Capillary microfluidic platform for sulfite determination in wines

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1. Reagents and materials

The reagents sodium hydrogen sulfite, sodium chloride, lactic acid, acetic acid, tartaric acid, ethanol, fructose, glucose, sucrose, citric acid, sulfuric acid, sodium hydroxide, sodium carbonate, iodine, ethylenediaminetetraacetic acid, starch, arsenic trioxide, used for the device calibration and characterization, were all analytical-grade unless stated otherwise, and purchased from Sigma Aldrich (Madrid, Spain). All aqueous solutions were prepared using purified water (resistance 18.2 MΩ·cm) obtained from a Milli-RO 12 plus Milli-Q station (Millipore, Bedford, MA, USA).

The thread used was a commercial white cotton thread (caliber 12 and NTex 94) from Finca (Presencia Hilaturas S.A. Alzira, Valencia, Spain) measuring some 600 μm in diameter and made up of 250 ± 10 fibres. Different filter papers were used as separation membrane (ref. 1238, 1240, 1242, 1244, 1246, 1248, 1249, 1254 and PC1) and were from Filtros Anoia, S.A. Barcelona, Spain Lab (www.fanoia.com). Double-sided adhesive tape from Miarco (http://www.miarco.com) and transparent sheets from Schwan-Stabilo (http://www.schwan-stabilo.com) were used to manufacture the devices.

For the synthesis of chromoreactand 4-[4-(2-hydroxyethanesulfonyl)-phenylazo]-2-formylnaphthalen-1-ol (GJM-530), the 2-(4-aminobenzenesulfonyl) ethanol hydrochloride was obtained from Aglycon Dr. Spreitz KG (Allerheiligen bei Wildon, Austria). 1-Hydroxy-2-naphthaldehyde, hydrochloric acid, sodium hydroxide, sodium carbonate, acetic acid, sodium nitrite, silica gel 60, acetone and dichloromethane were from Merck (Vienna, Austria). Whatman grade 1 Chr cellulose chromatography paper in a size of 20x20 cm^2 was from Bartelt GmbH (Graz, Austria).

2. Apparatus, instruments and software

The digitalization of μTPAD and μPAD was done using a Sony DSC-HX300 (Sony, Tokyo, Japan) digital camera, and photos were taken in a light box with two panels containing 50 LEDs each (550 lumens; colour temperature: 5600K). Pictures files obtained in JPG format were analysed using ImageJ (National Institutes of Health), and Avidemux 2.6 (Mean) was used to obtain single frames from video files. To perform statistical and mathematical treatment, the statistical software OriginPro v.8 (OriginLab Corporation, USA) and Microsoft Excel spreadsheets were used. The lamination of the devices was done by a FM-480 laminator using bioriented polypropylene (BOPP)

lamination film. Cutter plotter CSV 1350 – II series (Refine, China) was used to print the μPAD shape.

^{1}H and ^{13}C NMR spectra were recorded at room temperature on a Varian Direct Drive 500 MHz spectrometer. Chemical shifts were reported in ppm using solvent resonance as the internal standard ($CDCl_3$ at 7.26 ppm and 77.16 ppm).

3. Synthesis of chromoreactand

For the synthesis of GJM-530, 1.1 g (4.6 mmol) of 2-(4-aminobenzenesulfonyl)ethanol hydrochloride was suspended in 1.4 mL (8.4 mmol) of 6 $mol \cdot L^{-1}$ hydrochloric acid and cooled to below 5°C. To this, a solution of 0.28 g (4.1 mmol) sodium nitrite in 2 mL of purified water was added and the resulting yellow–orange solution stirred for 10 minutes at 5°C. This diazotization solution was slowly added to an ice-cooled solution of 0.68 g (3.9 mmol) of 1-hydroxy-2-naphthaldehyde in 30 mL of acetic acid. The solution was stirred at around 10°C, and stepwise at four subsequent intervals of 20 minutes, each 0.33 mL of an aqueous 30% (w/w) sodium hydroxide solution was added. The solution was stirred for 3 more hours, and then 1.0 mL of 6 M hydrochloric acid and 50 mL of water were added. The resulting oil, which solidified in the refrigerator overnight, was purified by flash chromatography on 90 g of silica gel 60 with dichloromethane/acetone (v/v=4:1) as the eluent, giving 0.5 g of the product (yield 33%).

4. Chromoreactand characterization

GJM-530 was characterized by ^{1}H NMR (Figure S1), ^{13}C NMR (Figure S2) and HSQC and Figure S3) as follows:

^{1}H NMR (500 MHz, $CDCl_3$+Methanol-d4) d 10.09 (d, J = 2.0 Hz, 1H), 8.97 – 8.87 (m, 1H), 8.54 (d, J = 8.4 Hz, 1H), 8.23 – 8.06 (m, 5H), 7.89 (ddt, J = 8.5, 6.9, 1.6 Hz, 1H), 7.71 (ddt, J = 8.4, 6.9, 1.5 Hz, 1H), 4.03 (t, J = 6.0 Hz, 2H), 3.46 (td, J = 6.0, 2.0 Hz, 2H).

^{13}C NMR (126 MHz, $CDCl_3$+ Methanol-d4) δ 197.09, 164.44, 155.83, 140.81, 140.36, 135.71, 132.03, 129.30, 127.12, 124.85, 124.52, 123.57, 123.23, 114.07, 113.54, 55.80.

GJM-530 molar extinction coefficients in methanol were calculated at the maxima at 458 nm (protonated form) and 530 nm (deprotonated form), respectively, where the acid and base form were obtained by adding trifluoroacetic acid and trimethylamine, respectively. The molar extinction coefficients were 32,400 (458 nm) and 25,400 (530 nm).

