

New autonomous and self-signaling biosensing device for sarcosine detection

Carolina S. Hora^{a,b}, Ana P.M. Tavares^c, Liliana P.T. Carneiro^c, Dzmitry Ivanou^{a,b}, Adélio M. Mendes^{a,b,*}, M. Goreti F. Sales^{c,**}

^a LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465, Porto, Portugal

^b ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, Porto, 4200-465, Portugal

^c BioMark@UC, Department of Chemical Engineering, Faculty of Sciences and Technology, University of Coimbra, Rua Silvio Lima-Polo II, Coimbra, 3030-790, Portugal

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ABSTRACT

An early diagnosis is the gold standard for cancer survival. Biosensors have proven their effectiveness in monitoring cancer biomarkers but are still limited to a series of requirements. This work proposes an integrated power solution, with an autonomous and self-signaling biosensing device. The biorecognition element is produced *in situ* by molecular imprinting to detect sarcosine, a known biomarker for prostate cancer. The biosensor was assembled on the counter-electrode of a dye-sensitized solar cell (DSSC), simultaneously using EDOT and Pyrrole as monomers for the biomimetic process and the catalytic reduction of triiodide in the DSSC.

After the rebinding assays, the hybrid DSSC/biosensor displayed a linear behavior when plotting the power conversion efficiency (PCE) and the charge transfer resistance (R_{CT}) against the logarithm of the concentration of sarcosine. The latter obtained a sensitivity of 0.468 Ω /decade of sarcosine concentration, with a linear range between 1 ng/mL and 10 μ g/mL, and a limit of detection of 0.32 ng/mL. When interfacing an electrochromic cell, consisting of a PEDOT-based material, with the hybrid device, a color gradient between 1 ng/mL and 10 μ g/mL of sarcosine was observed. Thus, the device can be used anywhere with access to a light source, completely equipment-free, suitable for point-of-care analysis and capable of detecting sarcosine within a range of clinical interest.

1. Introduction

Prostate cancer is the second most commonly occurring cancer in men and the fourth most common cancer worldwide [1]. The early detection of prostate cancer is usually made based on rectum digital examination, endorectal ultrasonography, prostatic puncture biopsy, computed tomography, and magnetic resonance imaging [2]. These standard examination tests are invasive, expensive and do not meet current point-of-care needs, thus stimulating the development of biosensors. Lately, biosensing devices have become ubiquitous in the point-of-care detection of any target analyte [3,4]; the most common and widely available biosensors are the glucose meters, pregnancy tests and now, the COVID antigen tests [5–7]. Biosensors can also be used to monitor cancer biomarkers [8–10], and sarcosine is a known biomarker

for prostate cancer [11,12]. Healthy patients have normal sarcosine levels of *ca.* (1.4 $\pm$ 0.6) μ M, and these levels are significantly increased during the progression and the metastatic process of prostate cancer [13, 14]. High values of sarcosine are also known indicators of Alzheimer, sarcosinemia, dementia, colorectal and stomach cancer, among others [15].

A biosensor is mainly composed of two elements: a bioreceptor or biorecognition element and a transducer. The biorecognition element determines the selectivity of the biosensor towards the target analyte. The bioreceptors can either be biological or synthetic. Although biological bioreceptors ensure simplicity and high selectivity of the device, they are very expensive, require special storage conditions and have limited shelf-life [16]. A different pathway considers the use of plastic antibodies [17], which simulate the recognition properties of biological

* Corresponding author. LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465, Porto, Portugal.

** Corresponding author.

E-mail addresses: mendes@fe.up.pt (A.M. Mendes), goreti.sales@gmail.com (M.G.F. Sales).

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materials. Plastic antibodies are usually prepared using molecularly imprinted polymers (MIPs). This technique involves imprinting a specific molecule, also known as template, in a 3D structure [18], so that a polymeric network is grown around the template. When the template is removed from the polymeric matrix, it reveals the binding positions displaying analogous behavior to natural antibodies. These binding positions should match the size and shape of the target. This technique can be challenging, but research on MIPs for protein recognition has been already addressed successfully [19,20].

Some biomimetic sensor approaches for sarcosine detection have been described in the literature. For example, Nguy et al. [21] reported a biomimetic sensor for sarcosine detection using the molecularly imprinting technique with aminothiophenol monomer electropolymerized onto carbon electrodes with gold-nanoparticles interlayer. The biosensor was assessed by Electrochemical impedance spectroscopy (EIS) and the authors reported a sensitivity of 1.437 k Ω /decade of $C_{\text{sarcosine}}$, over a linear range between 1 ng/mL to 1.6 μ g/mL. Another example of a biosensor developed using the MIP technique was proposed by Sheydaei et al. [22], using methacrylic acid as a functional monomer to imprint sarcosine onto a carbon paste electrode. This time the device was evaluated through differential pulse voltammetry, and the sensitivity reported was 0.0438 μ A/ μ M, over a concentration range of 5 μ M–1.1 mM (0.44–98 μ g/mL). In contrast to these previous potentiometric examples of sensors, Kumar et al. [14] suggested an amperometric sarcosine biosensor based on covalent immobilization of sarcosine oxidase onto gold electrodes. The linear range extended from 0.1 to 100 μ M (8.9 ng/mL – 8.9 μ g/mL). Additionally, Narwal et al. [23] and Deswal et al. [24] used similar approaches to also develop amperometric sarcosine biosensors, immobilizing sarcosine oxidase onto carbon/chitosan composites, electrodeposited onto gold electrodes. Both biosensors showed high sensitivity of 277.5 μ A/ μ M, with a linear range between 0.1 – 100 μ M and 0.001–100 μ M, respectively.

