Sensors & Actuators: B. Chemical 417 (2024) 136214

Contents lists available at ScienceDirect

Sensors and Actuators: B. Chemical

journal homepage: www.elsevier.com/locate/snb

# Portable fiber-optic sensor for simple, fast, cost-effective, and environmentally friendly quantification of total acidity in real-world applications

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ARTICLE INFO

*Keywords:*
Optical fiber sensor
Fluorescent pH-probe
Paper membrane
Food analysis
Vinegar
Total acidity

ABSTRACT

In this work, an innovative fiber-optic sensor for measuring total acidity (TA: expressed as the % w/v of acetic acid) in beverages was developed. For this purpose, we relied on the kinetic principles of heterogeneous solid-phase reactions (slow reactions due to diffusion processes) to develop a novel pH-sensitive fluorescent membrane calibration method for the quantification of TA. In the proposed calibration method, a linear relationship between TA and the protonation rate of a pH-sensitive fluorescent membrane with a pKa $\geq$ 9 (Paper-FM) is established. Paper-FM was synthesized by the covalent immobilization of the pH-probe Nile Blue on a porous paper membrane. In addition, a 3D-printed interface was designed to implement Paper-FM into a 0.5 mm diameter optical fiber. The fiber-optic sensor developed in this work was tested by analyzing 21 vinegar samples of different origins, and the results were successfully validated with a reference titration method. The results presented in this work show that our fiber-optic sensor allows a direct, simple, and fast (15 s response time) quantification of TA in vinegar samples. In addition, it is environmentally friendly, because, unlike titration methods, it does not generate toxic waste, is reversible, and is extremely stable (a 0.9 mm diameter circle of Paper-FM allows at least 50 consecutive measurements).

## 1. Introduction

The acidity of food and beverages plays a fundamental role in the food industry and its control and quantification is essential. The main short-chain soluble organic acids present in vinegar are tartaric, citric, malic, succinic, and acetic (these acids are produced naturally during fermentation processes), with acetic acid being the one found in the highest proportion, and thus the main responsible of TA of vinegar. In addition to being natural components of many foods and beverages, tartaric, citric, malic, succinic, and acetic acids are also widely used in the food industry as antioxidants, flavor enhancers, or additives to control pH [1,2]. Depending on the raw material (wine, cider, fruit, malt, malt distilled spirit, cereal, honey, etc.) and the method used for its production (type of fermentation) [1,3–7], many kinds of vinegar are currently available worldwide.

As stated above, acetic acid is the major acid component of vinegar, with a percentage of the total mass of acid (sum of all the acids present in vinegar) ranging between 50 % and 98 %, depending on the type of vinegar [8,9]. A community method for the analysis of the TA of vinegar (expressed as acetic acid concentration) consists of direct titration with sodium hydroxide in the presence of phenolphthalein [10]. However, titration has some drawbacks, such as the visual identification of the

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https://doi.org/10.1016/j.snb.2024.136214
Received 9 April 2024; Received in revised form 25 June 2024; Accepted 28 June 2024
Available online 1 July 2024

endpoint can lead to quantification errors, is time-consuming, and non-eco-friendly (generating corrosive and toxic sodium hydroxide solutions containing disposable pH colorimetric dyes) [10]. Alternatively, conventional instrumental methods such as capillary electrophoresis, ion exclusion chromatography with conductimetric detection, and liquid and gas chromatography coupled with mass spectrometry can also be used to determine the concentration of different organic acids and TA in vinegar [8]. However, these techniques have many drawbacks, including tedious sampling methods, sample transport and storage, very expensive equipment, specialized technicians, tedious and time-consuming sample and analysis treatments, large quantities of not environmentally friendly solvents, and they do not offer continuous monitoring at real-time [11]. Therefore, the development of simple and compact sensors capable of quantifying TA in real samples would provide the food industry with a valuable tool for process and quality control [12]. Gas sensor devices are a promising alternative for real-time monitoring of volatile organic acids in food samples [13]. Most gas sensors are based on a metal oxide semiconductor (MOS), whose electrochemical response is quite fast and reproducible [14]. However, these gas sensors are commonly very sensitive to humidity and, other volatile compounds such as alcohols, aldehydes, ketones, etc, which greatly affect the precision and accuracy of the measurements [15]. Optical sensors have some advantages over electrochemical sensors, such as the absence of electrical interferences, and measurement at different wavelengths with a wide variety of possibilities: transmittance, reflectance, luminescence (with its multiple modes: intensity or polarization, decay time, energy transfer, and quenching efficiency), infrared and Raman spectroscopy, interferometry, and surface plasmon resonance. Other advantages include the possibility to working in humid/aggressive/corrosive/radioactive environments where electrochemical sensors are not operational, cost-effectiveness, the possibility of measuring remotely and at a distance, they can be read without physical contact with the sample, easy miniaturization, etc [16,17]. Therefore, portable fiber-optic sensors are, an attractive solution for real-time monitoring of many parameters in the food industry [18]. Many fiber-optic sensors have been developed to sense gaseous acetic acid and other similar volatile compounds [18,19]. However, to the best of our knowledge, only two fiber-optic sensors have been developed for the quantification of the concentration of short-chain soluble organic acids in solutions, but none of them meet the requirements for application in real samples (e.g. vinegar, wine, kombucha, etc.). *Kurauchi* et al. [20] reported a fiber-optic sensor having a chitosan/poly(vinyl alcohol) cladding for the detection of the concentration of organic acids in an aqueous solution. However, this fiber-optic sensor presents a strong interference from ethanol, and the authors cannot use it to measure the acetic acid concentration or TA of real samples. On the other hand, Jesus et al. [12] have developed a sensing probe based on a fiber Bragg grating (FBG) Fabry-Perot cavity, coated with a thin film of sol-gel-PVP (polyvinylpyrrolidone) composite material. The polymeric thin film renders the interferometric output sensitive to the presence of carboxylic acid species. This optical sensor has a linear and reversible response and short response times with adequate reproducibility and repeatability. However, as the authors state, their results show that the sensor responds non-specifically to both short-chain carboxylic species and other types of molecules of similar size such as alcohols, aldehydes, etc., which does not allow its use for the analysis of real samples.