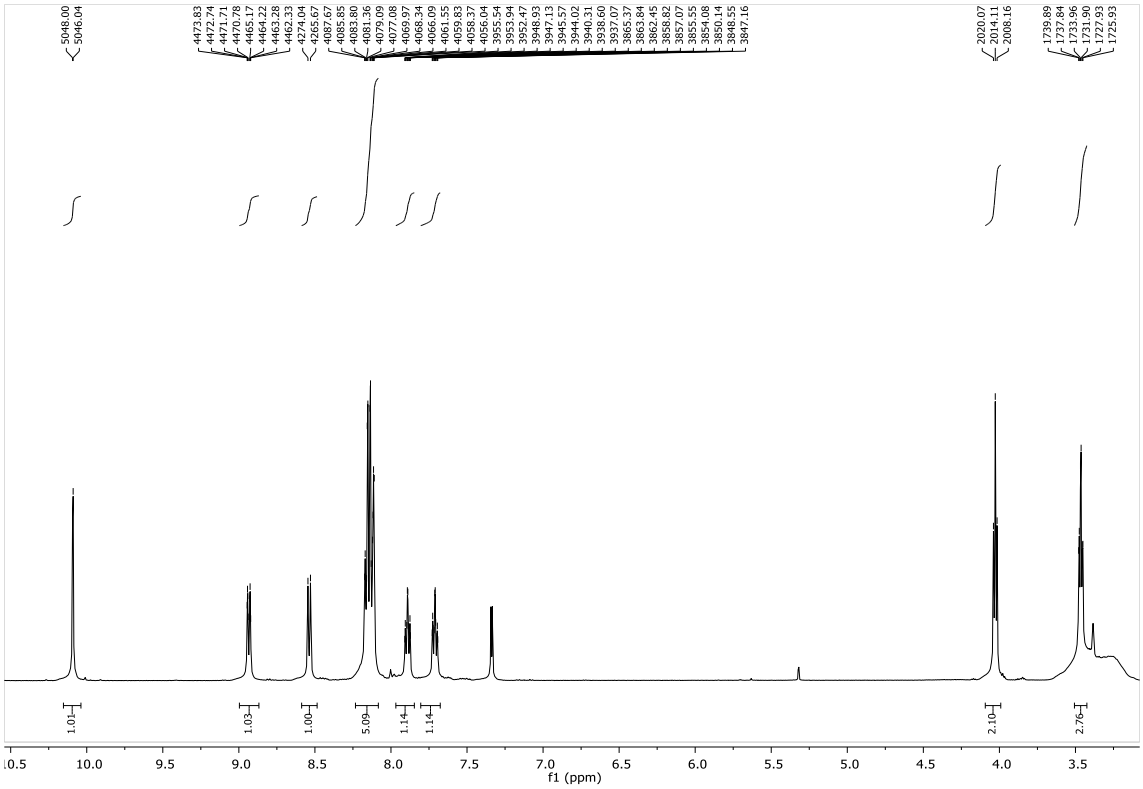

Figure S1. ^{1}H NMR of compound GJM-530.

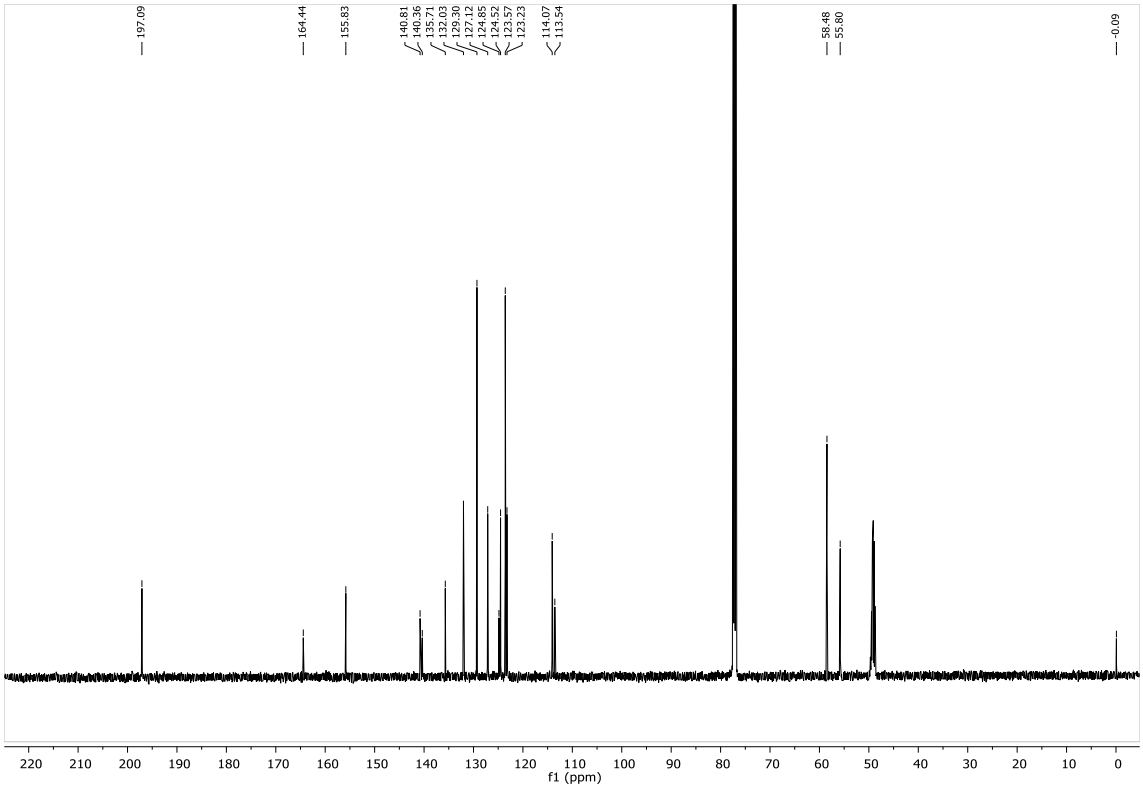

Figure S2. ^{13}C NMR of compound GJM-530.