Electrochemical biosensors, such as described previously, are known to be small, fast, cheap and, most importantly, they may replace the time-consuming laboratory analyses [15]. Yet, they remain electrically dependent, since they need batteries to operate. Photovoltaic cells, such as dye-sensitized solar cells (DSSCs), convert photons into electrical energy and they can be used as electrochemical transducers, while providing electrical power to biosensing devices. The simplest approach for merging biosensors with DSSCs is to replace the counter-electrode material with the biosensing film [25–27]. Moreover, aiming at developing a fully autonomous and self-signaling device, an electrochromic cell will be interfaced in the external circuit of the hybrid DSSC/biosensor [28,29]. This work proposes an integrated power solution for a device that will be able to detect sarcosine in urine samples within a range of clinical interest, which, to the best of our knowledge, has not been reported yet.

2. Experimental procedure

2.1. Reagents and solutions

The chemicals used throughout this work were of analytical grade and the water was ultrapure Milli-Q laboratory grade. Screen-printable TiO₂ pastes (30NR-D and 18NR-AO) and EL-HPE iodine electrolyte were acquired from Greatcell Solar Materials®. Dye N719 (ruthenizer 525-bisTBA), and chenodeoxycholic acid (CDA) were ordered from Solaronix®. TiCl₄ (99.9%) and Sarcosine were from Acros Organics. Absolute ethanol, titanium diisopropoxide bis(acetylacetonate), anhydrous isopropyl alcohol and dimethyl sulfoxide (DMSO), potassium hydroxide (KOH), lithium perchlorate (LiClO₄, 98%) and sodium perchlorate hydrate (NaClO₄, 99.99%) were purchased from Sigma Aldrich®. Potassium Chloride (KCl) was acquired from Merck; acetonitrile from Carlo Erba; 3,4-Ethylenedioxythiophene (EDOT, 98%) from TCI; pyrrole (Py, 98%) from Alfa Aesar, phosphate buffered saline (PBS) tablets from Fisher BioReagents; potassium di-hydrogen phosphate (KH₂PO₄) and

Dipotassium hydrogen phosphate (K₂HPO₄) were purchased from Pan-reac; creatinine was from Fluka; urea was from Fagron; and Nafion™ in alcoholic solution (20 wt%) was ordered from QuinTech.

2.2. Apparatus and materials

Electrochemical measurements were performed with an Autolab (PGSTAT302 N, Metrohm) electrochemical station, equipped with a frequency response analysis (FRA) module controlled by the NOVA 2.1 Software. Current vs. applied potential (*I*-*V*) response of the DSSCs was obtained under simulated solar light (AM1.5G, 100 mW•cm⁻²) using a Solar Simulator MiniSol (LSH-7320, Newport). The electrochemical impedance spectroscopy (EIS) was performed in dark conditions at -0.65 V; a sinusoidal perturbation with an amplitude of 10 mV and a frequency range of 100 kHz-0.1 Hz was applied.

Throughout the work, fluorine-doped tin oxide (FTO)-coated glasses (TEC-7; 7 Ω /sq) acquired from Greatcell Solar Materials® were used as conductive substrates for the construction of the biosensor, DSSCs and electrochromic device. The digital images of the electrochromic devices were obtained with a 17 Mp smartphone camera (Xiaomi), with the aid of tripod to always use the same lighting conditions, and the color coordinates of these images were collected using ImageJ in three different points. The color coordinates were analyzed according to the Hue, Saturation and Luminousness – HSL – color system. The quantitative color calibration was achieved by plotting the Saturation vs. the logarithm of the analyte concentration, where the Saturation represents the intensity of the color of the image.

2.3. Preparation of the photoanode

The photoanode was prepared following the work of Hora et al. [30], which started with the deposition of a blocking layer with a thickness of 80 $\pm$ 5 nm by spraying a TiO₂ precursor solution (7.0 mL of anhydrous i-PrOH + 0.6 mL titanium diisopropoxide bis(acetylacetonate) + 0.4 mL of acetylacetone) on substrates heated up to 450 °C, for 45 min. Then, two commercial pastes of TiO₂ were deposited by a screen printer in the following configuration: two mesoporous layers (30NR-D) and one scattering layer (18NR-AO), resulting in a thickness of 21 $\pm$ 1.0 μ m. The thickness was assessed by analyzing the cross-section image with Scanning Electron Microscopy (SEM, FEI Quanta 400 FEG ESEM). Each layer was dried in a hotplate at 120 °C for 10 min before printing the next layer; the photoelectrodes had an active area of 0.196 cm². Afterwards, the photoanodes were sintered for 1 h at 500 °C, with a heating rate of 2 °C/min. To improve the adhesion of the titania layer and suppress recombination losses, the photoanodes were treated in a 40 mM TiCl₄ aqueous solution for 20 min at 70 °C, dried and annealed at 500 °C for 1 h. Subsequently, the photoanodes were dipped in a fresh solution of 0.5 mM N719 dye +5 mM CDA in absolute ethanol for 20 $\pm$ 1 h. Finally, the identical photoanodes were washed with absolute ethanol, dried in N₂ flow, and immediately used.

