on-line preparation by multicommutation of tartaric acid standard solutions of 0.05, 0.10, 0.20, 0.30 and 0.40 g L^{-1}. Analytical characterization of the microsystem was carried out. Fig.1.B shows the obtained signal record for one of the calibrations. The obtained equation (n=6 and 95% confidence) was Abs = - 0.442 (± 0.002)[tartaric acid] - 0.0045 (± 0.0007), with r^2 = 9995, and detection and quantification limits of 0.01 g L^{-1} and 0.03 g L^{-1} of tartaric acid, calculated as three and ten times the standard deviation of the blank, respectively. The linear working range was established from 0.05 g L^{-1} to 0.50 g L^{-1} of tartaric acid.

In order to demonstrate the proper microsystem operation, 25 wine samples were analysed, and the results were compared to those obtained by the reference method (Table 1). The results showed very good agreement for white and rosé wines, while red wines presented a slight underestimation.

CONCLUSIONS Obtained results demonstrate that the developed analytical microsystem can be used for the automatic quantification of titratable acidity in wines. Further investigation is being carried out for the improvement of the determination for red wines. Additionally, taking profit of the achieved automation of the analytical microsystem, it will be installed in a winery for the on-line continuous monitoring of the titratable acidity during wine production the next harvest season.

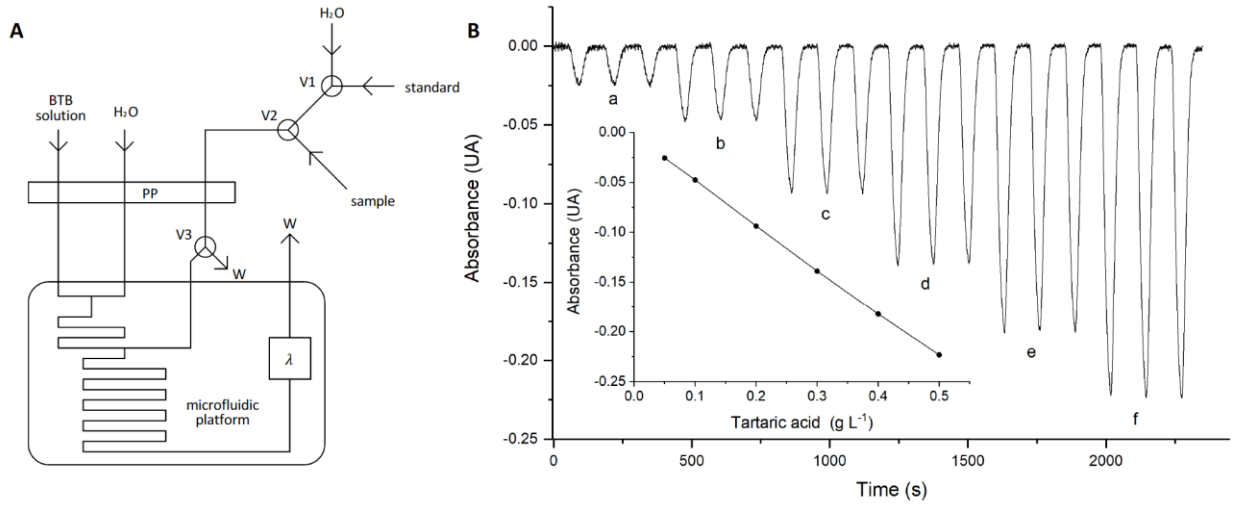

Figure 1. A) Scheme of the fluidic set-up. V: valve, PP: peristaltic pump, W: waste, λ: detection chamber. B) Signal recording and obtained calibration curve for tartaric acid standard solutions of 0.05 g L^{-1} (a), 0.10 g L^{-1} (b), 0.20 g L^{-1} (c), 0.30 g L^{-1} (d), 0.40 g L^{-1} (e) and 0.50 g L^{-1} (f). The obtained equation (n=6, 95% confidence) was Abs = - 0.442 (± 0.002)[tartaric acid] - 0.0045 (± 0.0007), r^2 = 9995.

Table 1. Titratable acidity, expressed as g L^{-1} of tartaric acid, for 25 wine samples.

Sample	Reference method*	Microanalyzer		% Error**
White wine 1	5.6	6.08	± 0.06	9
White wine 2	6	5.9	± 0.1	-2
White wine 3	5.4	5.5	± 0.1	2
White wine 4	6.2	6	± 0.2	-3
White wine 5	4.3	4.3	± 0.1	0
White wine 6	5.7	5.6	± 0.1	-2
Rosé wine 1	4.3	4.3	± 0.1	0
Rosé wine 2	5.7	5.81	± 0.09	2
Red wine 1	5.2	4.93	± 0.04	-5
Red wine 2	5.6	5.4	± 0.3	-4
Red wine 3	5.3	4.7	± 0.2	-11
Red wine 4	5.1	4.5	± 0.06	-12
Red wine 5	5.7	5.3	± 0.2	-7
Red wine 6	6.2	4.8	± 0.2	-23
Red wine 7	6.1	5.3	± 0.1	-13
Red wine 8	6.9	6.3	± 0.1	-9
Red wine 9	7.7	7.2	± 0.2	-6
Red wine 10	6.2	5.9	± 0.1	-5
Red wine 11	7.2	6.5	± 0.1	-10
Red wine 12	5.2	4.71	± 0.09	-9
Red wine 13	5.2	4.7	± 0.2	-10
Red wine 14	5.5	5.03	± 0.08	-9
Red wine 15	5.4	4.49	± 0.08	-17
Red wine 16	6	5.32	± 0.06	-11
Red wine 17	5.9	5.2	± 0.1	-12

* A commercial kit from Biosystems (Spain) was used as the reference method for measuring the titratable acidity.
** % Error is given with the corresponding sign to highlight the underestimation of titratable acidity in red wine samples.

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