In this work, we have developed, for the first time, to the best of our knowledge, a portable, simple, reversible, reusable, fast, cost-effective, and environmentally friendly fiber-optic sensor for the quantification of the total acidity in vinegar. More than 20 samples of vinegar of different origins (wine, apple, rice, Sherry, and Modena) were analyzed, and the results were successfully validated with a reference titration method. Therefore, the reported fiber-optic sensor developed in this work would be a clean and cost-effective device for the food industry, ideal to replace titration methods in monitoring the total acidity of many real samples such as vinegar, wine, juices, milk, etc.

## 2. Materials and methods

### 2.1. Chemicals and materials

Divinyl sulfone (DVS), ethylene diamine (TEA), Nile blue chloride (NB), methanol (MeOH), glacial acetic acid (AA), 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. 13058: 73 $gm^{-2}$ and 170 μm thickness) from FILTER·LAB® (Barcelona, Spain), and decolorizing-carbon (Cod. 434507) 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).

### 2.2. Synthesis of Paper-FM

Paper-FM was prepared by functionalizing porous filter paper (FILTER·LAB 1305) with the pH probe Nile Blue (NB). Functionalization was carried out in two steps [21,22] (see Fig. 1 and Supporting Information (SI): Protocol-S-1): 1) Activation of the hydroxyl groups of the paper with vinyl sulfone groups, 2) Covalent immobilization of NB by Michael reaction between the vinyl sulfone groups of the activated membrane and the amine groups of NB.

### 2.3. Equipment and measurement protocol

The fiber optic setup used in this work to conduct the fluorescence measurements is based on a 0.5 mm diameter bifurcated optical probe (from Ocean Insight) connected to a portable high-resolution spectrometer (measurement range: 100 nm to 1000 nm) equipped with seven excitation LED sources with central wavelengths of: 310 nm (power: 11.5 μW), 365 nm(power: 645.9 μW), 405 nm (Power: 991.6 μW), 457 nm (Power: 1071.9 μW), 523 nm (Power: 331.9 μW), 590 nm (Power: 86.8 μW), and 660 nm (Power: 568.1 μW) from Spyroistech company (Calle Tajonar 22, Edificio Jerónimo de Ayanz. 31006 Pamplona, Navarra, Spain). To reproducibly fit a circular piece 0.9 mm diameter of Paper-FM at a distance of 1 mm the optical fiber, a homemade interface based on three assembled components was designed and fabricated (by 3D printing) (see Fig. 2).

The excitation and emission wavelengths used for Paper-FM were $\lambda_{exc} = 523$ nm and $\lambda_{em} = 630$ nm, respectively. The measurement protocol is described in the SI (see Protocol-S-2). The TA of the non-coloured samples (white wine, apple, rice, and cider vinegar) was measured by introducing the membrane directly into the sample for 15 s. However, in the case of coloured samples (red wine, Sherry, and Modena vinegar), colour significantly interferes with the TA measurement. The interference due to colour was eliminated by decolorizing the samples with activated carbon (see decolorizing protocol in SI: Protocol-S-3).

### 2.4. Sensor validation

To validate the results obtained in the analysis of TA of the vinegar samples using the fiber-optic sensor proposed, the samples were also analyzed by the established reference method, which consists of direct titration with sodium hydroxide. The titration was carried out using a METTLER TOLEDO® Compact G20S according to the following protocol: Initially, the solution of NaOH was standardized with potassium hydrogen phthalate (KHP). For titration, the sample (2 mL of vinegar) was introduced into the sample cup, distilled water was added until the electrode was covered, and then titration was performed. The operation was repeated three times for each sample, and the volumes of NaOH spent were recorded and used for the calculation of total acidity,

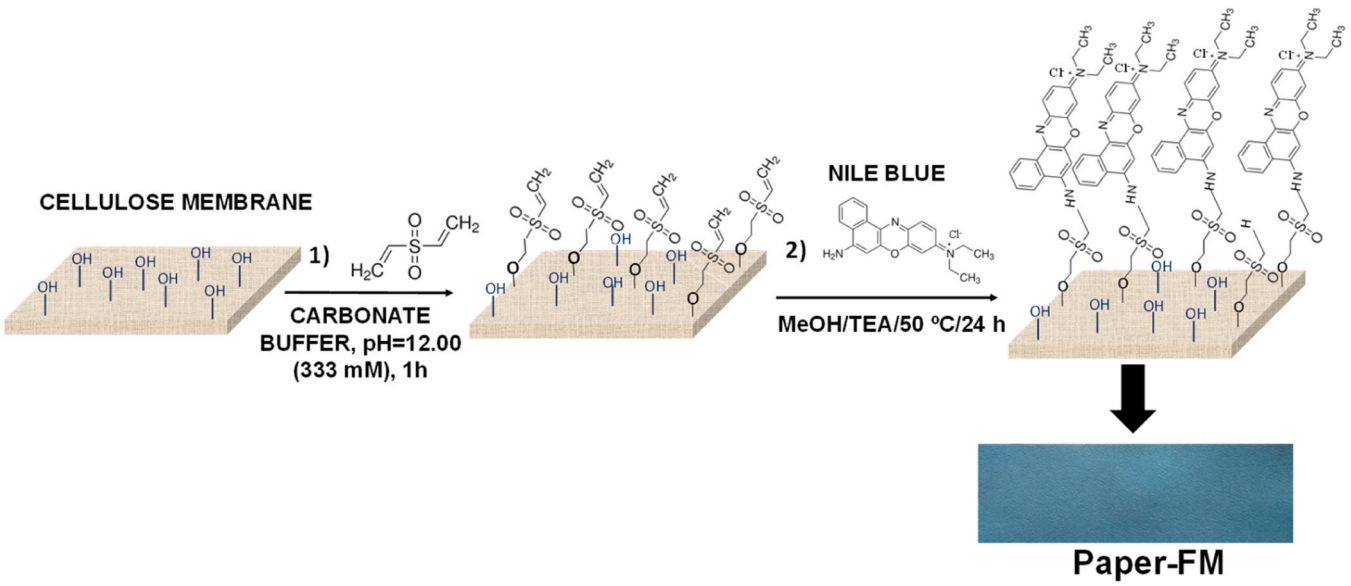


**Fig. 1.** Synthesis of Paper-FM: Activation of the hydroxyl groups of the paper with vinyl sulfone groups (1), covalent immobilization of NB by Michael reaction between the vinyl sulfone groups of activated membrane and the amine groups of NB (2).