2.4. Preparation of the biosensor

The glass substrates were cleaned in an ethanol solution of KOH (2.67 M in EtOH 70 wt%) in an ultrasonic bath, washed with distilled water, and dried. The steps involved in the construction of the sarcosine biosensor through molecular imprinting technique are illustrated in Fig. 1.

The construction of the biosensor was made in a three-electrode configuration, with the FTO glass acting as the working electrode, Ag/AgCl (0.1 M KCl) acting as the reference electrode and a platinum wire acting as a counter-electrode. To build the biosensor, two conductive polymers were electropolymerized: EDOT and Py. Both have been reported earlier as effective catalysts in DSSCs [31–36], albeit PEDOT presents a much higher conductivity (300–750 S•cm⁻¹) than PPy (2–100 S•cm⁻¹) [37–39]. After cleaning the FTO glasses, a solution of

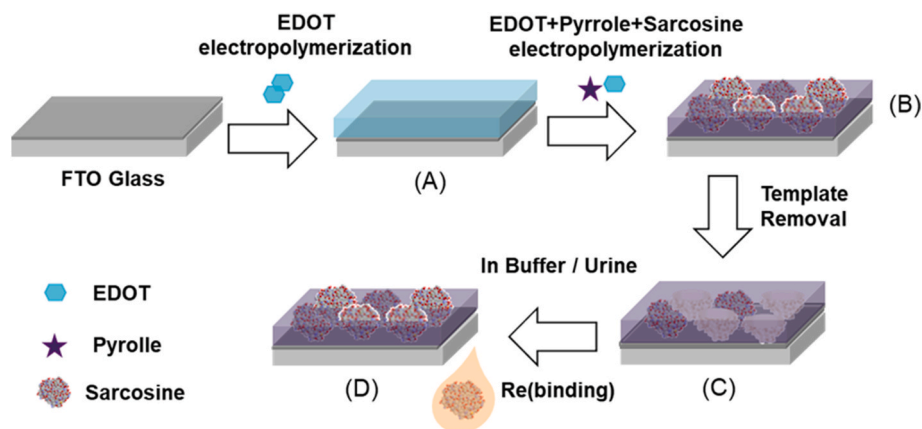


Fig. 1. Schematic illustration of the several steps for the sarcosine biosensor construction through molecular imprinting polymer technique.

EDOT (10 mM in KCl) was electropolymerized at a constant potential of 1.2 V for 15 s, and the FTO glass turned to a bright blue (step A in Fig. 1). Then, the PEDOT-coated glasses were immersed into a solution of EDOT (10 mM), Py (2 mM) and sarcosine (0.1 mM) in PBS buffer (pH 7.4) and subjected to further electropolymerization by applying 10 cycles of electrode potential sweep (50 mV/s) from -0.3 V to 1.0 V and back to -0.3 V. The resulting MIP sensor was visible to the naked eye because the glass substrate changed from bright blue to dark purple (step B in Fig. 1).

After imprinting sarcosine, the amino acid template was removed from the polymeric matrix by incubating the biosensing film with an EtOH:H₂O solution (7:3, V/V) for 90 min and a pure H₂O solution overnight (step C in Fig. 1). To check non-specific interactions between the polymeric matrix and sarcosine, a non-imprinted polymer (NIP) was prepared in parallel following the same protocol without the sarcosine template. The rebinding of the target template was performed on both MIP and NIP sensors and accomplished by incubating either blank buffer or standard solutions prepared in the buffer in the modified area of the electrode for 30 min (step D in Fig. 1). The sarcosine standard solutions were prepared either in phosphate buffer or urine (diluted 10 times). The sarcosine standard solutions were incubated, starting from the lowest (1 ng/mL) to the highest concentration (10 μ g/mL), with a washing step between each standard.

2.5. Assembly of hybrid device, sarcosine rebinding and electrochemical characterization

The set-up of the hybrid DSSC/biosensor is represented in Fig. S1 in Supplementary information. In a conventional DSSC the photoanode and the counter-electrode are sandwiched together with a thermoplastic sealant in the middle for a permanent sealing of the solar cell. The DSSC used herein had a more temporary option as sealant – scotch magic tape, so that the biosensor could be easily accessible to the rebinding assays. This unorthodox sealant worked due to the presence of acetonitrile in the commercial iodine electrolyte (EL-HPE) used. The acetonitrile slightly melted the scotch tape and created a temporary seal, lasting ca. 1 h, time enough to conclude the necessary characterizations. After each rebinding stage, where the standard solution of sarcosine prepared either in buffer or in urine (diluted 10 times) was incubated on the counter-electrode, the DSSC was assembled with one of the identical photoanodes produced as described in Section 2.3. The hybrid device was evaluated by recording I - V curves in linear sweep voltammetry from -0.8 V to 0.1 V as well as EIS in dark conditions at -0.65 V from a frequency range of 100 kHz-0.1 Hz. All assays were conducted in triplicate.