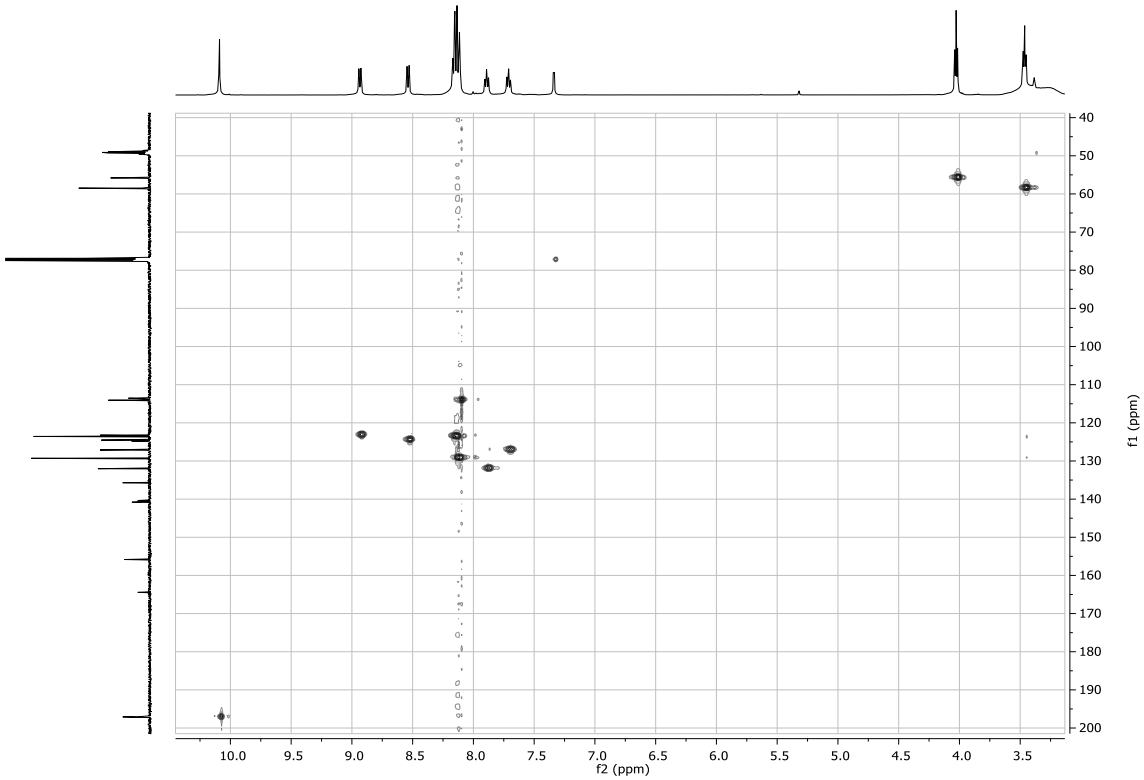

Figure S3. HSQC experiment of compound GJM-530.

5. Immobilization of GJM-530 on paper

For the covalent immobilisation of GJM-530 on chromatographic paper, 50 mg of the dye was treated with 0.5 mL concentrated sulfuric acid for 30 min at room temperature. The mixture was then poured into 400 mL of purified water and neutralised with 1 mL of 30% aqueous sodium hydroxide solution, and subsequently made alkaline by adding another 2.5 mL of 30% aqueous sodium hydroxide solution and a solution of 12.5 g of sodium carbonate in 100 mL of water. A sheet of Whatman grade 1 paper was placed into this solution, and gently stirred to facilitate colouration, but avoiding disintegration of the paper. After 30 min, the coloured paper was removed from the dyeing bath and gently rinsed with purified water to remove both the unbound dye and the alkaline reagents. Then, the sensor paper was dried at room temperature by pressing it between several sheets of filter paper to obtain flat indicator layers.

6. Analytical characterization of chromoreactand

As the analytical parameter, a chromatic coordinate obtained from a photograph of the membrane with GJM-530 immobilized after reacting with sulfite was used. To choose

the colour coordinate containing the most analytical information, a set of 2.5 mm diameter discs was prepared from the sensor paper. Those paper discs were soaked for 2 min. in sulfite solutions of different concentrations (n = 3) (10^{-7}, 10^{-6}, 10^{-5}, $5 \cdot 10^{-4}$, 10^{-4}, $5 \cdot 10^{-3}$ $2.5 \cdot 10^{-3}$, 10^{-3}, 10^{-2} and 10^{-1} M) and pH 4.9. The coordinates of the RGB and HSV colour space were calculated from the images obtained. Figures S4 and S5 show the evolution of the chromatic coordinates. The parameter that offered the best results was the tone (H) coordinate, taking into account the greater signal variation that it presented and the best precision for its use as an analytical parameter. The H coordinate was used rescaled from 0 to 1 instead directly as a degree.

The influence of pH was studied by immersing 2.5 mm diameter sensor paper disks in 1 M citric acid / citrate buffers at pH 4.0, 4.9, 6.0 and 7.0 which, after drying, reacted with sulfite solutions of increasing concentration. Figure S6 shows that pH 6 produced the largest range of variation of H.

A study was carried out to check whether the sensor paper exhibited a reversible behaviour. To do this, three circles of the sensor paper were reacted for two minutes with sulfite standards of increasing concentrations first and then decreasing concentrations. The results (Figure S7) show that the sensor paper was not reversible, meaning that the device is a single-use device.

