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Dual optically responsive paper-membrane for simple, portable, and versatile control of total and volatile acidity in wines

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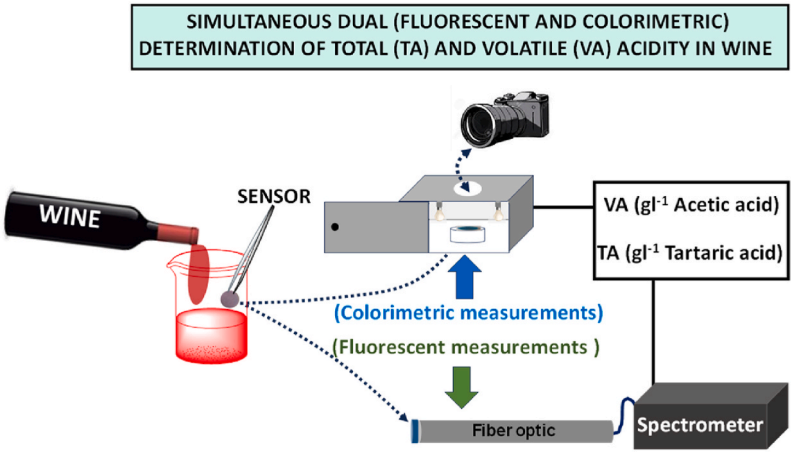
HIGHLIGHTS

- A novel optical sensor for total (TA) and volatile (VA) acidity was developed.
- The sensor allows simultaneous dual (fluorescent and colorimetric) quantification.
- TA and VA of wine samples were successfully determined.
- The sensor is simple, portable, fast, robust, cheap, reversible, and reusable.
- The sensor does not require additional reagents.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: The high consumption of expensive and polluting reagents and the use of tedious reactions with long analysis times are key problems in the quantification of total (TA) and volatile acidity (VA) in wine by official methods (titration with sodium hydroxide and enzymatic method).
Results: We have exploited the advantages and versatility of our optically responsive paper-membrane (Paper-FM) to perform the dual simultaneous determination (fluorescent and colorimetric) of TA and VA in wine. The technology was tested by performing the simultaneous double quantification (fluorescence and colorimetry) of TA and VA of wine samples from different origins, obtaining an excellent correlation between the results obtained by colorimetry and those obtained by fluorescence. Furthermore, the results were successfully validated

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Optical sensors
Fluorescence

using official reference methods (titration with sodium hydroxide and enzymatic method) in an accredited laboratory.
Significance: We have developed the first optical sensing method that allows the quantification of the TA and VA in wine. The results presented in this work demonstrate that our optical sensing technology enables direct, simple, reversible, reusable, fast, cost-effective, and environmentally friendly simultaneous dual quantification (fluorescent and colorimetric) of TA and VA in wine samples without the use of additional reagents.

1. Introduction

There are six main organic acids in wine, and their concentrations decrease in the following order: tartaric acid > malic acid > succinic acid > lactic acid > acetic acid [1]. Acidity is one of the most important parameters in winemaking because it not only gives the wine a fresh taste but also balances it and helps to stabilize its color. The acid of wine also acts as a natural preservative and is particularly important during fermentation, when acidity helps inhibit the growth of bacteria that could spoil the wine [1].

The procedure for determining the acidity of wines varies according to the official method used: the method of the Office International de la Vigne et du Vin (OIV, 1969), used in Europe, or the method of the Association of Official Analytical Chemists (AOAC), used in the United States [2]. The official methods proposed by the OIV [3–6] are based on titration against an alkaline standard solution. For the determination of total acidity (TA) in wine, the method describes direct titration with a comparison with an end-point color standard (OIV-MA-AS313-01); for volatile acidity (VA), the titration is carried out after steam distillation (OIV-MA-AS313-02). Acetic acid is the main component of VA in wines, and its concentration is critical for the quality of wines. In wine, the levels of VA (expressed as gl^{-1} of acetic acid) are usually below 0.72 gl^{-1}, above which the aroma of the wine begins to be affected, and the taste begins to deteriorate; at acetic acid concentrations of 0.90 gl^{-1} and above, the wine has a noticeably 'harsh', 'bitter' and 'sour' aftertaste [7]. According to European legislation, VA in wines should remain below 20 milliequivalents per Liter, i.e., 1.2 gl^{-1}, [4,8]. The concentration of carbonic acid and sulfur dioxide present in the wine does not contribute to the final TA and VA values; thus, these molecules are interferents present in most wines whose concentration must be removed or subtracted from the final TA and VA values, complicating titration-based analytical methods [3,9]. Although fast, simple, and inexpensive analytical techniques (sensors/biosensors) have made great strides in recent years, surprisingly, there is no such method for determining TA and VA in wine, leaving classical titration as the only method capable of accurately cost-effective determining TA and VA in a relatively short time [3,4,7–9]. However, titration-based analytical methods have some drawbacks, such as visual identification of the endpoint can lead to quantification errors, is non-eco-friendly (producing corrosive and toxic sodium hydroxide solutions containing disposable pH colorimetric dyes), does not allow for quick and easy analysis integrated into the production area, and is time-consuming: samples must be collected, transported, and stored, and the analysis must also be performed in a laboratory by a specialized technician [7,8]. Enzymatic methods have been developed as an alternative to titration to specifically determine the concentration of acetic acid (VA). However, enzymatic methods can have significant drawbacks, such as expensive and unstable reagents (enzymes), and long analysis times (due to the use of slow enzymatic cascade reactions). In addition, many molecules present in wine can interfere with or inhibit enzymatic reactions (enzyme selectivity is not 100 % in these cases), making enzymatic methods unreliable, poorly reproducible, and much less accurate than titration-based methods [10]. Therefore, the development of simple and compact sensors capable of easily monitoring TA and VA in wine samples would provide the wine industry with a valuable tool for process and quality control [11]. In our previous work [12], we developed, for the first time to our knowledge, an optically responsive paper membrane (Paper-FM) capable of providing a dual response (fluorescent and colorimetric) to the total acid concentration in solution (this technology has recently been protected by a European patent application: EP24382670.8). Paper-FM was