2.6. Assembly and performance of electrochromic cell

2.6.1. Preparation of electrochromic material

The electrochemical component was developed according to previous works [28,29,40]. By itself, EDOT has electrochromic properties but the 4-(2,5-di(thiophen-2-yl)-1H-pyrrol-1-yl)aniline (SNS-NH₂) was added to the mixture to enhance the color features of the final electrochromic device. The synthesis and chemical structure of SNS-NH₂ is described in the Supplementary information. To prepare the electrochromic cell, first, a solution of 0.25 mM of SNS-NH₂, 100 mM of EDOT, 100 mM of NaClO₄ and 100 mM of LiClO₄ were prepared in acetonitrile. Then, the electrochromic electrode was obtained by *in situ* electropolymerization of the solution previously described, in a three-electrode configuration, with a single sweep of electrode potential from 0 V to 1.3 V at a scan-rate of 50 mV/s; the FTO substrate turned blue. To ensure that the electrochromic electrode was ready to use and in its neutral state, a constant potential of 0 V was applied for 20 s with 0.1 M KCl as electrolyte, after which the glass electrode changed color from blue to purple.

2.6.2. Assembly of electrochromic device and interface with hybrid DSSC/biosensor

The electrochromic device was assembled by sandwiching two FTO glass substrates, one with the electrochromic material and one with bare FTO, using a Nafion™ electrolyte connecting both electrodes (Fig. 2B). Then, the electrochromic device was interfaced with the hybrid DSSC/biosensor by connecting the photoanode of the DSSC to the bare FTO electrode of the electrochromic device (black pathway in Fig. 2) and the counter-electrode with the biosensing film to the electrochromic electrode (red pathway in Fig. 2).

Under working operation, the hybrid DSSC/biosensor is illuminated by the solar simulator (or another light source available) and the current produced flows through the electrochromic device and causes a color change, which will be dependent of sarcosine concentration in the sample analyzed (Fig. 2C). When sarcosine is not present in the sample, *i.e.*, blank sample, the catalytic film won't have any obstacles for the charge transport, so the hybrid device will produce the maximum power/photovoltage and the electrochromic cell will turn blue. When the sample has a high concentration of sarcosine, the analyte will attach itself to the specific bindings and obstruct the charge transport, so the hybrid device will generate much less power/photovoltage and the electrochromic cell will turn purple.

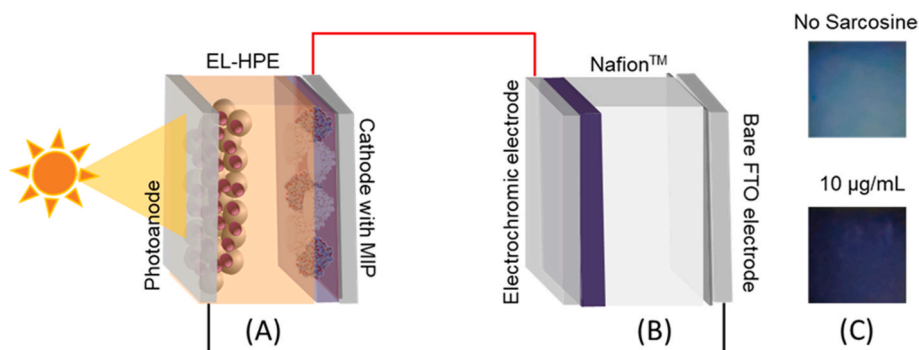


Fig. 2. Schematic representation of the interface between hybrid DSSC/biosensor (A) the electrochromic device (B) and two examples of electrochromic response depending on the concentration of sarcosine present in the sample tested (C).

3. Results and discussion

3.1. Photoelectrochemical performance of the DSSC/biosensor device

The hybrid DSSC/biosensor was electrochemically characterized after the deposition of each component layer. The characterization methods employed include: i) standard J - V curves, to determine the open-circuit potential (V_{OC}), the short-circuit current density (J_{SC}), the fill factor (η_{FF}), the maximum power point (MPP) and the power conversion efficiency (η_{PCE}); and ii) the EIS under dark conditions, to quantify the series resistance (R_s), the charge transfer resistance at the interface counter-electrode/electrolyte (R_{CT}) and the resistance to the recombination in the interface photoanode/electrolyte (R_k). These results are displayed in Fig. 3; the impedance spectra were fitted with the equivalent electrical circuit in Fig. 3c.

When the counter-electrode consists solely of PEDOT, a PCE of 7.3%

was achieved under 1-Sun illumination (Table 1). When the MIP layer was applied, the photovoltaic performance significantly dropped, mainly because the η_{FF} was more than 50% lower and the V_{OC} decreased 26 mV, resulting in a PCE of 2.7%. This decrease in η_{FF} and the shape of the J - V curve are most likely related to a substantial increase of the overall internal resistance of the whole device, particularly the R_{CT} . The copolymerized EDOT/Py film loaded with sarcosine (MIP) displays an interface with the electrolyte which exhibits a substantially higher R_{CT} ; the R_k is quite low, and this leads to higher recombination in the photoanode. After both sarcosine removal steps – 1.5 h and overnight, the overall PCE of the device recovered slightly, mostly because the R_{CT} at the counter-electrode interface decreased. The non-imprinted electrode (NIP) displayed a higher PCE than the imprinted material (MIP) due to a lower R_{CT} .