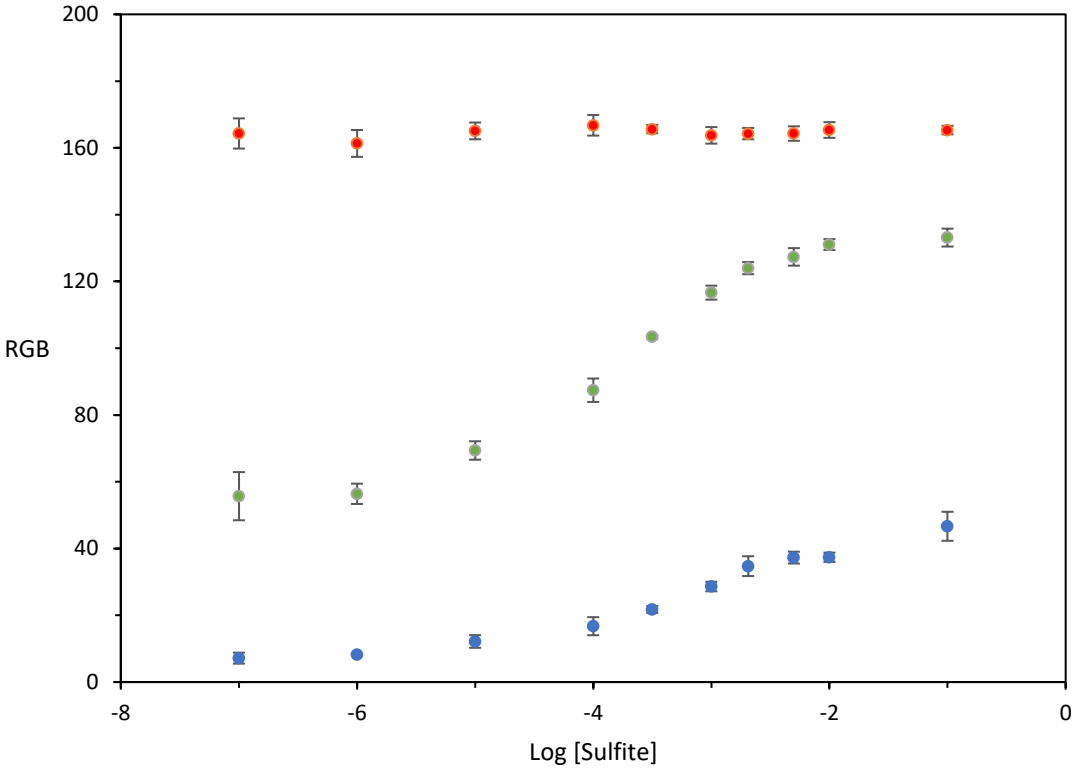

Figure S4. Evolution of RGB coordinates after equilibration for 2 min. with solutions of increasing sulfite concentrations. R (red data), G (green data) and B (blue data).

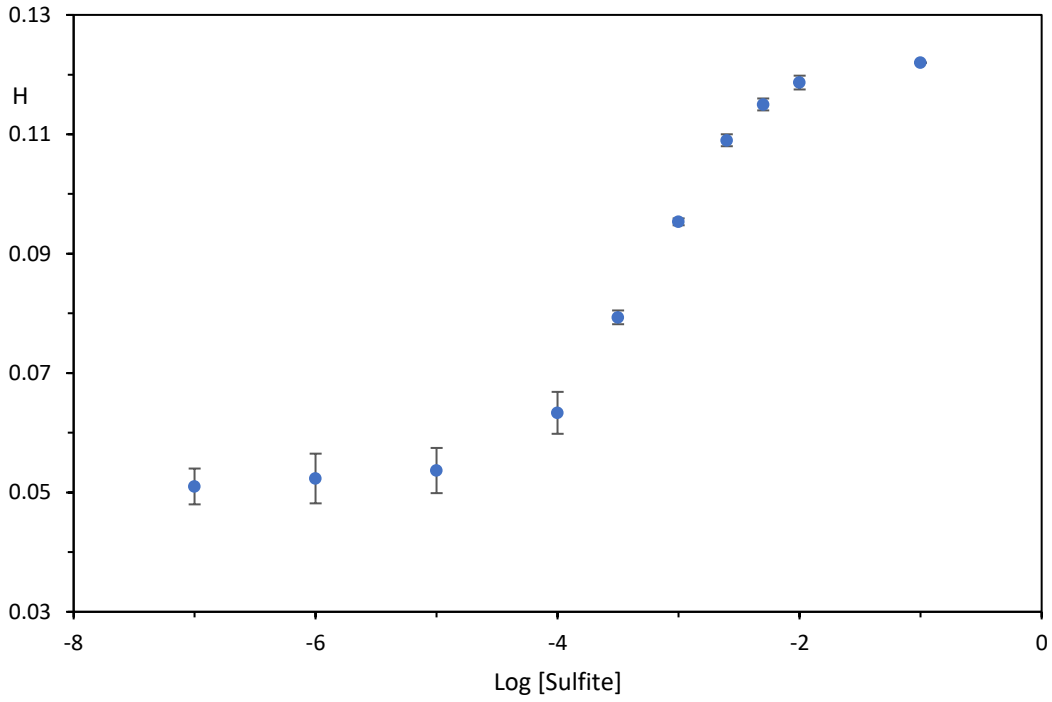

Figure S5. Evolution of H coordinate after equilibration for 2 min. with solutions of increasing sulfite concentrations.

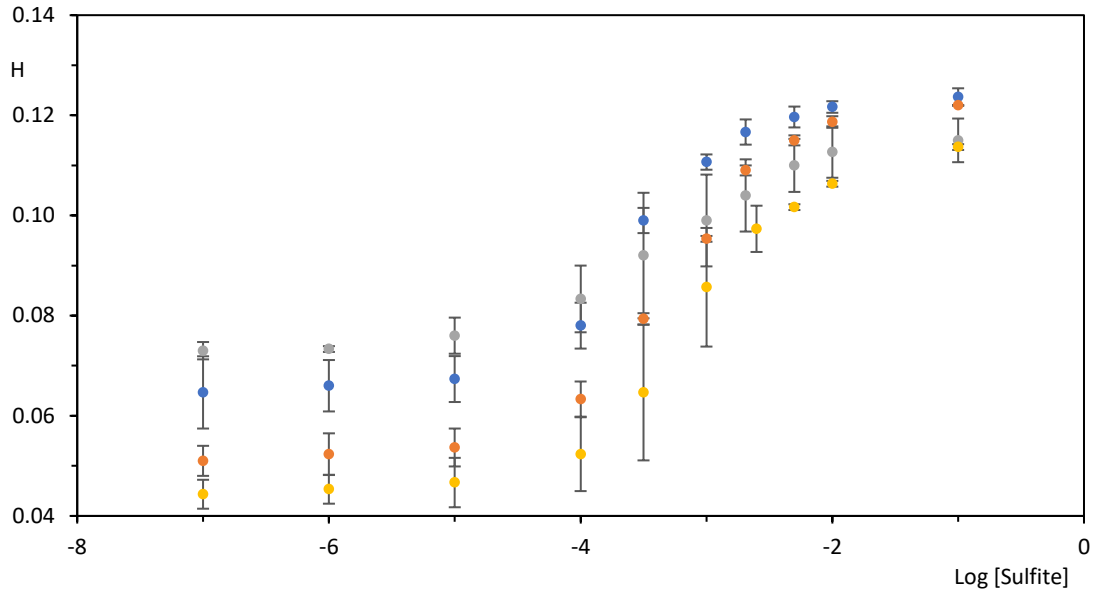

Figure S6. Evolution of the H coordinate of the sensor paper at different pH values and increasing concentrations of sulfite. pH 4.0 (grey points), 4.9 (blue points), 6.0 (orange points) and 7.0 (yellow points).