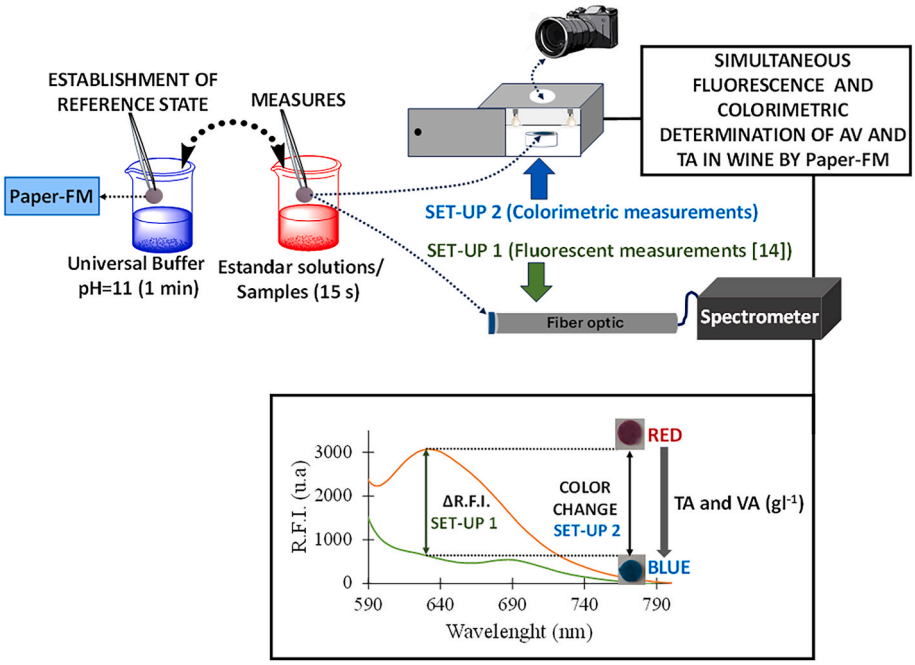


Fig. 1. Schematic of the measurement protocol of the TA and VA with both setsups (colorimetric and fluorescent) using Paper-FM.

successfully used to fabricate an innovative fluorescent fiber optic sensor for simple, reversible, reusable, fast, cost-effective, and environmentally friendly quantification of TA (expressed as gl^{-1} of acetic acid) in vinegar samples. In this work, Paper-FM has been implemented in two setups to perform the simultaneous double determination (fluorescent and colorimetric) of TA and VA in wine samples of different origins. In addition, the results were successfully validated by an externally accredited reference laboratory using the official methods: OIV-MA-AS313-01, OIV-MA-AS313-02, and the enzymatic method. The results reported in this work demonstrate both the optical versatility (double simultaneous response: colorimetric and fluorescence) of our innovative detection technology and its advantages over the current official methods (OIV-MA-AS313-01, OIV-MA-AS313-02, and enzymatic method) for determining TA and VA in wine samples.

2. Materials and methods

2.1. Chemicals and materials

Paper-FM was synthesized as described in our previous work [12], glacial acetic acid (AA), tartaric acid (TT), sodium carbonate (SC), citric acid (CA), potassium dihydrogen phosphate (PDP), tris(hydroxymethyl) aminomethane (TRIS), potassium chloride (KCl), sodium tetraborate decahydrate (STBDH), sodium hydroxide (NaOH), and hydrochloric acid (HCl) were purchased from Sigma-Aldrich, cellulose filter paper (ref. 13,058: 73 gm^2 and 170 μm thickness) was purchased from FILTERLAB® (Barcelona, Spain), and charcoal decolorizing (Cod. 434,507) was purchased from Grupo Montedison CARLO ERBA (Divisione Chimica/Milano/Italia). Throughout the experiment, all aqueous solutions were prepared using reverse osmosis-type quality water (Milli-RO 12 plus Milli-Q station from Millipore, conductivity 18.2 μΩ cm). Seven wine samples of different origins were purchased at different local stores.

2.2. Equipment

Two setups were used to measure the dual optical response (fluorescent and colorimetric) of Paper-FM (see Fig. 1).

1) Fiber optic setup for fluorescence measurements. The fiber optic setup designed and fabricated in our previous work for the fluorescent measurements of Paper-FM in the analysis of TA in vinegar was also used in this work to perform the fluorescence analysis of TA and VA in wine: The fiber optic setup is based on a 0.5-mm-diameter bifurcated optical probe connected to a portable high-resolution spectrometer equipped with seven excitation LED sources. Paper-FM was integrated into the setup using a homemade interface made by 3D printing [12]. The excitation and emission wavelengths used for Paper-FM were λ_{exc} = 523 nm and λ_{em} = 630 nm, respectively.
2) Setup for colorimetric measurements. The colorimetric analysis of TA and VA in wine was performed using a Canon Powershot G12 digital camera (Japan) placed inside of a homemade wooden enclosure [12] illuminated with two LED lamps (4.6 W, 6000 K, illumination inside of the box = 9680 Lx) positioned at 45° to the digital camera to minimize any interference from external light. The optimized settings used to photograph the sensing membrane were ISO 80, F 5.6, shutter speed January 1600 s, aperture value f/8, focal distance 11 mm; white balance, automatic; resolution, 3648 × 2432; mode, macro. To evaluate the color change, a photograph was taken in JPEG format. RGB and HSV color space coordinates were obtained from the region of interest (ROI) of the digitized membrane using Image J software, and the B coordinate was selected as the analytical signal.