— (1) PEDOT — (2) After MIP — (3) After NIP — (4) After removal — (5) After overnight removal

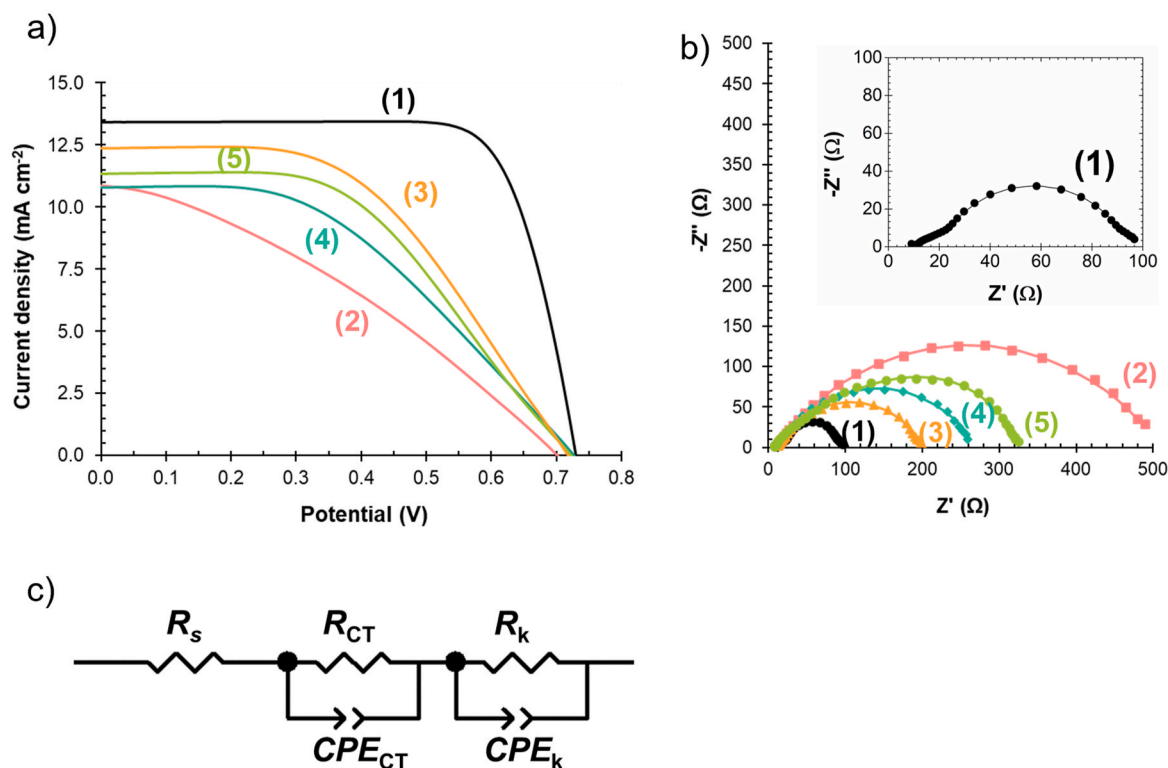


Fig. 3. J - V curve (a) and (b) EIS data, measured for the hybrid DSSC/biosensing device after EDOT electropolymerization (1), after MIP (2), after NIP (3), after template removal (4) and overnight removal (5) in the MIP electrode. (c) Equivalent electrical circuit used for fitting the spectra.

Table 1

Photovoltaic performance and electrochemical data obtained for the hybrid DSSC/biosensor at the various steps of biosensing film construction. Resistances ($\Omega\text{-cm}^2$) of the device were obtained by fitting the model (Fig. 3c) to the impedance spectra in Fig. 3b.

	η_{PCE} (%)	J_{SC} ($\text{mA}\cdot\text{cm}^{-2}$)	V_{OC} (mV)	η_{FF}	MPP ($\text{mW}\cdot\text{cm}^{-2}$)	R_{S} ($\Omega\cdot\text{cm}^2$)	R_{CT} ($\Omega\cdot\text{cm}^2$)	R_{k} ($\Omega\cdot\text{cm}^2$)
PEDOT	7.27	13.09	731	0.76	7.19	3.42	15.2	28.7
After MIP	2.72	10.30	705	0.37	2.69	4.44	167.0	12.7
After NIP	4.45	12.10	723	0.51	4.41	3.31	63.2	20.2
After Removal	3.54	10.79	728	0.44	3.50	4.15	109.9	15.4
After Overnight Removal	4.09	11.34	725	0.49	4.05	3.17	84.7	17.0

3.2. Rebinding assays of the hybrid DSSC/biosensor device

The analytical performance of the hybrid DSSC/biosensor was assessed by rebinding assays, as described in Section 2.5. The calibration of the device was performed using standard solutions that were first prepared in a phosphate buffer (Fig. S2 in Supplementary information) and then in human urine from healthy male volunteers, diluted $10 \times$ in phosphate buffer (Fig. 4).

The concentration of sarcosine spiked buffer solution ranged from 1 ng/mL to 10 $\mu\text{g/mL}$ and the electrochemical behavior was assessed based on the J - V curves and the EIS plots, using a commercial iodide

electrolyte in a DSSC configuration. From these two techniques, the $\Delta\eta_{\text{PCE}}$ and the ΔR_{CT} are determined, according to Eqs. (1) and (2), respectively.

$$\Delta\eta_{\text{PCE}} = \frac{\eta_{\text{PCE}_{\text{sarcosine_sample}}} - \eta_{\text{PCE}_{\text{blank}}}}{\eta_{\text{PCE}_{\text{blank}}}} \quad \text{Eq. 1}$$

$$\Delta R_{\text{CT}} = \frac{R_{\text{CT}_{\text{sarcosine_sample}}} - R_{\text{CT}_{\text{blank}}}}{R_{\text{CT}_{\text{blank}}}} \quad \text{Eq. 2}$$

When plotting the logarithm of $C_{\text{sarcosine}}$ against either $\Delta\eta_{\text{PCE}}$ and the Δ