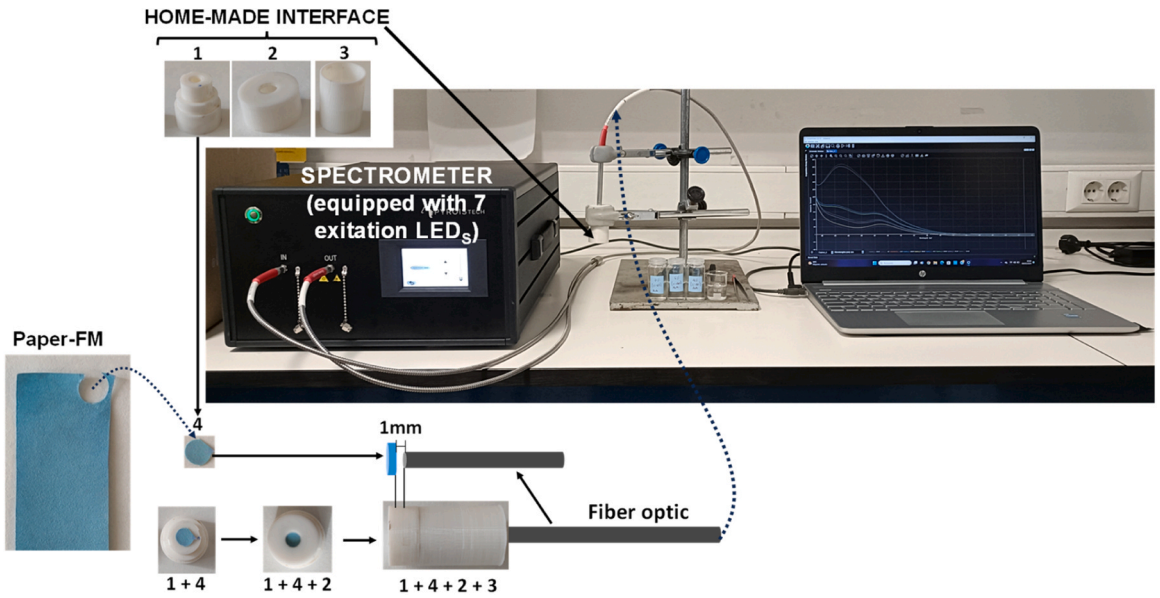


**Fig. 2.** Schematic of fiber optic set-up designed to measure the fluorescence changes in Paper-FM.

expressed as AA (%) w/v.

## 3. Results and discussion

### 3.1. Calibration method

Many fluorescent probes with different pKa values are available for measuring pH in acidic or basic media [23,24]. However, to perform a luminescent quantification of the total acidity in vinegar samples through pH changes, a fluorescent pH probe with a pKa around 2.5 and capable of detecting changes in the order of 0.01 pH units would be required. To the best of our knowledge, there are no fluorescent pH probes with such a high sensitivity in such a low pH range. Let us quantitatively analyze the previous argument. To do so, we first assume that the total acidity of vinegar is due only to acetic acid. This assumption is quite reasonable because the other acids of vinegar (tartaric, citric, malic, and succinic) are present in a much smaller proportion than acetic acid and are also weak acids with acidity constants on the order of that of acetic acid [8,9]. On the other hand, the total acidity of commercial vinegar ranges between 5 % and 8 % (w/v) [8,9], which can be translated into a minimum (0.83 M) and maximum (1.33 M) concentration of acetic acid, respectively. Considering the acidity constant of acetic acid ($K_a$=1.75 $\times 10^{-5}$), the minimum concentration (0.83 M) is translated into $[H_3O^+]$ = 0.0038 M→pH=2.41, and for the maximum concentration (1.33 M), we have $[H_3O^+]$ = 0.0048 M→pH=2.31. Hence, between the vinegar with the lowest acidity (5 % w/v) and the one with the highest acidity (8 %w/v), there is only a variation of 0.1 pH unit. Therefore, as mentioned above, to quantify the total acidity of vinegar by measuring pH changes with a fluorescent pH probe, it should be able to detect changes of 0.01 pH units in the pH interval [2,3], which, to the best of our knowledge, far exceeds the sensitivity limits of available pH probes.

In this work, we have developed an innovative strategy that allows simple, rapid, and clean quantification of total acidity in vinegar samples using a conventional pH-sensitive fluorescent membrane coupled to a portable fibre-optic device. It is well known that the protonation of a fluorescent pH probe in solution is an extremely fast reaction. However, when the pH probe is covalently immobilized on a porous membrane, the protonation reaction is much slower, because it becomes a heterogeneous solid-phase reaction, in which the proton must diffuse through the pores of the membrane where the pH probe is located. In these cases, the degree of protonation of the immobilized pH probe (fluorescent intensity) should be dependent on four experimental variables: i) Acid concentration in the solution (concentration of protons), ii) Reaction time, iii) Physicochemical properties of the support used for immobilization (type of material, polarity, porosity, thickness, etc.), iv) Temperature. Therefore, on the basis of these arguments, we can formulate the following hypothesis: *"Using a pH-sensitive fluorescent membrane with its maximum fluorescence intensity at basic pH, for a constant reaction time, it would be possible to establish a relationship (calibration curve) between the total acid concentration in solution and quenching of the luminescence of the membrane"*.

To validate our hypothesis, first, we focussed on the design of a pH-fluorescent-sensitive membrane (Paper-FM) with the above-mentioned characteristics (pKa $\geq$ 9). For this, the fluorescent pH-probe Nile Blue was covalently immobilized on a porous paper membrane (see Fig. 1 and SI: Protocol-S-1). Then, the characterization of Paper FM was carried out by introducing the membrane (0.9 mm diameter circle) into universal buffer solutions at different pH values: 7, 8, 9, 9.5, 9.8, 10, 10.5, 11, and 12. Subsequently, the fluorescence spectra were measured using the setup shown in Fig. 2, and the data were fitted using the following equation [25]:

$$I = \frac{I_0}{1 + (10^{pH-pKa})^{-1}} + B \quad (1)$$

where $I$ is the fluorescence at each pH value, $I_0$ is the fluorescence intensity of the basic form, and $B$ is the fluorescence intensity of the acid form. Fig. 3 shows the results of the optical characterization of Paper-FM.