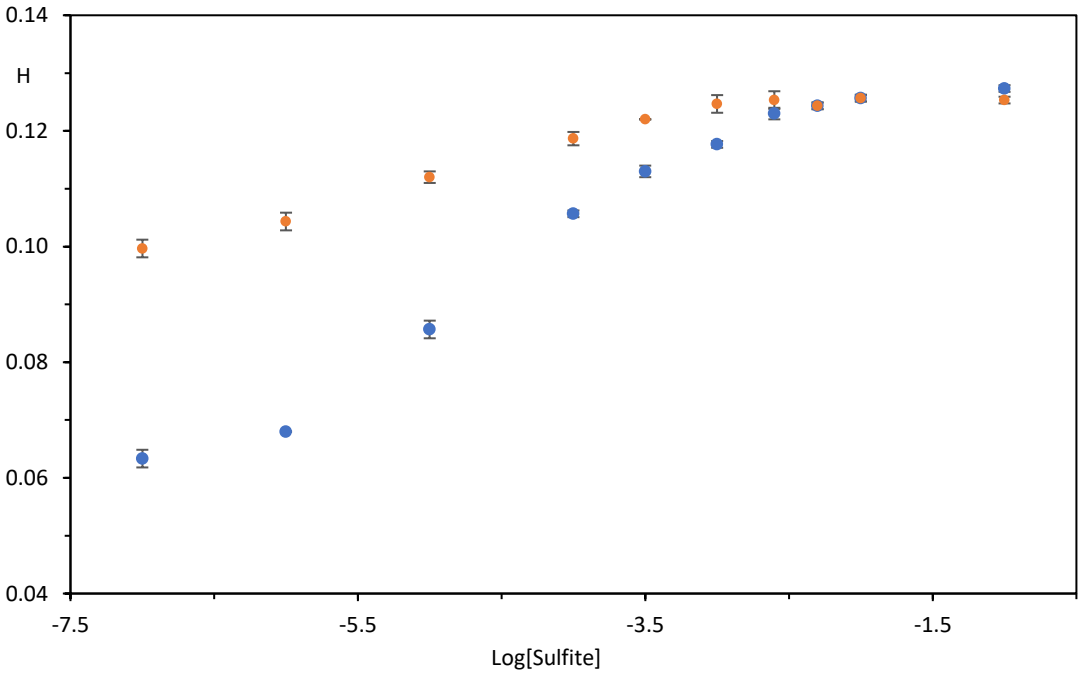

Figure S7. Evolution of the H coordinate after successive immersions of a sensor paper in solutions of increasing sulfite concentration (blue points) and subsequently decreasing concentrations (orange points).

7. µTPAD study

7.1. µTPAD design

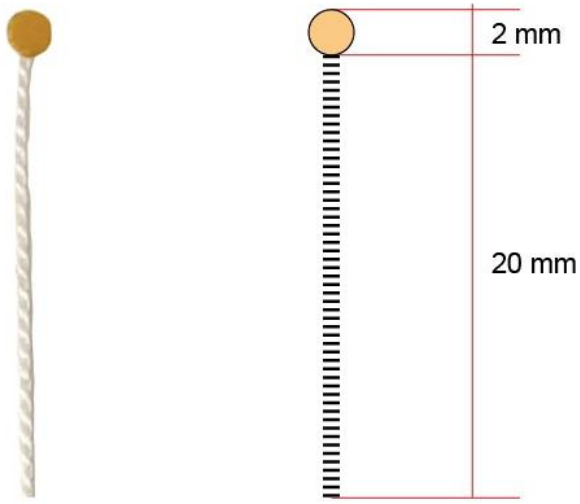

Figure S8. µTPAD design and size

7.2. Sample volume in the µTPAD

The optimal sample volume necessary to homogeneously wet the paper disk was studied using increasing volumes of a dye solution (Brilliant Blue) from 5 to 10 µL, calculating the coefficient of variation (CV) of the H coordinate of the pixels contained in the image of the disk, with 9 µL being the minimum volume required (Figure S9).

We then studied the volume of the sulfite solution to obtain the best signal. Different volumes of sulfite solutions were tested from 9 µL to 15 µL of different sulfite solutions (10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} and 1 M) and the results were compared with those obtained by immersing the sensor paper in those same solutions (Figure S10). The volumes of 10 and 11 µL present the best values with respect to the H value of the immersion method. Higher volumes saturate the µTAD and higher concentrations make the differences between the different volumes less obvious.

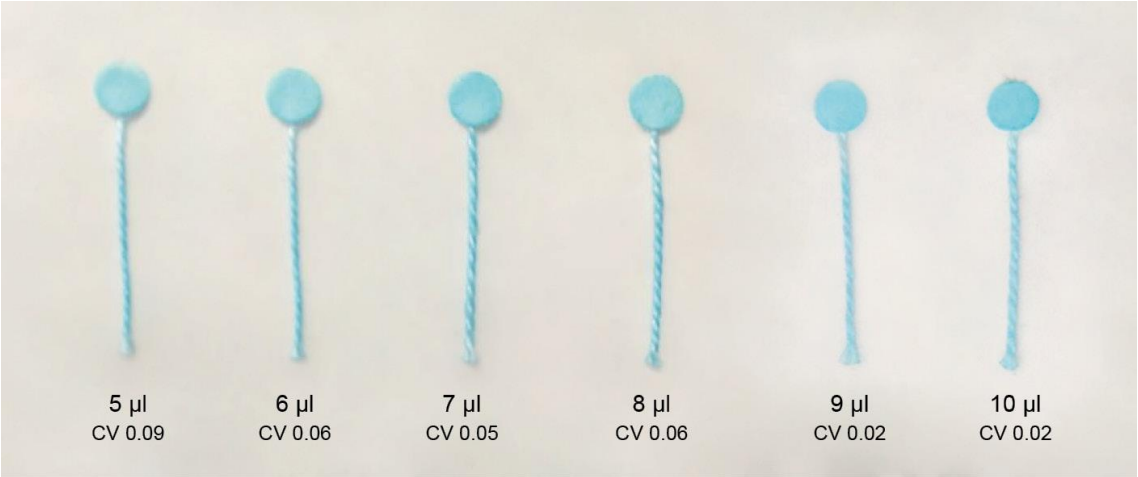

Figure S9. Volume required for homogeneous wetting of the sensor paper.

