ELECTRONIC SUPPLEMENTARY INFORMATION

Wireless wearable wristband for continuous sweat pH monitoring

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S1. Reagents, materials and instrumentation

All reagent and solvents were purchased and used as obtained from commercial sources (Sigma-Aldrich, Spain) unless otherwise indicated. Reagents for the μ CAD preparation were 10 mg·mL⁻¹ solution Na₂CO₃, methanol and Tris buffer pH=9.2 prepared from tris(hydroxymethyl)aminomethane. Calibration and characterization of μ CAD were performed using phosphate buffer solutions 0.01M which pH are ranged from 4.00 to 10.00 using KH₂PO₄ and K₂HPO₄. TLC were performed on Merck Silica gel 60 F254 aluminum sheets by staining the plates with ceric sulfate (1% w/v) and ammonium sulfate (2.5% w/v) in 10% (v/v) aqueous sulfuric acid or ethanolic sulfuric acid (10% v/v). For the μ CAD fabrication, white 100% cotton cloth was used as support and black plastisol ink was selected to define by screen printing the hydrophilic pattern. As passive pump, absorbent pads containing Flexicel[®] (P&G Spain, Madrid) were used. To apply sample and standards solutions, a syringe pump Model 11 (Hardvard Apparatus; Massachusetts, USA) together with a 5 mL plastic syringe was used to calibrate and test the μ CAD flowing the samples and standards.

Spectroscopic characterization by ¹H- and ¹³C-NMR was made using a Varian Direct Drive 400 MHz spectrometer. Spectra were recorded at room temperature. Chemical shifts are reported in ppm using solvent resonance as the internal standard (CDCl₃ at 7.26 ppm and 77.16 ppm). Electrospray ionization (ESI) mass spectra were recorded with a Waters LCT Premier XE spectrometer.

The following instrumentation was used during the system development: an Agilent diode array spectrophotometer (model 8453; Nortwalk, CT, US) commanded by UV-Vis ChemStation software, used to optically characterize the vinyl sulfone acidochromic dye AD-VS-1; a Samsung Galaxy S4 Zoom together with Open Camera App to fix the camera setting; and lately, video and images obtained were analyzed using Avidemux (Mean) and ImageJ (National Institutes of Health, Bethesda, Maryland, USA), respectively.

The development and electrical characterization of the readout and processing unit was carried out using the following laboratory instrumentation: a mixed signal oscilloscope (MSO4101, Tektronix, Oregon, USA), an 81/2-bit Digital Multimeter 3158A (Agilent Technologies, California, USA), a 15 MHz waveform generator 33120A (Agilent Technologies), and a DC power supply E3630A (Agilent Technologies). The PCB was fabricated on 1.5 mm thick FR4 substrate using a mechanical milling machine model ProtoMat S100 (LPKF Laser & Electronics AG, Garbsen, Germany). The FR4 substrate had a relative permittivity of ε_r =4.6 and a loss tangent of tan δ =0.015, while metallization layer was 35 µm thick copper with a conductivity of σ =4.6×10⁷ S/m. As software tools, MPLAB X IDE v5.40 (Microchip Technology Inc., Arizona, USA) was used to program the firmware in the microcontroller, and Altium Designer 19.1.7 (Altium Limited, NSW, Australia) was employed to design the circuit layout.

S2. Synthesis of vinyl sulfone acidochromic dye (AD-VS-1)



Fig. S1. Synthesis of AD-VS-1.

S2.1. Synthesis of 4-(2-(vinylsulfonyl)-ethylthioaniline (1)

To a solution of 4-aminothiophenol (1.6 g, 12.8 mmol) in dichloromethante:2-propanol (20 mL) was added divinyl sulfone (2,58 ml, 25.6 mmol) and triethylamine (1295 mg, 12.8 mmol). The obtained solution was stirred at room temperature for 3 h. After this time, the solvent was removed under vacuum. The crude purified by column chromatography (diethylether-hexane 4:1) to afford compound 1 as a syrup (2.174 g, 70%). ¹H NMR (400 MHz, CDCl₃) δ 7.23 (d, *J* = 8.6 Hz, 2H), 6.61 (d, *J* = 8.6 Hz, 2H), 6.57 (m, 1H), 6.39 (d, *J* = 16.6 Hz, 1H), 6.15 (d, *J* = 9.8 Hz, 1H), 3.93 (bs,2H), 3.27 – 3.12 (m, 2H), 3.09 – 2.90 (m, 2H).¹³C NMR (101 MHz, CDCl₃) δ 147.25, 135.69, 135.01, 133.85, 131.05, 119.34, 115.56, 115.18, 53.97, 28.56.