2.3. Measurement protocols

2.3.1. Fluorescent and colorimetric quantification of TA in wine samples

First, standard solutions (0.6 gl^{-1}, 1.30 gl^{-1}, 2 gl^{-1}, 2.70 gl^{-1}, 3 gl^{-1}, and 3.30 gl^{-1}) of TT in purified water were prepared. Subsequently, the respective calibration curves (fluorescent and colorimetric) were performed according to the protocol described in our previous work [12]. First, a piece (8 × 2.5 cm) of Paper-FM was synthesized and cut into circles of 0.9 cm diameter. The fluorescent and colorimetric reference states ($F_{(Ref.Est)}$ and $B_{(Ref.Est)}$) of Paper-FM were established by introducing the membrane in universal buffer (pH 11, 0.05 M) for 1 min (*Universal buffer 0.05 M: Tris (3.02 g), citric acid (5.25 g), sodium tetraborate decahydrate (9.53 g), potassium dihydrogen phosphate (3.54 g), and potassium chloride (1.86 g) were dissolved in* 500ml*of water, and then the* pH*was adjusted to 11 using a solution of NaOH*).

Both calibrations (fluorescent and colorimetric) were simultaneously carried out as follows: A 0.9-cm diameter circle of Paper-FM was immersed in universal buffer (pH 11, 0.05 M) for 1 min, and the fluorescent and colorimetric referent states were measured using the respective setups described in section 2.2. Paper-FM was then immersed in the corresponding TT standard solution for 15 s, and both responses (fluorescent: F_x and colorimetric: B_x; x = gl^{-1} of TT of the standard solution) were measured using the setups described in section 2.2. The measurement cycle universal buffer pH 11 (1 min) ↔ TT standard solution (15 s) was repeated with all the TT standard solutions. Subsequently, calibration curves (fluorescent and colorimetric) were obtained by representing the optical responses of Paper-FM (fluorescent: $\Delta F = F_{(Ref.Est)} - F_x$ and colorimetric: $\Delta C = B_{(Ref.Est)} - B_x$) versus the concentration of TT in the standard solution (x).

The TA measurements of wine samples were performed following the same procedure used for the calibration curve: Firstly, fluorescent and colorimetric reference states were obtained by introducing a 0.9-cm diameter circle of Paper-FM into the universal buffer (pH 11, 0.05 M) for 1 min. The membrane was then introduced into the diluted wine sample (1/3.5, wine/distilled water, v/v) for 15 s, and its fluorescence and colorimetric response (F_{Sx} and B_{Sx}; S_x = sample x) were measured using the setups described in section 2.2. The results of TA (expressed as gl^{-1} of TT) were obtained by extrapolating the optical responses of Paper-FM in the wine samples (fluorescent: $\Delta F = F_{(Ref.Est)} - F_{Sx}$ and colorimetric: $\Delta C = B_{(Ref.Est)} - B_{Sx}$) on the corresponding calibration curve. White wine samples were directly measured without any sample pre-treatment. However, in the case of red wine samples, color interferes negatively with the quantification of TA, but color interference was eliminated by a simple and short decolorization protocol (see **Protocol-S-1**).

2.3.2. Fluorescence and colorimetric quantification of VA in wine samples

Considering that the main volatile acid in wine is acetic acid, for the quantification of VA, the calibration curve was performed with AA standard solutions (0.3 gl^{-1}, 0.50 gl^{-1}, 0.8 gl^{-1}, 1 gl^{-1}, 1.3 gl^{-1}, 1.8 gl^{-1}, 2.00 gl^{-1} and 2.3 gl^{-1}) following the same protocol as described above for the calibration with TT standard solutions. Then, VA measurements of the wine samples were performed in two steps: **i)** According to the official method (OIV-MA-AS313-02), the volatile acids present in the wine (mainly acetic acid) were previously separated by distillation, using the following protocol: *1.25 g of tartaric acid was dissolved in* 50ml*of wine, and then the wine sample was distilled until* 40ml*of distillate were collected*, **ii)** After distillation, VA (expressed as AA gl^{-1}) was directly measured in the distillate (without any dilution) following the same protocol as that used above to measure TA. In the case of VA, all wine distillates are practically colorless, so any sample pre-treatment is necessary, and VA measurements were directly performed on the distillates. All measurements were performed in triplicate and the measurement error was calculated as $st/\sqrt{n}$ [12]: where *s* is the standard deviation of the three measurements, *t* is the *Student's t-test* at 95 % probability and *n-1* degrees of freedom, where *n* is the total number of

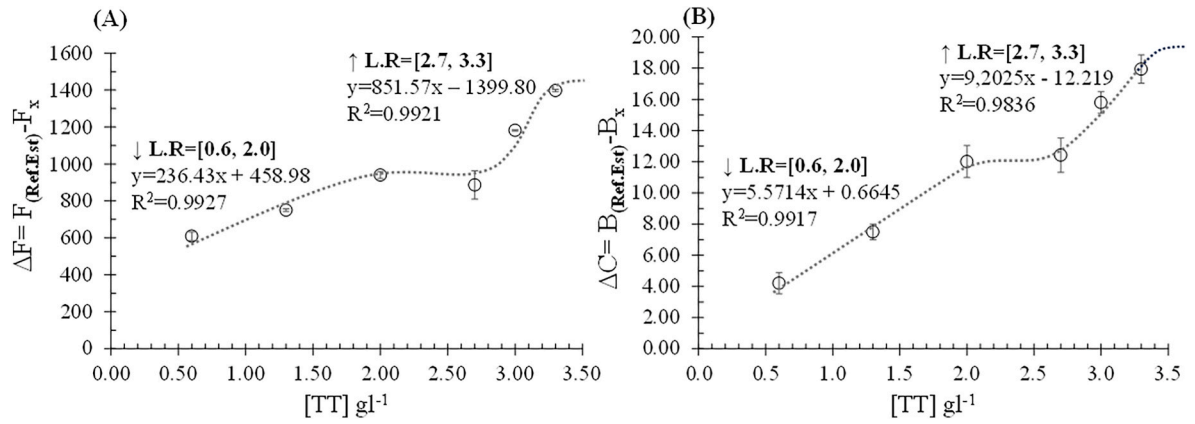


Fig. 2. Fluorescent response ($\Delta F=F_{(Ref.Est)}-F_x$) vs. TT gl^{-1} (A), and colorimetric response ($\Delta C=B_{(Ref.Est)}-B_x$) vs. TT gl^{-1} (B).