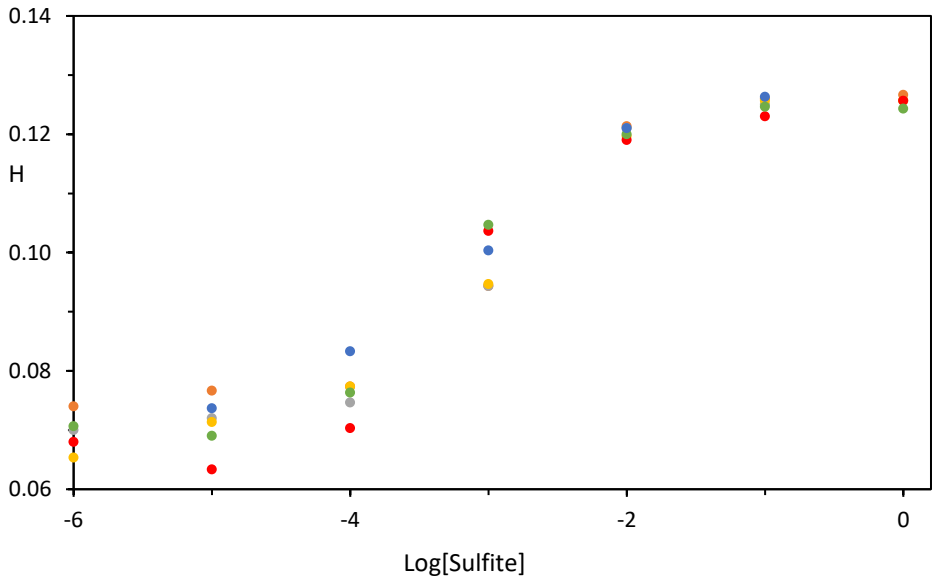

Figure S10. Influence of the sample volume of sulfite solutions (10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} and 1 M) added to the thread. 9 µL (orange dots), 10 µL (grey dots), 11 µL (yellow dots), 13 µL (red dots), 15 µL (green dots), immersion (blue dots).

7.3. Reaction time

The reaction time was studied using different concentrations of sulfite (10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} and 1 M) obtaining images every 30 s (Figure S11). 60 s was selected as the working time.

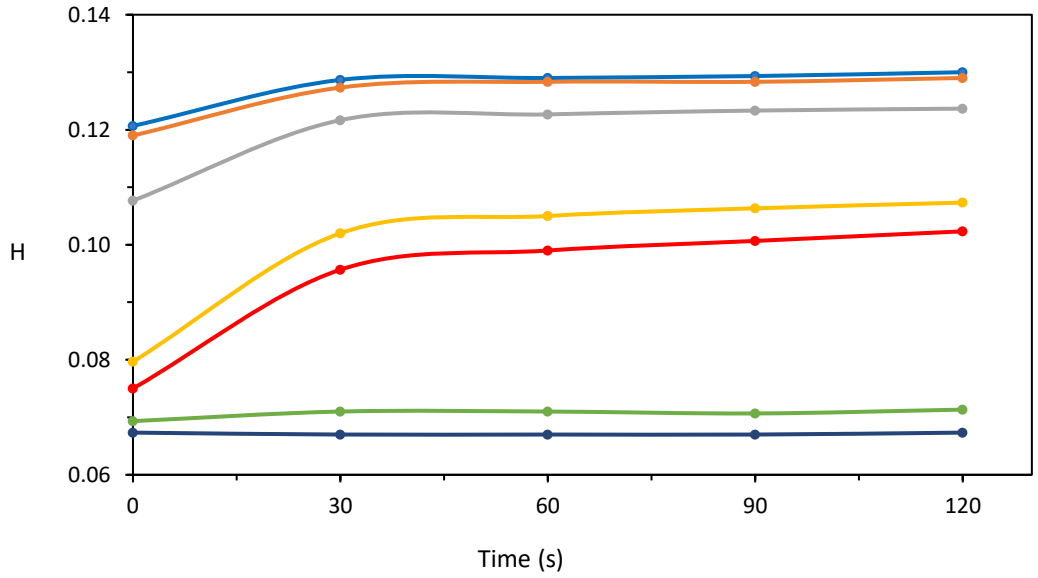

Figure S11. Evolution of H value over time for 10^{-6} (dark blue), 10^{-5} (green), 10^{-4} (red), 10^{-3} (yellow), 10^{-2} (grey), 10^{-1} (orange) and 1 M (blue) sulfite concentrations.

7.4. Buffering method

To adjust the pH, 10 µl of pH 6.0 citric acid / citrate buffer 0.5 M was deposited directly on the thread of the µTPAD, which was left to dry under ambient conditions. Three sulfite solutions of different concentrations (10^{-6}, 10^{-3} and 1 M) were measured after depositing 10 µL of each standard on the µTAD with the same buffer. The results were compared with those obtained with the same buffered sulfite solutions (see Figure S12).

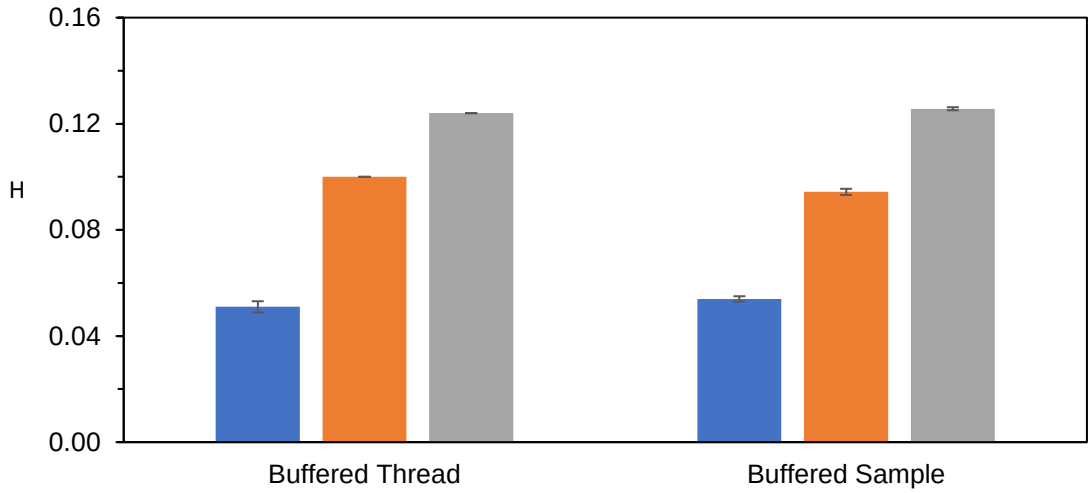

Figure S12. Hue values obtained by buffering the sample versus buffering the thread for 1 M (grey), 10^{-3} M (orange) and 10^{-6} M (blue) sulfite concentration.