Fig. S2. ¹H-NMR (400 MHz, CDCl₃) of compound 1.



Fig. S3. ¹³C-NMR (101 MHz, CDCl₃) of compound **1**.

S2.2. Synthesis of (E)-2,6-dimethoxy-4-((4-((2-vinylsulfonyl)ethyl)thio)phenyl) diazenyl) phenol AD-VS-1)

To a solution of 4-(2-(vinylsulfonyl)-ethylthioaniline (1) (1.2 g, 4.9 mmol) in HCl 6N (1.6 mL, 9.8 mmol) cooled at -15°C was added an aqueous solution of NaNO₂ (2 mL, 340 mg, 4.9 mmol). The obtained mixture was added to a solution of 2,6-dimethoxyphenol (680 mg, 4.41 mmol) and Na₂CO₃ (1.35g, 12.73 mmol) in water (5 mL), and was stirred at room temperature for 1h. After this time, the solvent was removed under vacuum and the crude purified by column chromatography (dichloromethane-AcOEt 2:1) to afford AD-VS-1 as an orange syrup (276 mg, 14%). ¹H NMR (400 MHz, CDCl₃) δ 7.84 (d, *J* = 8.5 Hz, 2H), 7.44 (d, *J* = 8.5 Hz, 2H), 7.29 (s, 2H), 6.66 (dd, *J* = 16.5, 9.8 Hz, 1H), 6.49 (d, *J* = 16.6 Hz, 1H), 6.23 (d, *J* = 9.8 Hz, 1H), 5.95 (s, 1H), 4.00 (s, 6H), 3.37 – 3.31 (m, 2H), 3.30 – 3.24 (m, 2H).¹³C NMR (101 MHz, CDCl₃) δ 151.40, 147.37, 145.61, 138.23, 136.73, 136.02, 131.65, 129.80, 123.54, 100.71, 56.53, 53.82, 25.70. HR-MS (ESI⁺): *m*/*z*= 409,0901calcd. for [M+H]⁺ (C₁₈H₂₁N₂O₅S₂): 409,0892



Fig. S5. ¹³C-NMR (101 MHz, CDCl₃) of compound 2.

S3. Optical characterization of AD-VS-1



Fig. S6. Absorbance spectra of AD-VS-1 solved in DMSO. Blue: $0.313 \text{ mg} \cdot \text{mL}^{-1}$ Green: 0.156 mg·mL⁻¹; Yellow: 0.078 mg·mL⁻¹; Red: 0.078 mg·mL⁻¹; Purple: 0.020 mg·mL⁻¹.



Fig. S7. Molar extinction coefficient of AD-VS-1 calculated in DMSO at 400 nm (Blue dots, $\varepsilon = 1.32 \cdot 10^4$) and 259 nm (Red dots, $\varepsilon = 1.01 \cdot 10^4$).

S4. Cotton cloth characterization

Cotton cloth selected as support to fabricate the μ CAD was characterized by terms of Washburn constant. For this purpose, the beginning of a 1cm x 10 cm piece of cotton cloth was introduced in a 10 mg·mL⁻¹ Brilliant Blue solution while being recorded using the smartphone. Frames at different times were cropped from the video and calculated the area covered by the Brilliant blue solution. As expected in this kind of studies (Fig. S8), the response presents an exponential behavior, showing a fast wicking at the beginning and slowing down as the area covered with colorant increases (Fig. S8(a)).



Fig. S8. Wicking behavior of cotton cloth. A) Area vs time; B) Area vs \sqrt{time} .