As deduced from the results reported in Fig. 3, Paper-FM has an apparent $pK_a$ of 9.60 $\pm$ 0.08, which is ideal for developing our calibration method. Subsequently, the relationship between luminescence quenching of the membrane and acetic acid concentration for a fixed reaction time of 15 s was studied (see SI: point 3 of Protocol-S-2). For this purpose, stock solutions with different AA (%) w/v were prepared (2 %, 4 %, 6 %, 8 %, and 10 %). On the other hand, Paper-FM was cut into circles of 0.9 mm diameter, and the fluorescent intensity at pH=11 was selected as the maximum reference fluorescence value (Ref.$IF_{max}$). To set the Ref.$IF_{max}$ value, the membrane was introduced in 10 mL of universal buffer pH 11, 0.05 M for 1 min, and then its fluorescence was measured using the setup shown in Fig. 2. Then, the membrane was introduced into the acetic acid stock solutions for 15 s, and its fluorescence ($IF_x$) was also measured with the set-up shown in Fig. 2. The sensor response ($\Delta IF$=Ref.$IF_{max}$-$IF_x$) was the same for all acetic acid stock solutions. This result may be because the selected AA concentration range (2 %-10 %) is too high, and thus the protonation rate of Paper-FM is also extremely high over the entire calibration range, causing the luminescence of Paper-FM to drop to zero in an extremely short reaction time (much less than the selected 15 s). Therefore, to determine the optimal concentration range of acetic acid, stock solutions were progressively diluted, and the sensor began to respond when the stock solutions were diluted 40 times (1/39, acetic acid stock solution /distilled water, v/v). Fig. 4 shows the sensor response ($\Delta IF$=Ref.$IF_{max}$-$IF_x$) versus the concentration of AA (%) w/v after dilution: 0.05 %, 0.10 %, 0.15 %, 0.20 %, and 0.25 %.

The experimental results reported in Fig. 4 agree well with the proposed hypothesis: by selecting a constant reaction time within the interval [10 s–39,40 s] a relationship (calibration curve) between the concentration of acetic acid in solution and the luminescence quenching of Paper-FM can be established. To represent the calibration curve (see SI: point 3 of Protocol-S-2), stock solutions of acetic acid with the following concentrations (AA (%) w/v): 1 %, 2 %, 3 %, 4 %, %5, 6 %, 7 %, 8 %, and 9 %, were prepared, and subsequently, they were diluted 40 times with purified water (1/39, AA stock solution/purified water, v/v) to obtain the following standard solutions: 0.03 %, 0.05 %, 0.08 %, 0.10 %, 0.13 %, 0.15 %, 0.18 %, 0.20 %, and 0.23 %. Then, the sensor response ($\Delta IF$=Ref.$IF_{max}$-$IF_x$) was measured for all standard solutions, and the calibration curve was obtained by representing $\Delta IF$= Ref.$IF_{max}$-$IF_x$ versus AA (%) w/v. Fig. 5 shows a schematic of the measurement procedure and the calibration curve.

As shown in Fig. 5**A**, the response of the sensor versus the concentration of AA corresponds to a type of sigmoidal curve in which two linear ranges are observed, one at low concentrations (% w/v) of AA [0.03 %-0.08 %] and, the other at higher concentrations [0.1 %-

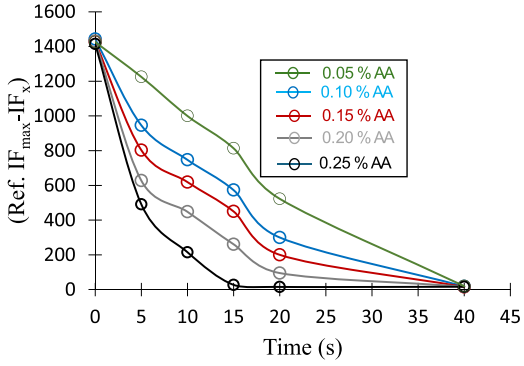


**Fig. 4.** Protonation rate of Paper-FM versus the % of AA.

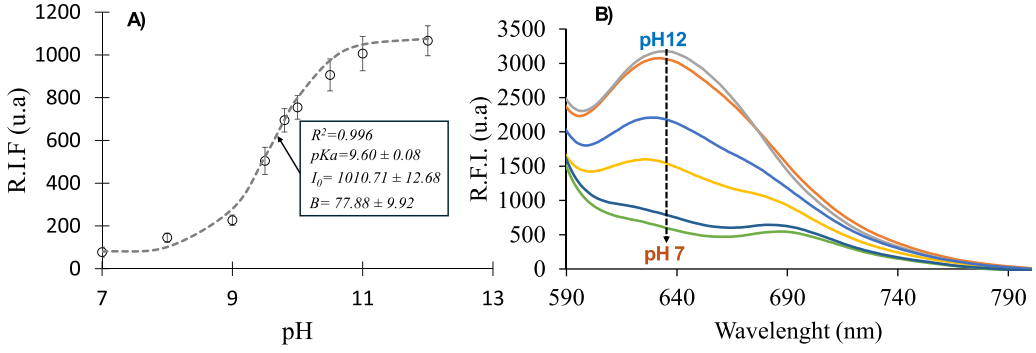


**Fig. 3.** Optical characterization of Paper-FM: Fitting the data to Eq. 1, where experimental data are denoted with the symbol (○) and the theoretical plot is indicated with a grey dashed line (A), fluorescence emission spectra at different pH values of Paper-FM (B).