7.5. Detection area size

The influence of the detection area was tested using 2, 2.5 and 3 mm diameter devices against 10^{-5}, 10^{-4}, $5\cdot10^{-3}$, $2.5\cdot10^{-3}$,10^{-3}, $5\cdot10^{-2}$, $2.5\cdot10^{-2}$, 10^{-2}, $5\cdot10^{-1}$, $2.5\cdot10^{-1}$ and 10^{-1} M sulfite solution, performing the study in triplicate (Figure S13). The results show that the 2 mm detection area provides the largest range of variation of H.

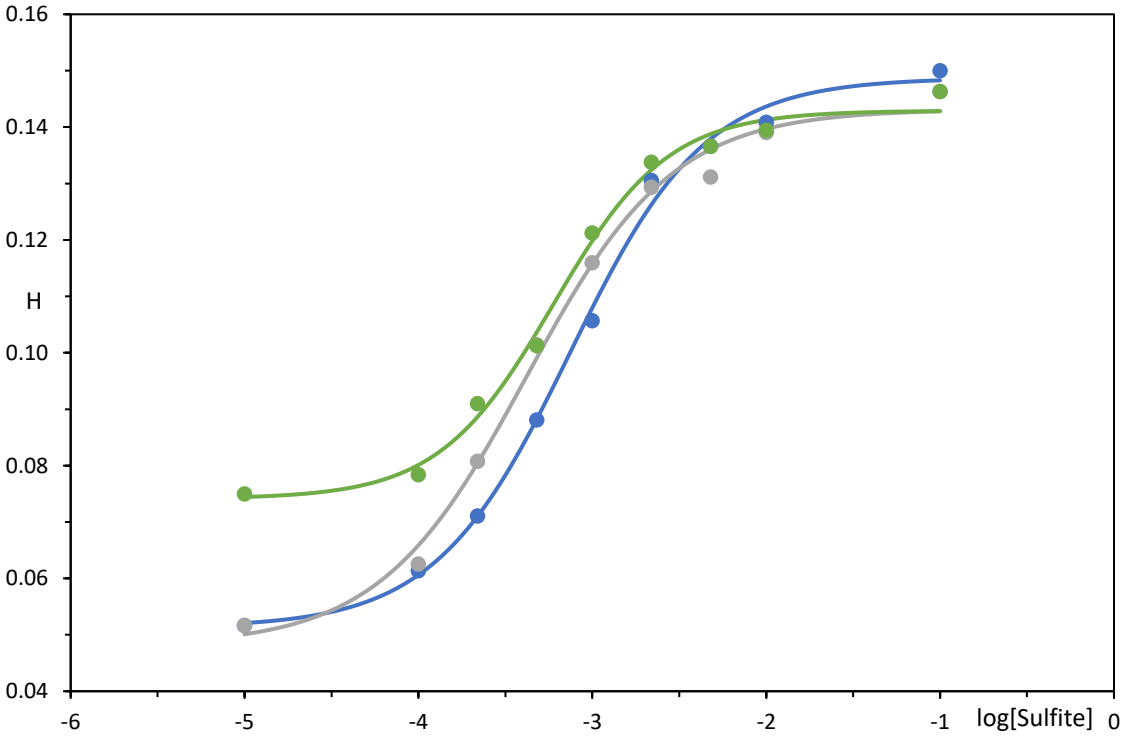

Figure S13. Influence of the diameter of the sensor paper. 2.0 mm (blue line), 2.5 mm (grey line), and 3.0 mm (green line).

7.6. Study of interferences

For the first interference study, µTPADs were used as described above with typical concentrations in wine: 1 $g\cdot L^{-1}$ acetic acid, 3 $g\cdot L^{-1}$ lactic acid, 5 $g\cdot L^{-1}$ tartaric acid, 40 $mg\cdot L^{-1}$ cysteine, 100 $mg\cdot L^{-1}$ glutathione and a 13% (v/v) ethanol. The results obtained were compared with a blank solution without interferents and a 0.1 $g\cdot L^{-1}$ (1.0 mM) of sulfite concentration.

Later, four different solutions with 5, 18, 45 and 67.5 $g\cdot L^{-1}$ each of monosaccharide were prepared, measured and compared with the signal of a 0.1 $g\cdot L^{-1}$ (1.0 mM) sulfite solution. Finally, solutions containing 67.5 $g\cdot L^{-1}$ of each monosaccharide and 0.1 $g\cdot L^{-1}$ (1.0 mM) of sulfite were tested. The used concentrations are those typically found in the different types of wines [1].

8. µPAD study

8.1. µPAD design

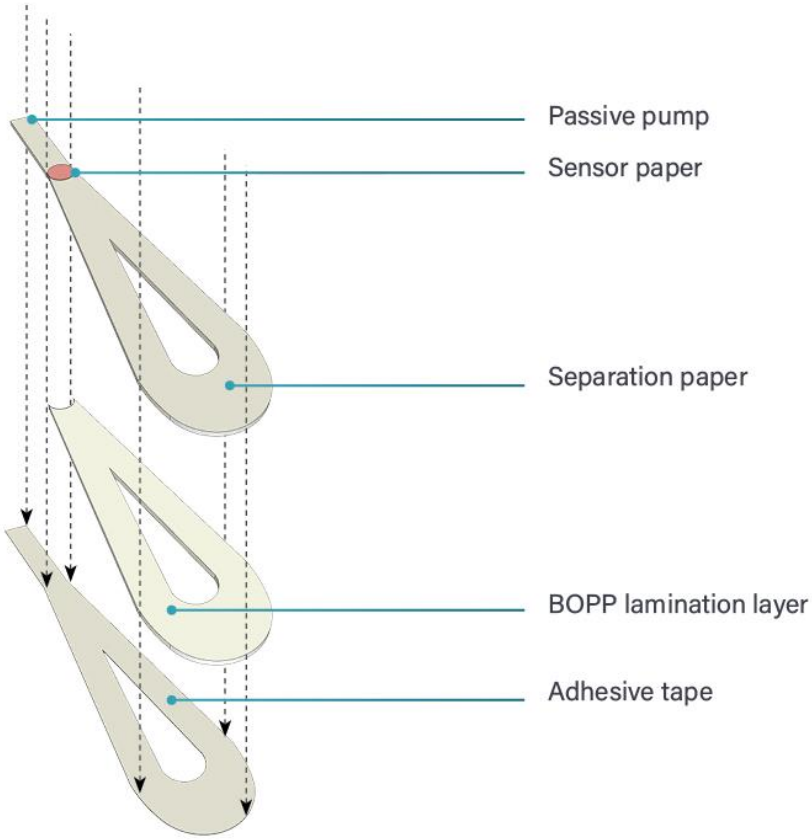

Figure S14. Exploded view of the µPAD

8.2. Colorant separation from red wine

Table S3. Properties of paper tested as support for µPADs.