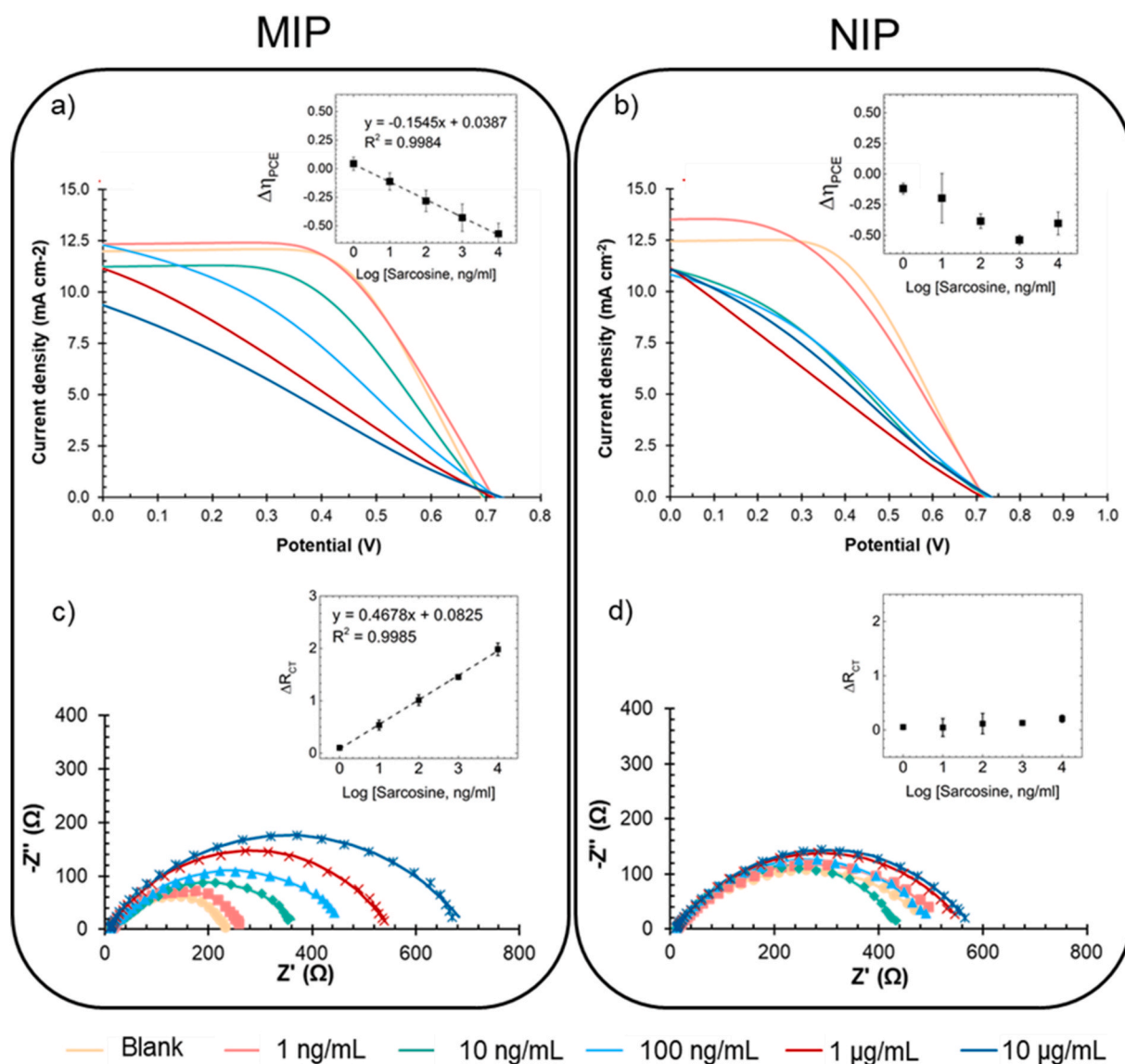


Fig. 4. J - V characterization (a), (b), and Nyquist plots (c), (d), for the rebinding assays in human urine diluted in phosphate buffer for the MIP (a), (c), and NIP electrodes (b), (d), performed in a DSSC configuration. Inset: typical regression lines plotting the $\Delta\eta_{\text{PCE}}$ / ΔR_{CT} against the logarithm of sarcosine concentration.

R_{CT} , the slope of the linear regression will determine the sensitivity of the device.

After successive incubations, the sarcosine present in the spiked solutions adsorbed on the imprinted cavities, making the electrochemically active surface area substantially smaller, and consequently the R_{CT} increased as shown in Fig. S2c and Fig. 4c. The increase in the overall resistance of the device has a very negative effect on the η_{FF} , affecting the shape of the J - V curves for both calibration mediums as it can be observed on Fig. S2a (buffer) and Fig. 4a (urine).