measurements (3). Fig. 1 shows a schematic of the measurement protocol with both setups (colorimetric and fluorescent).

2.4. Validation

To validate the results obtained with our technology, the accuracy of the method was determined by studying trueness and precision. To check the trueness of the TA and VA methods, all the wine samples were also analyzed using official methods (titration and enzymatic method) by an international laboratory accredited and authorized to carry out official analysis in the wine sector (Dolmar laboratory, Tentamus company, https://www.tentamus.es/). Repeatability and reproducibility studies were carried out to check the precision.

3. Results and discusión

3.1. Fluorescent and colorimetric calibration of Paper-FM for quantifying TA and VA in wine

In this work, we intend to go one step further to demonstrate how the innovative optical technology (Paper-FM), which was previously developed for the fluorescent quantification of TA in vinegar [12], can also become an extremely useful and versatile tool for the wine industry because it presents important advantages in the determination of both TA and VA compared to the classical official methods [5,6]. Paper-FM exhibits a dual optical response by simultaneously changing its fluorescence and color based on the total acid concentration of the sample (see Fig. 1). In our previous work, we only exploited the fluorescence response of Paper-FM to perform a simple, rapid, and reagent-free in situ determination of TA in vinegar (expressed as gl^{-1} of AA) [12]. In this work, we go further andexploit the optical versatility of Paper-FM to perform simultaneous colorimetric and fluorescence detection of TA and VA in wine. Considering that the main acid in vinegar is AA, in our previous work, we used standard solutions of AA to establish a calibration curve for the quantification of TA in vinegar [12]. However, the major acid in wine is TT, so for the determination of TA in wine, both calibration curves (colorimetric and fluorescent) were studied using TT standard solutions. The colorimetric and fluorescent response of Paper-FM versus the concentration of TT was measured with the respective setups described in section 2.2 (see Fig. 1), following the measurement protocol described in section 2.3.1. In the case of colorimetric calibration, RGB and HSV color space coordinates were obtained and studied, and the B (blue) coordinate was the one that presented the best analytical performance (see Supplementary Material (SM): Fig. S-1). Therefore, B (blue) coordinate was selected as analytical signal. Fig. 2 shows the fluorescent ($\Delta F=F_{(Ref.Est)}-F_x$) and colorimetric ($\Delta C=B_{(Ref.Est)}-B_x$) responses of Paper-FM (see section 2.3) versus the concentration of TT in standard solutions (calibration curves). The calibration curves in Fig. 2 shows a sigmoidal behaviour. This behaviour is common in heterogeneous phase reactions where the immobilised fluorescent probe can be found in different physicochemical environments within the membrane. The calibration in Fig. 2 is based on the protonation rate of Nile Blue covalently immobilised on a porous paper membrane. This reaction is governed by complex mechanisms of proton diffusion through the membrane. Therefore, the sigmoidal behaviour of the calibration curve in Fig. 2 may be due to the presence of immobilised Nile Blue in two different physicochemical environments within the membrane. The lower linear range could correspond to Nile Blue immobilised inside large pores (micropores) with a very polar microenvironment in which water, and thus protons, can diffuse very easily,

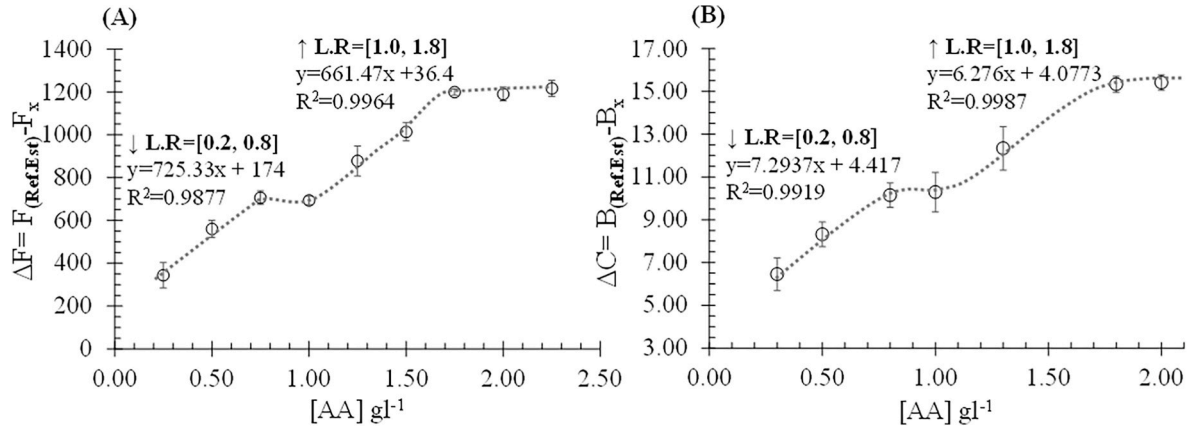


Fig. 3. Fluorescent response ($\Delta F=F_{(Ref.Est)}-F_x$) vs. AA gl^{-1} (A), and colorimetric response ($\Delta C=B_{(Ref.Est)}-B_x$) vs. AA gl^{-1} (B).