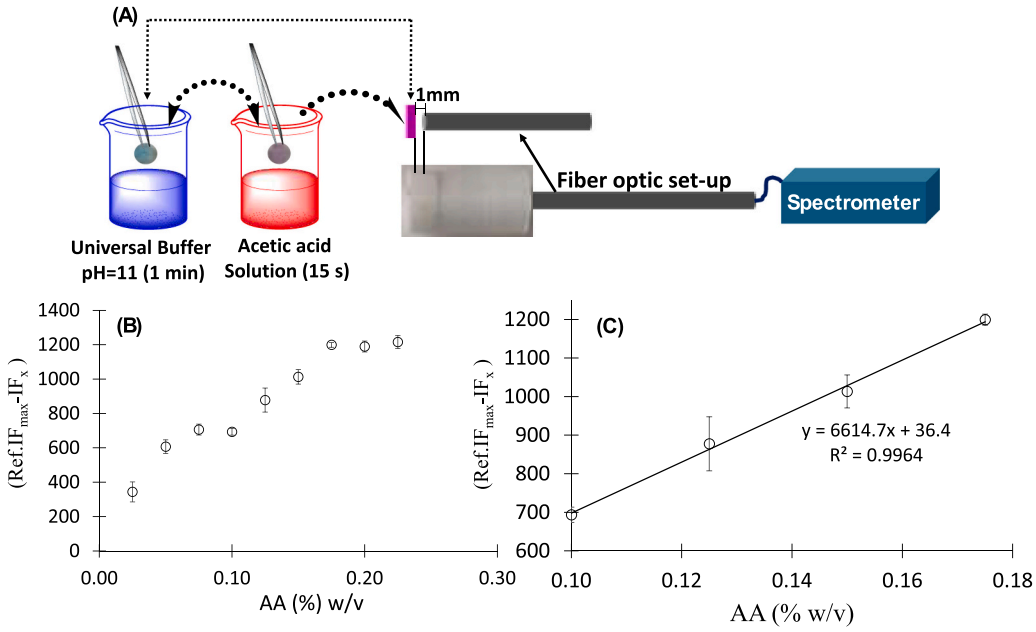


**Fig. 5.** Schematic of the measurement procedure (A), Sensor response: ΔIF=Ref.$IF_{max}$-$IF_x$ vs. AA (%) w/v of the standard solution for a constant reaction time of 15 s (B), and linear range (C).

0.18 %]. This behavior is common in heterogeneous phase reactions in which the immobilized fluorescent probe can be found in different physicochemical environments within the membrane [26]. The protonation rate of the immobilized Nile Blue in a porous membrane may be considered as a heterogeneous phase reaction governed by complex diffusion mechanisms that mainly depend on the following variables: i) Concentration of acid in solution (proton concentration), ii) Concentration of Nile Blue immobilized in the membrane, iii) Physicochemical properties of the membrane (polarity, type of pores, pore size distribution, membrane thickness, electrical charge of the membrane, etc.), iv) Temperature. Therefore, the non-linear behavior of the calibration curve of Fig. 5 may be due to the presence of the Nile Blue immobilized in two different environments within the membrane. The lower linear range could correspond to Nile Blue immobilized within large pores (micropores) with a very polar microenvironment in which water (and therefore protons) can penetrate very easily, whereas the second linear range could correspond to Nile Blue immobilized in smaller pores (nanopores) with a less polar environment in which water has more difficulty penetrating. Either way, the experimental data presented in Figs. 4 and 5 validated our initial hypothesis, leading to a novel and innovative calibration method based on the relationship between acid concentration in solution and quenching of the luminescence of a conventional pH-sensitive fluorescent membrane with a basic pKa.

Either of the two linear ranges in Fig. 5 could be used for the calculation of TA (expressed as AA (%) w/v), we have chosen the higher linear range (0.1 %-0.18 %) (see Fig. 5**C**) simply because of its greater amplitude, and because it is closer to the TA of the vinegar, thus requiring less dilution of the samples. The linear range (0.1 %-0.18 %) has a good correlation coefficient ($R^2$= 0.996) with detection and quantification limits of 0.0024 and 0.0083 %, respectively. The detection and quantification limits were determined using the IUPAC method (LOD = 3 $s_b$/m; LOQ=10 $s_b$/m). The standard deviation of the blank (sb) was obtained by measuring (10 measurements) the response of the sensor for a pH=11, 0.05 M universal buffer solution.

Considering that the proposed calibration method is based on the kinetics of the protonation reaction of immobilized pH-probe, the rate constants and diffusion coefficients of this reaction can be affected by temperature. Therefore, to check the influence of temperature, the calibration was carried out at different temperatures (15ºC, 20ºC, 25ºC, 30ºC, and 35ºC). As shown in Fig. 6 between 15ºC and 25ºC calibration is not affected by temperature, however, as expected, above 25ºC the rate of the protonation reaction increases considerably, and although good linearity is still maintained, the slope of the calibration begins to decrease (decrease in sensitivity). Even so, the sensitivity of the calibration at 35ºC (black line of Fig. 6**)** is still quite good, with a detection limit (0.004 AA% w/v) and quantification limit (0.013 % AA w/v) well below the TA values in the vinegar samples (TA in vinegar is between 5 % AA w/v and 8 % AA w/v).

### 3.2. *Stability of Paper-FM*

To analyse the stability and reusability of the Paper-FM, short-term precision (repeatability) and long-term precision (reproducibility) were studied. For short-term precision, the sensor response (ΔIF= Ref.$IF_{max}$-$IF_x$) was measured for four concentration levels: AA (%) w/v: 0.10 %, 0.13 %, 0.15 %, and 0.18 % (10 measurements were performed for each concentration), and all the measurements (50) were performed consecutively in less than 2 h using a single membrane of 0.9 mm diameter. As can be seen in Fig. 7, Paper-FM is extremely stable: each membrane of 0.9 mm diameter can be used to perform at least 50 measurements.

The long-term stability was studied by measuring the sensor response for a single concentration level (0.14 AA (%) w/v) for 18 days (after each measurement, the membrane was dried at room temperature (RT) and stored in the absence of light). All measurements were performed using the same 0.9 mm diameter membrane. The results were interpreted using a Shewhart diagram (Long-term stability was defined as the signal that remains within the control lines on the Shewhart chart). As shown in Fig. 8**,** after 18 days, the response of the membrane remained within the established control limits, showing excellent long-term stability.