The fit to the Washburn equation was obtained by fitting the experimental data with a square root function (equation S1) where A is the area covered by colorant, t time in seconds and the Washburn constant. Experimental data fits to the Washburn equation (Fig. S8(b)) with a R^2 = 0.998 and a constant a= 4.19.

$$A = a\sqrt{t}$$
 (Equation S1)

S5. µCAD design



Fig. S9. Screen-printed pattern on the cotton cloth.

S6. Selection of the analytical parameter

Several pieces of cotton with immobilized AD-VS-1 were prepared following a small variation of the procedure described in Materials and Methods (Section 2.1), by using 5 μ L of AD-VS-1 solution 2.5 mg·mL⁻¹ in DMSO. Once prepared, the samples were subjected to different pH values by adding 10 μ L of 8 different pH standards (n=3) solutions. The color was digitalized using the smartphone as described in Section S8 (see Fig. S10).



Fig. S10. Color change of the AD-VS indicator immobilized on cloth depending on the pH.

From the obtained pictures, the ROI was selected and analyzed to get the R, G, B color coordinates from the RGB color space and the H parameter from the HSV color space. As observed from Fig. S11, the H parameter achieves the best performance in terms of precision, since error bars are smaller than for the RGB color coordinates. In addition, the H parameter changes at lower pH than the other three coordinates. Because of this, the H parameter was selected to be used as the analytical parameter to detect the color change due to the pH variation.



Fig. S11. Variation of R (red dots), G (green dots), B (blue dots) (left Y-axis) and H (brown dots) (right Y-axis) depending on the pH.



Fig. S12. Dependence of H parameter with pH

S7. Optimization assays for the preparation of the sensing area



Fig. S13. Aspect of the sensing area after addition of different volumes of Brilliant Blue solution: a) 0.1 μ L; b) 0.2 μ L; c) 0.3 μ L; d) 0.4 μ L; and e) 0.5 μ L

Different concentrations of AD-VS-1 solution in DMSO (5, 10, 15 and 20 mg·mL⁻¹) were tested to achieve the most reproducible sensing area and the highest possible signal variation. Immobilization was performed following the procedure described in Materials and Methods (Section 2.1). Once prepared, the μ CAD was dipped for 30 s in 10 different standard solutions whose pH's ranged from 4 to 10 (n=5) and the change of color digitalized are showed in Fig. S14 and Table S1.



Fig. S14. Optimization of AD-VS-1 solution used for μ CAD preparation. 5 mg·mL⁻¹ (blue dots), 10 mg·mL⁻¹ (red dots), 15 mg·mL⁻¹ (purple dots) and 20 mg·mL⁻¹ (green dots) (n=5).

Concentration	CV (n=5)
5 mg⋅mL ⁻¹	4.9
10 mg⋅mL ⁻¹	3.8
$15 \text{ mg} \cdot \text{mL}^{-1}$	2.9
$20 \text{ mg} \cdot \text{mL}^{-1}$	2.5

Table S1. Average CV from the 10 standard solutions tested with each AD-VS-1 solution (n=5).

S8. Imaging and color analysis

For the μ CAD development, a Samsung Galaxy S4 Zoom was used to digitalize the color change of the AD-VS-1 immobilized in cotton cloth. For that purpose, an Open Camera App (available in Google Play Store) we used, which allows a fully control of the camera settings to a resolution of 4608×3456 pixels, aperture value of f/3.3, exposure time of 1/800 s, ISO-800 and 2800K white balance, and format saving in *jpg*. In the case of video recording, the camera was set to 1280×720 pixels, 30 fps, 2800K white balance, and format saving in *mp4*. In all the cases, the digitalization was conducted inside a cube light box in a fixed place, illuminated by two bulbs whose color temperature was 3000K.

Once the recordings and pictures were obtained, Avidemux software was used to crop different frames from the videotape, and they were analyzed together with the camera pictures using ImageJ software. This software allows the manual selection of a region of interest (ROI) that lately will be analyzed obtaining the red (R), green (G), and blue (B) coordinates from the RGB color space, as well as the Hue parameter (H) from the HSV color space. To obtain this one, the pictures were converted from the RGB color space to HSV color space by using a color space converter included in the same software.