Table 1
Analytical parameters of the calibrations performed with the linear ranges of Figs. 2 and 3.

Ref.	R^2	LOD[a] (gl^{-1})	LOQ[a](gl^{-1})
↓L.R, Fluorescence (TT gl^{-1}); (Fig. 2A)	0.9927	0.04	0.13
↑L.R, Fluorescence (TT gl^{-1}); (Fig. 2A)	0.9921	0.05	0.15
↓L.R, Colorimetry (TT gl^{-1}); (Fig. 2B)	0.9917	0.05	0.17
↑L.R, Colorimetry (TT gl^{-1}) (Fig. 2B)	0.9836	0.03	0.11
↓L.R, Fluorescence (AA gl^{-1}) (Fig. 3A)	0.9877	0.04	0.14
↑L.R, Fluorescence (AA gl^{-1}) (Fig. 3A)	0.9964	0.05	0.15
↓L.R, Colorimetry (AA gl^{-1}) (Fig. 3B)	0.9919	0.04	0.14
↑L.R, Colorimetry (AA gl^{-1}) (Fig. 3B)	0.9987	0.05	0.17

[a] Detection and quantification limits were determined using the IUPAC method (LOD = $3s_b$/m; LOQ = 10 s_b/m). The standard deviation of the blank (sb) was obtained by measuring (10 measurements) the fluorescent and colorimetric response of Paper-FM for a pH = 11, 0.05 M universal buffer solution.

while the second linear range could correspond to Nile Blue immobilised in smaller pores (nanopores) with a less polar environment in which water (protons) has more difficulties to diffuse.

On the other hand, VA in wine is defined as the total concentration of volatile acids expressed as gl^{-1} of AA. Therefore, the determination of VA is carried out in two steps (see section 2.3.2): **i)** Volatile acids of wine are previously separated by distillation, **ii)** VA is directly measured in the distillate. Considering that the main volatile acid of wine is AA, in the case of VA, calibration curves (fluorescence and colorimetric) were obtained using standard solutions of AA. The colorimetric and fluorescent response of Paper-FM versus the concentration of AA was measured using the respective setups described in section 2.2 (see Fig. 1), following the measurement protocols described in section 2.3.2. In the case of colorimetric calibration, the B (blue) coordinate was selected as the analytical signal. Fig. 3 shows the calibration curves for VA: fluorescent ($\Delta F=F_{(Ref.Est)}-F_{Sx}$) and colorimetric ($\Delta C=B_{(Ref.Est)}-Bs_x$) responses of Paper-FM (see section 2.3), versus the concentration of AA in standard solutions.

In Figs. 2 and 3 it can be observed that for AA and TT, both responses of Paper-FM (fluorescent and colorimetric) show two different linear ranges, one of them in a low acid concentration interval (**↓L.R**) and another one in a higher concentration interval (**↑L.R**). Table 1 shows the analytical parameters (correlation coefficient: R^2, detection limit: LOD, and quantification limit: LOQ) of the calibration curves corresponding to all linear ranges of Figs. 2 and 3. Since the values of TA and VA in wine are typically between 3 and 5 gl^{-1} of TT, and between 0.2 and 0.6 gl^{-1} of AA, respectively [7], any of the linear ranges in Figs. 2 and 3 would be useful (in terms of linearity and sensitivity: see Table 1) for the determination of TA and VA in wine.

3.2. Stability study of Paper-FM

In our previous work, the repeatability and reproducibility of the fluorescent response ($\Delta F=F_{(Ref.Est)}-F_x$) of Paper-FM in AA solutions were studied, demonstrating excellent reutilization capability: a single 0.9-cm diameter membrane can be used for more than 50 consecutive fluorescent measurements of AA concentration, and can also be stored and reused for at least 18 days [12]. In order to extend the stability study, in this work, we studied the repeatability and reproducibility of the colorimetric response of Paper-FM in AA solutions, and the repeatability and reproducibility of both fluorescent and colorimetric responses in TT solutions. Repeatability was studied at four concentration levels (10 measurements were performed for each concentration), and all measurements (50) were performed consecutively using a single membrane with a diameter of 0.9 cm. Reproducibility was studied for a single concentration level for several days (all measurements were performed using the same 0.9-cm membrane, and after each measurement, the membrane was dried and stored in the absence of light), and the results were interpreted using a Shewhart diagram (reproducibility was defined as the signal that remains within the control lines on the Shewhart chart). As shown in Fig. 4, the colorimetric response of Paper-FM in AA solutions shows excellent stability, similar to that found in our previous work for the fluorescent response in AA solutions [12]: a single 0.9-cm diameter membrane can be used to perform at least 50 consecutive colorimetric measurements of the concentration of AA with a repeatability of less than or equal to 5 %, and can be stored and reused for at least 25 days without the need for recalibration (although after 25 days the limits set in the Shewhart control chart are exceeded, the membrane is still responsive, and thus it could be recalibrated and used further).

However, for the colorimetric and fluorescent response of Paper-FM in TT solutions, only seven consecutive measurements with repeatability less than or equal to 5 % can be made; from the seventh measurement onwards, both responses (colorimetric and fluorescent) showed coefficients of variation greater than 10 %. In terms of reproducibility, in TT solutions, on the second day both responses (fluorescence and colorimetric) exceed the limits established in the Shewhart control chart. Therefore, from a reusability point of view, Paper-FM is less efficient in TT solutions than in AA solutions. This may be because TT is a non-volatile dicarboxylic acid that can interact more strongly than acetic acid with the Nile blue molecule immobilised on the membrane (Nile blue has two tertiary amines and one secondary amine in its structure). An increase in the acid-base interaction strength between Nile blue and TT would lead to a progressive accumulation of TT on the Paper-FM after each measurement, leading to a decrease in the number of effective consecutive measurement cycles. Anyway, from a commercial point of view, the reusability of Paper-FM in TT solutions can be considered quite efficient (seven measurements per membrane).