The results reported in Figs. 7 and 8 show extraordinary stability and reusability of Paper-FM: a single 0.9 mm diameter membrane can be used to perform more than 50 consecutive measurements, and can also be stored and reused for at least 18 days (although after 18 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). Therefore, with commercial use in mind, Paper-FM is extremely efficient and cost-effective.

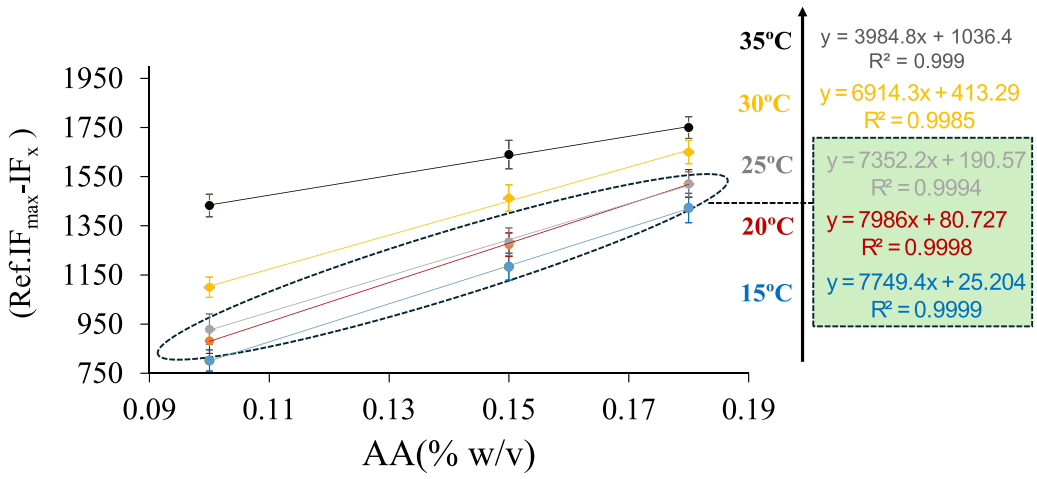


**Fig. 6.** Effect of temperature on the calibration curve.

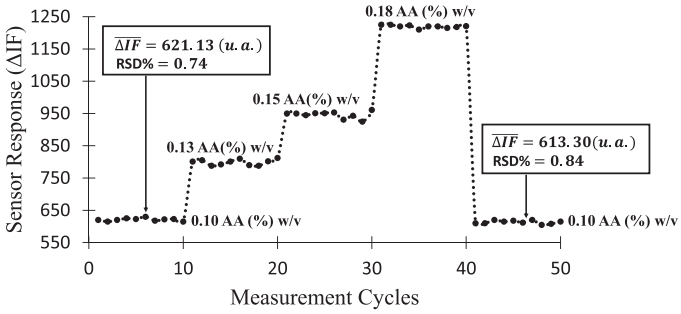


**Fig. 7.** Short-term repeatability of Paper-FM: 10 measures for four levels of AA (%) w/v: 0.10 %, 0.13 %, 0.15 %, and 0.18 %).

### 3.3. Real-world application and validation

To evaluate the efficiency in the analysis of real samples, 21 samples of vinegar of different origins were analysed by both the proposed fiber-optic sensor and the reference titration method (see section *2.4.4. Sensor validation*). In the case of coloured samples (red wine, Sherry, and Modena vinegar), colour significantly interferes with the TA measurement. The interference due to colour was eliminated by decolorizing the samples with activated carbon using the protocol described in section *2.4.3*. Each membrane of 0.9 mm in diameter was used for 24 measurements (four points for the calibration curve and three vinegar samples: three replicates for each measure). Table 1 summarizes the results of TA (expressed as % w/v of acetic acid) given by the manufacturer, obtained with our fiber-optic sensor, and those obtained by the reference method (titration).

A correlation coefficient of 0.985 was calculated (Fig. S1 shows the calculation of the correlation coefficient) [27] by comparing the TA obtained with the proposed fiber-optic sensor, and with those obtained by titration, which indicates excellent correlation between the two methods.

The results reported in Figs. 5, 6, 7, 8, Fig. S1, and Table 1 demonstrate, that our fiber-optic sensor allows simple, fast, cost-effective, and environmentally friendly quantification of TA in vinegar samples.

## 4. Conclusions

In this work, a novel fiber-optic sensor for measuring TA in real samples has been developed. For this purpose, an innovative calibration method of a pH-sensitive fluorescent conventional membrane (with a