S9. µCAD patterning procedure

Two different types of μ CAD were prepared following two different procedures. The first one was prepared as described in Section 2.1 of the manuscript, while the other type was prepared by swapping the immobilization and patterning steps. As it can be observed in Fig. S15, the response obtained for both μ CADs was pretty similar in terms of H value but, on the other side, the errors associated to the μ CADs prepared adding first the AD-VS-1 are much higher than if the screen-printing of the cloth is done first and then AD-VS-1 is immobilized.



Fig. S15. μ CAD response depending on the making procedure. Patterning and then AD-VS-1 immobilization (red dots); AD-VS-1 immobilization and then patterning (blue dots) (n=5).

S10. 3D-printed wristband design

A two-piece case was designed and fabricated (Fig. S16b) with a Witbox 2 desktop 3D printer (BQ, Madrid, Spain) using coal black polyurethane Filaflex filament (Fig. S16a) and polylactic acid filament (Fig. S16c), both of them with a diameter of 1.75 mm. This low-cost 3D printer was operated using *Cura* open-source software to command and control the process parameters. Apart from the attachment of the system to the user's wrist, the 3D-printed case ensures the correct alignment and positioning of the pH sensor and the sensing module, while providing a uniform dark environment for the colour measurements. The *.stl* design file has been provided as supplementary material.



Fig. S16. 3D-printed wristband case design.

S11. AndroidTM application development

To transmit the data from the wristband to a smartphone, a user-friendly Android[™] application was developed. Android Studio 3.5.3 was used as the integrated development environment (IDE) to code the application, which was designed and tested against API level 27 (Android 8.1). However, it supports different Android versions as the lowest API level compatible with the application is API 14 (Android 4.0).



Fig. S17. Screen captures of the custom-developed app showing (a) RGB coordinates, (b) H values and (c) pH values; (d) menu bar with the options offered to the user; (e) option *Share* where the user can send the logged data and graph through different messaging and/or cloud services.

The application takes control of the Bluetooth interface of the smartphone to communicate with the Bluefruit LE module. Once the wristband is paired with the smartphone, the BLE sends in real time the measured color data obtained with the color sensor (RGB coordinates) from the MCU to the smartphone app. The obtained RGB data are displayed on the screen as shown in Fig. S17(a). The RGB coordinates are then

converted to Hue value (H) in the HSV color space. The graph of the H values can be obtained in the middle tab of the app interface, as depicted in Fig. S17(b). Finally, the pH values are computed from the normalized H values as per the obtained calibration (see Section 3.3), and the graph is displayed in the last tab of the app as shown in Fig. S17(c). For data visualization, *MPAndroidChart* open source charting library version 3.1.0 was used (Jahoda, 2016). The user can scroll, pan, and zoom in and out in the displayed graphs. Fig. S17(d) shows the menu bar with the different options offered by the app: *Save chart*, *Clear chart*, *Save data*, *Share* and *About*. Through the *Save chart* and *Save data* options, the application provides a datalogger function to save the measurements in a text file and the graph as an image file that are stored in the smartphone's memory. The results (both graphical views and stored values) can be shared through email and/or different cloud or messaging services using the *Share* option as illustrated in Fig. S17(e), thus allowing the possibility to connect to remote health care providers or medical experts.

S12. Dipping calibration



Fig. S18. µCAD calibration from acidic to basic pH (blue dots) and from basic to acidic pH (red dots) (n=3).



Fig. S19. µCAD calibration and fitting to a Boltzmann equation (n=6).

Boltzmann equation:

$$y = A_2 + \frac{(A_1 - A_2)}{1 + e^{\frac{(x - A_3)}{A_4}}}$$
(Equation S2)

S13. Absorbent pad characterization

Round pieces of absorbent with different diameters (5.0, 7.5, 10.0, 12.5 and 15.0 mm) were cut using a laser engraving printer and later weighted. Afterwards, all of them were dipped into a 500 mL baker full of water for 5 minutes, and then taken from the inside and weighted again. The increment in mass of the adsorbent material is due to the water that it can absorb. Fig. S20 showed the increase in weight depending on the mass and area of the material used. From the slope of the Fig. S20(a), it is proved that the material can absorb 20 times its own weight.