The optical detection mechanism of Paper-FM is based on the kinetics of the protonation reaction of the immobilised pH probe (Nile blue) and therefore, as was demonstrated in our previous work, the kinetic parameters (rate constants and diffusion coefficients) of the Nile blue protonation reaction can be affected by temperature [12]. In our

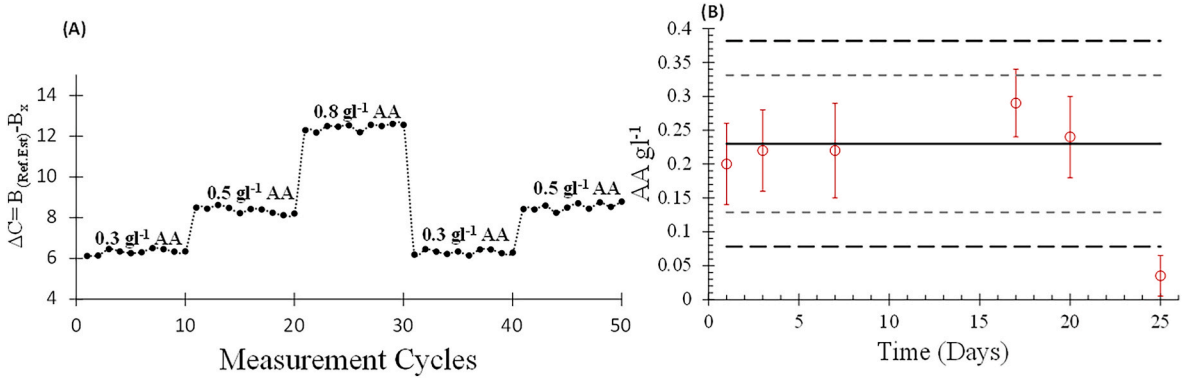
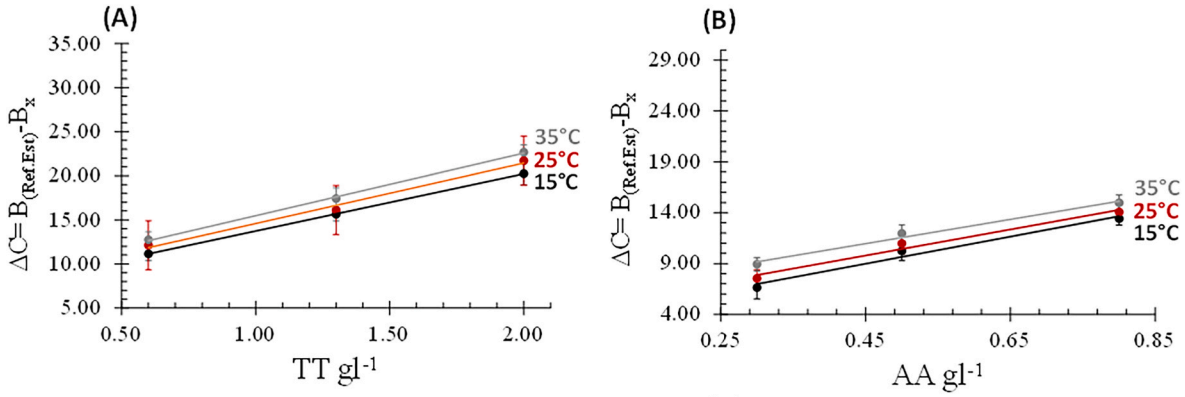


Fig. 4. Repeatability and reproducibility of the colorimetric response ($\Delta C=B_{(Ref.Est)}-B_x$) of Paper-FM in AA solutions.



(D)

*S.R	T (°C)	b	o.o	R^2
$\Delta C=B_{Ref.Est}-B_x$ (TT)	15	6.49	7.25	0.9999
$\Delta C=B_{Ref.Est}-B_x$ (TT)	25	6.85	7.74	0.9904
$\Delta C=B_{Ref.Est}-B_x$ (TT)	35	7.09	8.39	0.9990
$\Delta C=B_{Ref.Est}-B_x$ (AA)	15	13.35	2.99	0.9776
$\Delta C=B_{Ref.Est}-B_x$ (AA)	25	12.82	4.02	0.9789
$\Delta C=B_{Ref.Est}-B_x$ (AA)	35	11.94	5.59	0.9863
$\Delta F= F_{Ref.Est}-F_x$ (TT)	15	197.86	474.12	0.9981
$\Delta F= F_{Ref.Est}-F_x$ (TT)	25	201.79	526.85	0.9956
$\Delta F= F_{Ref.Est}-F_x$ (TT)	35	164.29	712.76	0.9992

*S.R.= bx + o.o: S.R=sensor resoponse; x= acid concentration (TT or AA).