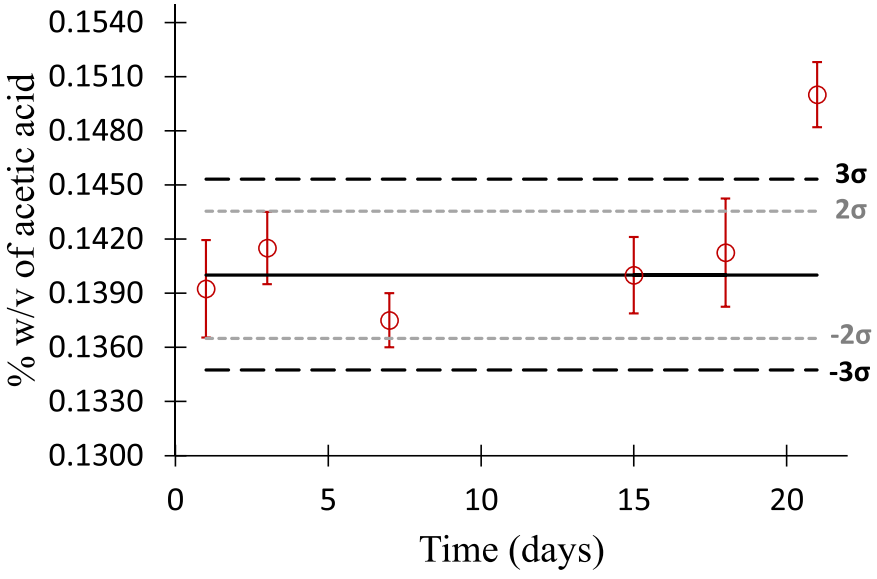


**Fig. 8.** Shewhart control chart for checking the long-term stability of Paper-FM.

**Table 1**
Results of TA by manufacturer, the proposed fiber-optic sensor, and titration method.

| Type of vinegar | Brand | TA (Indicated by the manufacturer) | TA (By Proposed fiber-optic sensor) | TA (By Titration Method) |
|---|---|---|---|---|
| **Apple** | Hacendado | 5 | 4.94±0.17 | 4.80±0.15 |
| | Consum | 5 | 5.05±0.05 | 4.95±0.03 |
| | Kania | 5 | 4.78±0.05 | 4.68±0.16 |
| **White wine** | Hacendado | 6 | 5.92±0.14 | 6.04±0.43 |
| | Consum | 6 | 6.28±0.16 | 6.50±0.35 |
| | Kania | 6 | 5.75±0.14 | 5.60±0.20 |
| **Rice** | Blue Dragon | NSM* | 3.30±0.16 | 3.45±0.08 |
| | Tiger Khan | NSM* | 4.40±0.17 | 4.31±0.08 |
| | Lockcheng | NSM* | 4.41±0.03 | 4.53±0.14 |
| **Cider** | Corte Inglés | 5 | 4.56±0.24 | 4.66±0.20 |
| | Coviran | 5 | 4.17±0.04 | 4.20±0.22 |
| | Femua | 5 | 4.71±0.22 | 4.65±0.17 |
| **Modena (PGI)** | Hacendado | 6 | 5.38±0.22 | 5.41±0.02 |
| | Comsum | 6 | 5.42±0.19 | 5.51±0.27 |
| | Ferranini | 6 | 5.99±0.05 | 5.96±0.13 |
| **Sherry (PDO)** | Hacendado | 8 | 7.01±0.13 | 7.15±0.15 |
| | Comsum | 7 | 6.21±0.10 | 6.31±0.09 |
| | Corte Inglés | 7 | 6.34±0.20 | 6.36±0.31 |
| **Red Vine** | Consum | 6 | 5.54±0.16 | 5.41±0.28 |
| | Corte Iglés | 6 | 4.82±0.22 | 4.80±0.03 |
| | Rioja Corte Inglés | 6 | 5.60±0.09 | 5.54±0.18 |

NSM*: not specified by manufacturer.

basic pKa) for the quantification of TA has been developed for the first time. The fiber-optic sensor was used to analyze 21 vinegar samples of different origins, and the results were successfully validated with the community titration method. In comparison with the titration method, the fiber-optic sensor combined with a decolouration protocol (only in the case of coloured samples) offers several important advantages such as 1) It is a simple method in which the measurements are performed by introducing the membrane directly into the sample, 2) It is fast, the sensor response is obtained in 15 s, 3) It is environmentally friendly as it does not require additional reagents (titration methods generate corrosive and toxic sodium hydroxide solutions containing disposable pH dyes), 4) It is cost-effective, as Paper-FM is reversible, reusable and, extremely stable (a membrane of 0.9 mm diameter can be used to carry out at least 50 consecutive measurements), 5) It is portable and could be easily implemented in a miniaturized optoelectronic device. Therefore, the fiber-optic sensor developed in this work would be a clean and cost-effective device for the food industry, ideal for in situ monitoring the total acidity of real samples (e.g., vinegar, wines, juices, etc.).

## CRediT authorship contribution statement

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

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data Availability

Data will be made available on request.

## Acknowledgements

This work was supported by the Grants C. EXP. 030. UGR23 and C. EXP. 103. UGR23 funded by Consejería de Universidad, Investigación e Innovación and by ERDF Andalusia Program 2021–2027, and by Project European Union's Horizon 2020 research and innovation program under the Marie Sklodowska-Curie-RISE Grant Agreement "SUSTAINABLE" No. 101007702 (http://www.projectsustainable.eu/). Project PID2022–138727OB-I00 funded by MCIN/AEI/10.13039/501100011033 and, by ERDF "A way of making Europe", by the "European Union. Antonio. L. Medina-Castillo. acknowledges funding by Plan Propio-investigación y Transferencia de la Universidad de Granada*: "Programa 9. Proyectos de investigación para la incorporación de jóvenes Doctores a Nuevas Líneas de investigación´en Grupos de la UGR".*

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.snb.2024.136214.