Fig. S20. Water absorbed by Flexicel material depending on: a) wheight, and b) area of material used (n=3).



Fig. S21. Signal variation along time: a) from pH 4 to 10; b) from pH 10 to 4.



Fig. S22. Reversibility of the μ CAD. Acidic-basic-acidic cycle (blue line); basic-acidic-basic cycle (orange line).

S15. Stability

Three different μ CADs just prepared were kept in a black desiccator. Its color was monitored over time using the digital camera as described in Section S7. The obtained results were plotted in a Shewart graph to determine the stability of the μ CAD in dark conditions. It can be observed that after 8 weeks (Fig. S23), the H value deviates away from the control area.



Fig. S23. Lifetime of the μ CAD. Dotted line: upper and lower control lines at $\pm 2\sigma$.

S16. Comparison with commercial pH-meter

Once the μ CAD was fully characterized, seven different samples were analyzed using the μ CAD and the obtained results were compared to the ones provided by a pH-meter Crison, mod. micro pH2000 (Barcelona, Spain). Table S2 shows the obtained results, being the error in the determination around 2% in almost all the cases.

Sample	pH-meter	μCAD	Error (%)
Pool water	7.5	7.6	1.3
Tap water 1	7.7	7.9	2.6
Tap water 2	7.3	7.4	1.4
Standard buffer solution 1	8.4	8.0	4.8
Standard buffer solution 2	6.3	6.5	3.2
Contact lens fluid 1	7.3	7.3	0.0
Contact lens fluid 2	7.6	7.5	1.3

Table S2. Validation of samples using the μ CAD and a pH-meter as reference method (n=3)

S17. Electronics performance

All the components in the readout and processing electronics were selected for low power consumption, thus allowing long battery life. The white LED has a very low power consumption of only 0.8 mW, while the S11059-02DT color detector only consumes 250 μ W in operational mode. The heart of the readout circuit, i.e. the PIC12LF1822 MCU was setup at 500 kHz low-power internal oscillator. Without considering the Bluetooth module, the consumption of the readout circuit including the MCU, color sensor and LED is 8.5 mW in operational mode. The Bluetooth module in transmission mode has an average current consumption of 2.11 mA, meaning that the power consumption at the battery voltage supply of 3.7 V is 7.81 mW. Therefore, the consumption of the whole wristband system including the wireless transmission is 16.31 mW. Considering the battery capacity, this means that the expected battery life is around 34 hours in continuous operational mode, which is enough for the proposed application.

Even so, the battery life was further extended by implementing a non-continuous sensing and transmission protocol in the MCU program. Due to the nature and rate of the human sweat, it is not necessary to sense and transmit the data in a continuous way. Instead, in a real-life application it would be enough to conduct the sensing measurements and transmit the data every 5 seconds. During the rest of the time, the system can be entered in a power-down mode. This means that the number of wakeups per hour is 720. The measured time needed by the system to take, process and transmit the color measurements in each wake up is roughly 1 second. In power-down mode, both the LED and the color sensor are switched off, while the MCU is configured to sleep mode with a power consumption of only 74 nW. During the non-operational periods, the Bluetooth

module is set to a slow advertising mode. Altogether, the power consumption in powerdown or sleep mode is reduced down to 5.25 mW. Considering the capacity rating of the battery (150 mAh), the power consumption of the wristband in each mode, and the number and duration of wakeups, the estimated battery life following this approach would be 2.63 days. In any of the cases (continuous or non-continuous operation), the wristband presents a battery life much longer than the absorption capacity of the Flexicel pad, which is currently limited to 1058 min (Section 3.2). Depending on the sweating rate and the required application, the absorbent pad could be replaced to allow longer operations thanks to the low power consumption of the associated electronics. As an illustrative example, if the wristband was used daily during 100 min for a training session, the electronics would allow operation for more than one month before requiring the battery charging. In this case, only the disposable absorbent pad in the μ CAD would require a replacement every 1058 min, i.e. more than 17 hours. In the case of sessions of 100 min duration, this would mean a replacement every 10 sessions approximately.