Fig. 5. Thermal stability of the colorimetric response ($\Delta C=B_{(Ref.Est)}-B_x$) of Paper-FM in TT solutions **(A)**, in AA solution **(B)**, thermal stability of the fluorescent response ($\Delta F= F_{(Ref.Est)}-F_x$) of Paper-FM in TT solutions **(C)**, and fitting parameters (ordinate at the origin, (*o.o*), slope (*b*), and R^2 of the calibration curves at each temperature **(D)**.

previous work, we studied the effect of T on the fluorescent response of Paper-FM in AA solutions. In this work, we have completed the study of the effect of T on the response of Paper-FM. For this purpose, the following cases have been studied: **i)** Effect of T on the colorimetric response of Paper-FM in AA solutions, **ii)** Effect of T on the fluorescent response of Paper-FM in TT solutions, **iii)** Effect of T on the colorimetric response of Paper-FM in TT solutions. Surprisingly, as shown in Fig. 5, the colorimetric response ($\Delta C=B_{(Ref.Est)}-B_x$) of paper-FM in both AA and TT solutions is very robust, and it was not significantly affected by temperature. However, as in the case of the fluorescent response in AA solutions [12], above 25 °C, the fluorescent response ($\Delta F= F_{(Ref.Est)}-F_x$) of Paper-FM in TT solutions is also affected by temperature: at 35 °C good linearity is still maintained, but the slope (sensitivity) change significantly. Nevertheless, the detection and quantification limits of the fluorescent response of Paper-FM in TT solution at 35 °C (grey line of Fig. 5C) were 0.05 TT $gl^{-1}$and 0.16 TT gl^{-1} respectively, which is well below the typical TA values in wine (TA in wine is between 3 and 5 TT gl^{-1}).

3.3. Determination of TA and VA in wine samples of different origins: validation

To evaluate the effectiveness (trueness) of our optical sensing technology in the analysis of TA and VA in wine, seven wine samples from different origins were analyzed simultaneously by fluorescence and colorimetry using Paper-FM, and the results were compared with those obtained by an accredited reference laboratory (Dolmar laboratory, Tentamus company, https://www.tentamus.es/) using the official methods: OIV-MA-AS313-01(**Vol.1**) for TA, and OIV-MA-AS313-02 (**Vol.2**) and the enzymatic method (**Enz**) for VA. The determination of the TA of red wines by Paper-FM is affected by the red color of the samples. The composition of red wine is much more complex than white wine. In red wine, in addition to the pigments that give it its characteristic red color (anthocyanins, flavonoids, and catechins: most of them are not only colored but also fluorescent), there may also be other polyphenols, organic acids, and minerals that are not present in white wines. Many of the compounds characteristic of red wines are adsorbed on Paper-FM and interfere with the membrane response to the total acid concentration. However, as in the case of strongly colored vinegars [12], interference due to color was easily and quickly removed by decolorizing the samples with activated carbon (see **SM**: **Protocol-S1)**. In the case of VA, all distillates (red and white wine distillates) are practically colorless, so there is no need to treat the samples, and measurements are made directly on the distillates.

Simultaneous determination (colorimetric and fluorescent) of TA and VA in the wine samples was performed according to the protocols described in section 2.3, using the fluorescent and colorimetric setups described in section 2.2. (see Fig. 1). As was demonstrated in section 3.2, in TT solutions, each 0.9-cm-diameter membrane can be used for only seven consecutive measurements. Therefore, to establish appropriate measurement conditions for the quantification of TA (expressed as gl^{-1} of TT) in the wine samples, a piece of 2.5 cm × 9 cm (22.5 cm^2) of Paper-FM was synthesized following the protocol described in Ref. [12] and was then cut into 18 membranes of 0.9 cm diameter. For the colorimetric and fluorescent calibration curves, three membranes were randomly selected from the batch, and each membrane was used to perform six replicates of each of the three TT concentrations of the calibration. The remaining 15 membranes from the batch were used to measure the wine samples (three replicates per sample). Due to the high reusability of Paper-FM in AA solutions, for the quantification VA (expressed as gl^{-1} of

Table 2
VA and TA values of wine samples obtained with Paper-FM by fluorescence and those obtained from an accredited reference laboratory.

WINE TYPE	BRAND	TA (TT gl^{-1}) by Paper-FM (FLUORESCENT)	TA (TT gl^{-1}) by official method Vol.1	VA (AA gl^{-1}) by Paper-FM (FLUORESCENT)	VA (AA gl^{-1}) by official methods: Enz and Vol.2
RED	Viña Albali, Rioja crianza 2019	4.58 ± 0.02	4.44 ± 0.08	0.58 ± 0.03	**Enz** = 0.49 ± 0.06 **Vol.2** = 0.55 ± 0.08
	Arnum, tinta de Toro, 2023	4.34 ± 0.16	4.39 ± 0.09	0.56 ± 0.04	**Enz** = 0.46 ± 0.06 **Vol.2** = 0.50 ± 0.08
	Viña Carpio, Ribera del Duero 2022	4.57 ± 0.15	4.78 ± 0.13	0.45 ± 0.07	**Enz** = 0.47 ± 0.06 **Vol.2** = 0.52 ± 0.08
	DaVida, La Mancha 2022	6.37 ± 0.10	6.19 ± 0.13	0.64 ± 0.04	**Enz** = 0.65 ± 0.08 **Vol.2** = 0.68 ± 0.10
WHITE	Casa de Luna, Rueda Verdejo, 2023	6.56 ± 0.30	6.82 ± 0.15	0.28 ± 0.06	**Enz** = 0.22 ± 0.04 **Vol.2** = 0.26 ± 0.04
	Beso de Luna, Rioja 2023	6.50 ± 0.17	6.70 ± 0.18	0.35 ± 0.06	**Enz** = 0.27 ± 0.04 **Vol.2** = 0.33 ± 0.05
	Vino Fidencio, La Mancha	4.89 ± 0.11	4.99 ± 0.23	0.33 ± 0.03	**Enz** < 0.10 ± 0.05 **Vol.2** < 0.20 ± 0.10

Table 3
VA and TA values of wine samples obtained with Paper-FM by colorimetry and those obtained from an accredited reference laboratory.