## References

[1] J.J. Román-Camacho, I. García-García, I.M. Santos-Dueñas, J.C. Mauricio, Latest trends in industrial vinegar production and the role of acetic acid bacteria: classification, metabolism, and applications—a comprehensive review, Foods 12 (2023) 3705, https://doi.org/10.3390/foods12193705.
[2] P. Mayser, S. Fromme, C. Leitzmann, K. Grunder, The yeast spectrum of the *'tea fungus Kornbucha*, Mycoses 8 (7-8) (1995) 3289–3295, https://doi.org/10.1111/j.1439-0507.1995.tb00410.x.
[3] S. Li, P. Li, F. Feng, L.X. Luo, Microbial diversity and their roles in the vinegar fermentation process, Appl. Microbiol. Biotechnol. 99 (2015) 4997–5024, https://doi.org/10.1007/s00253-015-6659-1.
[4] Z. Qi, H. Yang, X. Xia, W. Quan, W. Wang, X. Yu, Achieving high strength vinegar fermentation via regulating cellular growth status and aeration strategy, Process Biochem 49 (2014) 1063–1070, https://doi.org/10.1016/j.procbio.2014.03.018.
[5] R.M. Callejon, L. morales, A.Silva Ferreira, A. Troncoso, Defining the typical aroma of sherry vinegar: sensory and chemical approach, J. Agric. Food Chem. 56 (2008) 8086–8095, https://doi.org/10.1021/jf800903n.
[6] D. Bertelli, A. Maietti, G. Papotti, P. Tedeschi, G. Bonetti, R. Graziosi, V. Brandolini, M. Plessi, Antioxidant activity, phenolic compounds, and NMR characterization of balsamic and traditional balsamic vinegar of modena, Food Anal. Methods 8 (2015) 371–379, https://doi.org/10.1007/s12161-014-9902-y.
[7] R. Ríos-Reina, S. Elcoroaristizabal, J.A. Ocaña-González, D.L. García-González, J. M. Amigo, R.M. Callejón, Characterization and authentication of Spanish PDO wine vinegars using multidimensional fluorescence and chemometrics, Food Chem. 230 (2017) 108–116, https://doi.org/10.1016/j.foodchem.2017.02.118.
[8] M. Cocchi, P. Lambertini, D. Manzini, A. Marchetti, A. Ulrici, Determination of carboxylic acids in vinegars and in aceto balsamico tradizionale di modena by HPLC and GC methods, J. Agric. Food Chem. 50 (2002), https://doi.org/10.1021/jf020155l, 5255-526.
[9] A. Caligiani, D. Acquotti, G. Palla, V. Bocchi, Identification and quantification of the main organic components of vinegars by high resolution 1H NMR spectroscopy, Anal. Chim. Acta 585 (2007) 110–119, https://doi.org/10.1016/j.aca.2006.12.016.
[10] I. Dini, R.M. Di Lorenzo, A. Senatore, D. Coppola, S. Laneri, Validation of rapid enzymatic quantification of acetic acid in vinegar on automated spectrophotometric system, Foods 9 (2020) 761, https://doi.org/10.3390/foods9060761.
[11] A. Medvedovici, F. Albu, V. David, Handling drawbacks of mass spectrometric detection coupled to liquid chromatography in bioanalysis, J. Liq. Chromatogr. Relat. Technol. 33 (2010) 1255–1286, https://doi.org/10.1080/10826076.2010.484375.
[12] C. Jesus, S.F.O. Silva, M. Castanheira, G. Gonzalez Aguilar, O. Frazo, P.A.S. Jorge, J.M. Baptista, Measurement of acetic acid using a fibre Bragg grating interferometer, Meas. Sci. Technol. 20 (2009) 125201. ⟨https://iopscience.iop.org/article/10.1088/0957-0233/20/12/125201⟩.
[13] Q. Chen, J. Zhao, Z. Chen, H. Lin, D.A. Zhao, Discrimination of green tea quality using the electronic nose technique and the human panel test, comparison of linear and nonlinear classification tools, Sens. Actuators B: Chem. 159 (2011) 294–300, https://doi.org/10.1016/j.snb.2011.07.009.

[14] C. Di Natale, A. Macagnano, F. Davide, A. D'Amico, R. Paolesse, T. Boschi, M. Faccio, G. Ferri, An electronic nose for food analysis, Sens. Actuators B: Chem. 44 (1997) 521–526, https://doi.org/10.1016/S0925-4005(97)00175-5.
[15] J.H. Sohn, M. Atzeni, L. Zeller, G. Pioggia, Characterisation of humidity depend ence of a metal oxide semiconductor sensor array using partial least squares, Sens. Actuators B: Chem. 131 (2008) 230–235, https://doi.org/10.1016/j.snb.2007.11.009.
[16] M.D. Marazuela, M.C. Moreno-Bondi, Fiber-optic biosensors – an overview, Anal. Bioanal. Chem. 372 (2002) 664–682, https://doi.org/10.1007/s00216-002-1235-9.
[17] O.S. Wolfbeis, Materials for fluorescence-based optical chemical sensors, J. Mater. Chem. 15 (2005) 2657–2669, https://doi.org/10.1039/B501536G.
[18] O.S. Wolfbeis, Fiber-optic chemical sensors and biosensors, Anal. Chem. 80 (2008) 4269–4283, https://doi.org/10.1021/ac060490z.
[19] X.Dong Wang, O.S. Wolfbeis, Fiber-optic chemical sensors and biosensors (2008−2012), Anal. Chem. 85 (2013) 487–508, https://doi.org/10.1021/ac303159b.
[20] Y. Kurauchi, T. Ogata, N. Egashira, et al., Fiber-optic sensor with a dye-modified chitosan/poly(vinyl alcohol) cladding for the determination of organic acids, Anal. Sci. 12 (1996) 55–59, https://doi.org/10.2116/analsci.12.55.
[21] A.L. Medina-Castillo, L. Ruzic, B. Nidetzky, Juan M. Bolivar, Hydrophilic nonwoven nanofiber membranes as nanostructured supports for enzyme immobilization, ACS Appl. Polym. Mater. 4 (2022) 6054–6066, https://doi.org/10.1021/acsapm.2c00863.
[22] M.D. Fernández-Ramos, M. Bastida-Armesto, R. Blanc-García, L.F. Capitán-Vallvey, A.L. Medina-Castillo, Design of colourimetric nanostructured sensor phases for simple and fast quantification of low concentrations of acid vapors, Microchim Acta 190 (2023) 160, https://doi.org/10.1007/s00604-023-05723-0.
[23] C. Staudinger, J. Breininger, I. Klimant, S.M. Borisov, Near-infrared fluorescent aza-BODIPY dyes for sensing and imaging of pH from the neutral to highly alkaline range, Analyst 144 (2019) 2393–2402, https://doi.org/10.1039/C9AN00118B.
[24] A. Steinegger, O.S. Wolfbeis, S.M. Borisov, Optical sensing and imaging of pH values: spectroscopies, materials, and applications, Chem. Rev. 120 (2020) 12357–12489, https://doi.org/10.1021/acs.chemrev.0c00451.
[25] A.L. Medina-Castillo, J.F. Fernandez-Sanchez, A. Segura-Carretero, A. Fernandez-Gutierrez, Design and synthesis by ATRP of novel, water-insoluble, lineal copolymers and their application in the development of fluorescent and pH-sensing nanofibres made by electrospinning, J. Mater. Chem. 21 (2011) 6742, https://doi.org/10.1039/C1JM10209E.
[26] E.R. Carraway, J.N. Demas, B.A. DeGraff, J.R. Bacon, Photophysics and photochemistry of oxygen sensors based on luminescent transition-metal complexes, Anal. Chem. 63 (1991) 337–342, https://doi.org/10.1021/ac00004a007.
[27] T. Ramon-Marquez, A.L. Medina-Castillo, A. Fernandez-Gutierrez, J.F. Fernandez-Sanchez, A novel optical biosensor for direct and selective determination of serotonin in serum by Solid Surface-Room Temperature Phosphorescence, Biosens. Bioelectron. 82 (2016) 217–223, https://doi.org/10.1016/j.bios.2016.04.008.

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