Overall, when the concentration of sarcosine increases in the spiked solutions (both in buffer or in urine) the PCE decreases, and the $\Delta\eta_{PCE}$ displays a linear response vs. the logarithm of $C_{sarcosine}$ (Fig. S2a and Fig. 4a). The anionic slope of the $\Delta\eta_{PCE}$ regression is very similar between buffer and urine calibration medium; the obtained sensitivities were respectively -0.159% (Fig. S2a) and -0.154% (Fig. 4a) per decade of $C_{sarcosine}$, with a determination coefficient of $R^2 = 0.99$. Comparably, the cationic slope of the ΔR_{CT} regression vs. the log of $C_{sarcosine}$ also shows similar values for the calibrations performed with buffer and with urine; the obtained sensitivities were respectively 0.494Ω (Fig. S2c) and 0.468Ω (Fig. 4c) per decade of $C_{sarcosine}$, with a determination coefficient of $R^2 = 0.99$. Moreover, the LOD of the hybrid DSSC/biosensor calibrated in urine was 0.33 ng/mL . Repeated calibrations ($n = 3$), with

standard deviations $<12\%$ for all concentrations, were obtained indicating a good-precision and stable device. The control electrode (NIP) showed random responses with the target template concentration, validating the performance of the electrode to determine sarcosine in complex samples (Fig. 4b,d).

In general, the device shows that efficiency and charge transfer resistance are concentration dependent. Moreover, the system responds to sarcosine concentration ranges that are clinically relevant. The MIP and NIP show different electrochemical behaviors that demonstrate the ability of the MIP electrode to interact with sarcosine, mostly through its imprinted sites, displaying a small non-specific interaction with the non-imprinted sites at the polymer surface.

3.3. Selectivity of the hybrid device

The selectivity of the hybrid DSSC/biosensor was assessed when chemical compounds that could potentially interfere with the sarcosine detection are present. Creatinine and urea were the selected interferents since they are among the most abundant compounds in urine. Considering the reference values for these two compounds [41] and the dilution of urine ($10 \times$), the concentrations used for creatinine and urea were 0.1 mg/mL and 1 mg/mL , respectively. The sarcosine spiked

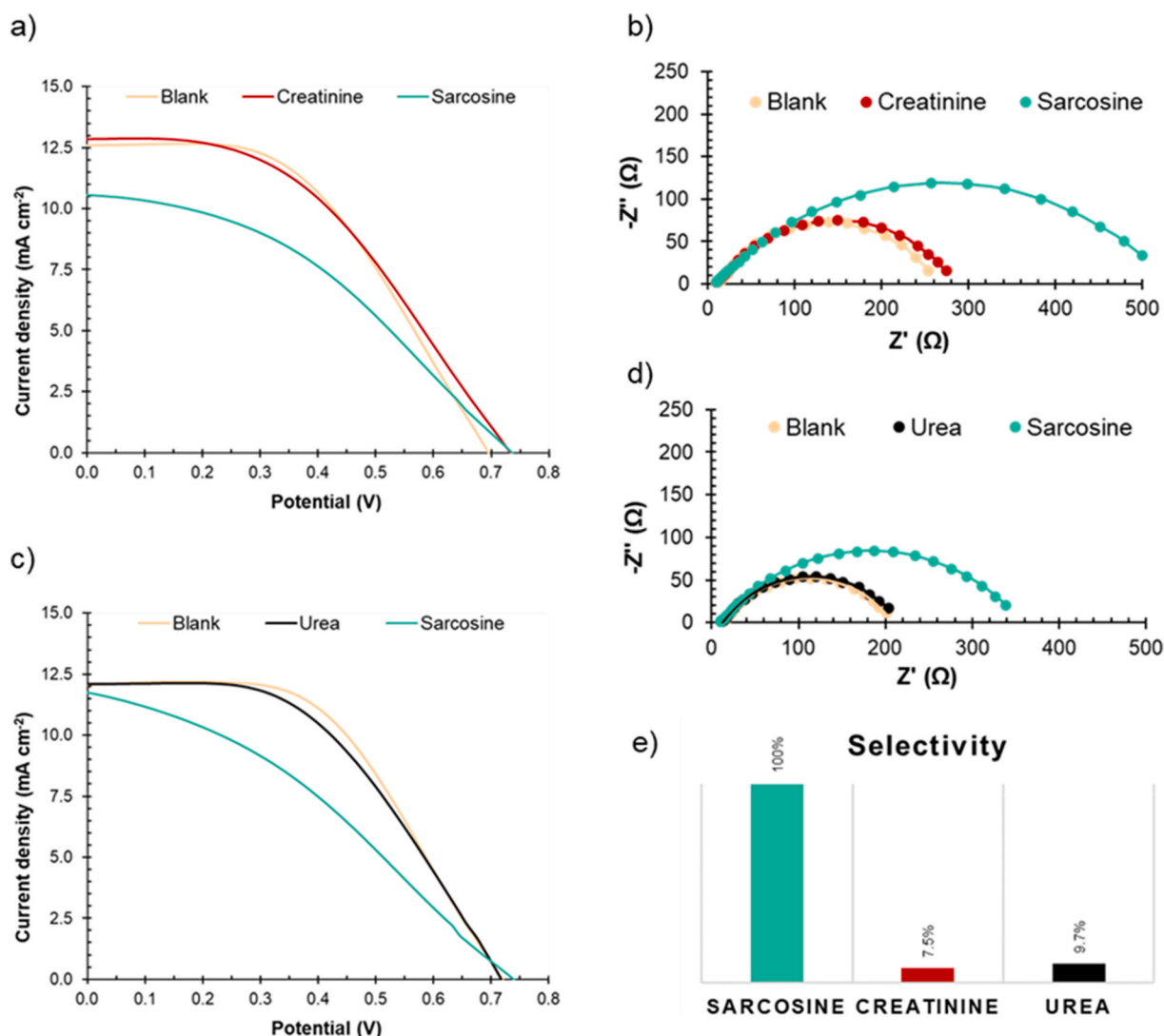


Fig. 5. J - V characterization (a) and (c) and Nyquist plots (b) and (d) regarding the selectivity of the hybrid DSSC/biosensor device when incubated with a single interfering compound, Creatinine (a) and (b), and Urea (c) and (d). The concentrations used upon the incubation of the biosensing film were 0.1 mg/mL , 1 mg/mL and 100 ng/mL for creatinine, urea, and sarcosine respectively. Selectivity study with single solution of sarcosine and interfering species (creatinine and urea) (e).

solution used in the selectivity test had a concentration of 100 ng/mL.