WINE TYPE	BRAND	TA (TT gl^{-1}) by Paper-FM (COLORIMETRY)	TA (TT gl^{-1}) by official method Vol.1	VA (AA gl^{-1}) by Paper-FM (COLORIMETRY)	VA (AA gl^{-1}): BY official methods: Enz and Vol. 2)
RED	Viña Albali, Rioja crianza 2019	4.50 ± 0.60	4.44 ± 0.08	0.51 ± 0.15	**Enz** = 0.49 ± 0.06 **Vol.2** = 0.55 ± 0.08
	Arnum, tinta de Toro 2023	4.39 ± 0.09	4.39 ± 0.09	0.55 ± 0.08	**Enz** = 0.46 ± 0.06 **Vol.2** = 0.50 ± 0.08
	Viña Carpio, Ribera del Duero 2022	4.78 ± 0.59	4.78 ± 0.13	0.52 ± 0.13	**Enz** = 0.47 ± 0.06 **Vol.2**= 0.52 ± 0.08
	DaVida, La Mancha 2022	6.09 ± 0.45	6.19 ± 0.13	0.71 ± 0.15	**Enz** = 0.65 ± 0.08 **Vol.2** = 0.68 ± 0.10
WHITE	Casa de Luna, Rueda Verdejo, 2023	6.06 ± 0.58	6.82 ± 0.15	0.20 ± 0.07	**Enz** = 0.22 ± 0.04 **Vol.2** = 0.26 ± 0.04
	Beso de Luna, Rioja 2023	6.49 ± 0.38	6.70 ± 0.18	0.38 ± 0.14	**Enz** = 0.27 ± 0.04 **Vol.2** = 0.33 ± 0.05
	Vino Fidencio, La Mancha	5.16 ± 0.23	4.99 ± 0.23	0.22 ± 0.05	**Enz** < 0.10 ± 0.05 **Vol.2** < 0.20 ± 0.10

Table 4
Correlation coefficients calculated by Ref. [13].

Paper-FM vs. Official method	R^2
TA by Paper-FM (FLUORESCENT RESPONSE) *vs.* **Vol.1**	0.9892
VA by Paper-FM (FLUORESCENT RESPONSE) *vs.* **Enz**	0.9762
VA by Paper-FM (FLUORESCENT RESPONSE) *vs.* **Vol.2**	0.9872
TA by Paper-FM (COLORIMETRY RESPONSE) *vs.* **Vol.1**	0.9901
VA by Paper-FM (COLORIMETRY RESPONSE) *vs.* **Enz**	0.9595
VA by Paper-FM (COLORIMETRY RESPONSE) *vs.* **Vol.2**	0.9835

AA), a single 0.9-cm diameter membrane was used for 21 consecutive measurements (three points for the calibration curve and 4 wine samples: three replicates for each measurement). Table 2, and Table 3 show the values of VA and TA of the wine samples calculated by colorimetry and fluorescent using Paper-FM, and those obtained by the accredited reference laboratory using the official methods.

Table 4 shows the correlation coefficients (calculated by Ref. [13]; see **SM**: Fig. S-2) obtained by comparing the VA and TA results obtained by fluorescence and colorimetry using Paper-FM and those obtained by the reference laboratory indicated an excellent correlation between our optical technology (in its two modes: fluorescence and colorimetry) and the official methods.

Therefore, the results presented in Figs. 2, 3, 4, and 5 and Tables 1, 2, and 3 demonstrate that Paper-FM allows simple, fast, cost-effective, and environmentally friendly on-site simultaneous quantification (fluorescent and colorimetric) of TA and VA in wine (without the need for additional expensive or contaminating reagents).

4. Conclusions

The optical sensing technology presented in this work represents the first optical sensor capable of carrying out the quantification of TA and VA in wine. The results reported demonstrate both the optical versatility (double simultaneous response: colorimetric and fluorescence) of the technology, and its advantages over the current official methods (titration methods: OIV-MA-AS313-01, OIV-MA-AS313-02, and enzymatic method) for determining TA and VA in wine. The technology has been used to analyze the TA and VA of wine samples of different origins, and the results have been successfully validated by an accredited reference laboratory using official methods. In comparison with the official methods, our technology offers several important advantages: **i)** It is a simple method in which the measurements are performed by introducing Paper-FM directly into the sample (only in the case of the quantification of TA in red wine, a fast, simple and inexpensive decolorization protocol is necessary), **ii)** The values of TA and VA are obtained in 15 s: the classical titration methods require 10–15 min per sample, and the enzymatic method up to 20 min, **iii)** It is environmentally friendly as it does not require additional reagents: titration methods consume many reagents and generate corrosive and toxic sodium hydroxide solutions containing disposable pH dyes, and enzymatic methods require extremely expensive and unstable reagents (enzymes and coenzymes), **iv)** It is cheap, reversible, reusable and, extremely stable, **v)** Paper-FM could be easily implemented in a miniaturized optoelectronic analytical device. Therefore, this work complements our previous work by demonstrating the optical versatility of Paper-FM (it is useful for both colorimetric and fluorescent measurements) and

extending its applicability to the wine industry. We are currently working on the implementation of Paper-FM in a *"miniaturized"*, compact and portable colorimetric measurement device with a built-in micro-distillation system (for distillation of sample volumes between 5 and 10 ml) to perform in situ quantification of TA and VA in wine.

CRediT authorship contribution statement

Melany G. López Aveiga: Writing – review & editing, Visualization, Validation, Investigation, Formal analysis, Conceptualization. **María Dolores Fernández Ramos:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. **Vanesa Martos Núnez:** Resources, Funding acquisition. **Antonio González Casado:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Conceptualization. **Antonio L. Medina Castillo:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Antonio L. Medina Castillo has patent OPTICAL KINETIC METHOD FOR THE DETERMINATION OF TOTAL OR VOLATILE ACIDITY pending to 2024_57 EP. There is nothing more to declare. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2025.344016.

Data availability

Data will be made available on request.

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