Support	Basis weight $g \cdot m^{-2}$	Thickness mm	Pore size µm	W_R
1238	85	0.200	20-25	0.184
1240	85	0.200	14-18	0.184
1242	70	0.160	7-9	0.187
1244	85	0.170	2-4	0.149
1246	100	0.200	1-3	0.099
1248	80	0.210	25-30	0.318
1249	88	0.180	20-25	0.240
1254	80	0.160	2-4	0.212
PC1	90	0.180	12	0.158

8.3. Buffering on µPAD

As with the µTPAD, a study was carried out to include the buffer in the device and thus simplify the treatment of the sample. Previous published work has shown that the paper can retain the salts from the buffer in place, making it possible to buffer the paper directly [2,3]. The test was carried out buffering directly the sensor paper with 1 µL of 1, 0.5, 0.25 and 0.15 M citric/citrate pH 6 buffer. After 5 min at room temperature, 10 µL of red wine was spotted onto each paper. The pH of the µPADs was measured by attaching a litmus paper (3.9 to 6.9) to the end of the device and visually monitoring the colour of the indicator paper. A concentration of 0.5 M was chosen and was tested again with different volumes from 1.5 to 0.5 µL. Finally, 0.5 µL of 0.5 M of citric/citrate pH 6 buffer was shown to be sufficient for buffering the sensor paper.

8.4. Calibration of µPAD for sulfite

A first µPAD calibration was done with a 21 mm mono-channel with 1248 paper, with both sides laminated, and a sensor paper with 2.5 mm of diameter at the end of the design. The sensor paper was first buffered by 0.5 µL of citric/citrate 0.5 M buffer at pH 6 and left to dry. For this calibration, 10 µL of a series of sulfite solutions (10^{-5}, 10^{-4}, $5\cdot10^{-3}$, $2.5\cdot10^{-3}$,10^{-3}, $5\cdot10^{-2}$, $2.5\cdot10^{-2}$, 10^{-2}, $5\cdot10^{-1}$, $2.5\cdot10^{-1}$ and 10^{-1} M) was used (n=3). A picture was taken in the light box after 1 min after the sample reached the sensor paper. The photos were converted from RGB space colour to HSV in ImageJ software. The selected area was then analysed to obtain the H values by a ROI selection to build a calibration curve (Figure S15). The result showed a low signal in the area of interest (between 10^{-4} and 10^{-3} M). The high LOD and the calibration function with too low sensitivity did not enable the recognition of sulfite in wine samples.

Consequently, a bi-channel µPAD was prepared and calibrated in the same way as a mono-channel µPAD, resulting in an improved sensitive range and lower LOD (Figure 3).

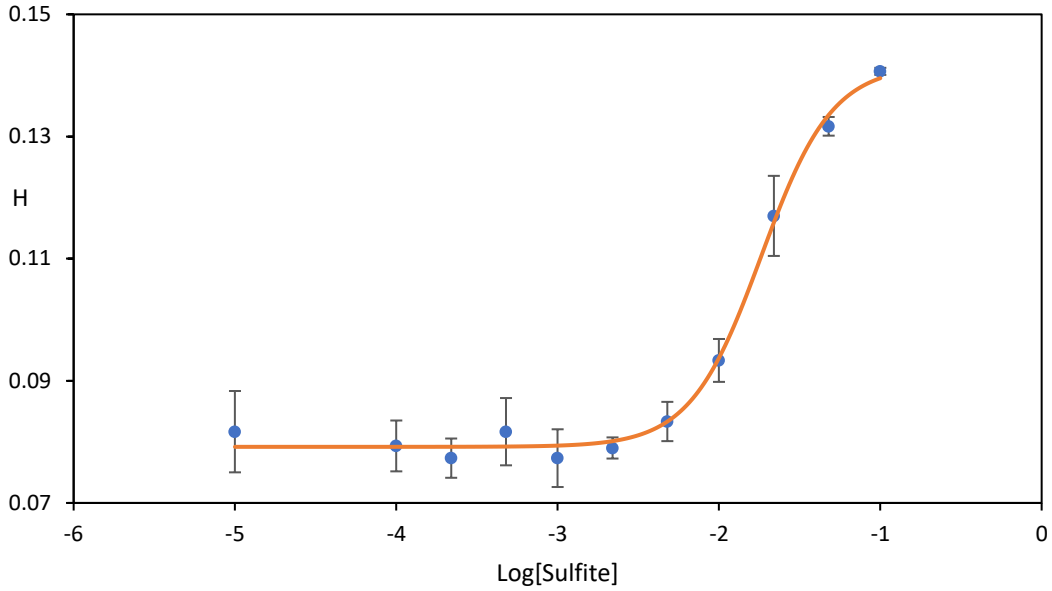

Figure S15. Mono-channel µPAD calibration.

9. References

[1] OIV, International standard for the registration of papers with or without watermarks, 2022. http://www.paperhistory.org/standard.htm.

[2] M.M. Erenas, B. Carrillo-Aguilera, K. Cantrell, S. Gonzalez-Chocano, I.M. Perez de Vargas-Sansalvador, I. de Orbe-Payá, L.F. Capitan-Vallvey, Real time monitoring of glucose in whole blood by smartphone, Biosens. Bioelectron. 136 (2019) 47–52. https://doi.org/10.1016/j.bios.2019.04.024.

[3] M.J. Arroyo, M.M. Erenas, I. De Orbe-payá, K. Cantrell, J.A. Dobado, P. Ballester, P. Blondeau, A. Salinas-castillo, L.F. Capitán-vallvey, Thread based microfluidic platform for urinary creatinine analysis, Sensors Actuators B. Chem. 305 (2020) 127407. https://doi.org/10.1016/j.snb.2019.127407.