The selectivity was obtained using a single component solution, with the interfering species. Each compound was incubated separately, for 30 min, and afterwards the *J-V* curves and EIS data were collected. The results are displayed on Fig. 5, and they indicate no significant interference with sarcosine detection. The single component approach used to evaluate the selectivity of the device demonstrated low rebinding properties for creatinine (7.5 ± 2.8) % and for urea (9.7 ± 1.7) %. In general, the device showed great selectivity of the biosensing film, confirming that the biosensor has a high affinity for the target analyte.

3.4. Addition of self-signaling device

The hybrid DSSC/biosensor device showed a clear dependence of performance vs. concentration, but to operate this device in an equipment-free environment it is necessary to establish a self-signaling approach. Since electrochromic cells change color intensity depending on the power input and the power produced by the DSSC/biosensor device decreases with the sarcosine concentration, combining the two devices allows to visually assess the sarcosine concentration in a biological sample using a fully autonomous device, cf. Fig. 2. The developed electrochromic cell varied its color from blue to purple for sarcosine concentrations of 1 ng/mL to 10 μ g/mL; the preparation of the electrochromic material is described in the supplementary information.

The rebinding assays of the new self-signaling device set-up were made for sarcosine concentrations between 1 ng/mL to 10 μ g/mL. The standard solutions used to calibrate the device were prepared using human urine diluted in a phosphate buffer, incubated for 30 min. The time established for the electrochromic cell to reach a stable color was set to 2 min.

The colors obtained for each standard in urine are displayed on Fig. 6a. The blank assay corresponds to the incubation of urine solution without the target analyte, which achieved the highest power output by the hybrid device; when connected to the electrochromic cell, the resulting color was a light blue. When the sarcosine concentration in the standard solutions increases, the power generated by the hybrid DSSC/biosensor slowly decreases and the electrochromic cell goes from light blue to darker blue, and for the final standard, at 10 μ g/mL, it turns purple. Thus, there is a noticeable color gradient which allows visual detection of pathogenic concentration of sarcosine, >1 ng/mL. The developed sensor displays a working concentration range similar to other published works [21,23,24]. However, the present work reports a fully autonomous device with self-signaling ability.

Quantitative data was extracted from the color calibration, following the approach reported first by Silva et al. [42]. The color coordinates of the HSL color system (Hue, Saturation and Luminance) were determined

using the image J software for each digital picture, taken in three different spots. When plotting the Saturation against the logarithm of the sarcosine concentration, a linear trend was achieved: $y = 9.4 \log(C_{\text{sarcosine}}) + 92.8$, with a corresponding determination coefficient of ca. $R^2 = 0.99$ (Fig. 6b).

4. Conclusions

A self-signaling and electrically autonomous biosensor was successfully assembled on the counter-electrode of a DSSC, interfaced with an electrochromic cell. The biosensor was first assessed in a three-electrode configuration, targeting the quantification of sarcosine in human urine samples from healthy male volunteers. The biosensing film was produced *in situ*, molecularly imprinting it on a polymerized EDOT and Py film. The biosensing film was evaluated assembled in the counter-electrode of a DSSC. It was found that the $\Delta\eta_{\text{PCE}}$ and the ΔR_{CT} of the DSSC were linearly dependent with the logarithm of sarcosine concentration, with a sensitivity in urine of -0.154% /decade of $C_{\text{sarcosine}}$ and 0.468Ω /decade of $C_{\text{sarcosine}}$, respectively. The device presented a linear range between 1 ng/mL to 10 μ g/mL and a LOD of 0.32 ng/mL. The biosensor was selective towards sarcosine, as it is apparent from the selectivity study performed with two of the most abundant compounds in urine: creatinine and urea.

Finally, an electrochromic cell was interfaced with the hybrid DSSC/biosensor and there was a clear color gradient permitting visual detection of pathogenic concentration of sarcosine present in an actual biological sample. The saturation vs. the logarithm of sarcosine concentration displayed a linear trend in the same concentration range tested herein and a $R^2 = 0.99$. Overall, the device reported in this article is equipment-free, fit for point-of-care analysis and able to screen sarcosine in real samples. This device only requires a light source to power the DSSC, to provide clinically relevant information anywhere. Furthermore, this simple integration of a biorecognition element with the DSSC can be applied to almost any cancer biomarker.

Credit author statement

Carolina S. Hora: Methodology, Conceptualization, Writing – original draft, Formal analysis, Data curation. **Ana P.M. Tavares:** Conceptualization, Investigation, Supervision, Writing – review & editing. **Liliana P.T. Carneiro:** Investigation, Formal analysis. **M. Goreti F. Sales:** Funding acquisition, Supervision, Investigation, Writing – review & editing. **Dzmitry Ivanou:** Investigation, Supervision, Data curation, Writing – original draft. **Adélio Mendes:** Funding acquisition, Supervision, Investigation, Writing – original draft.

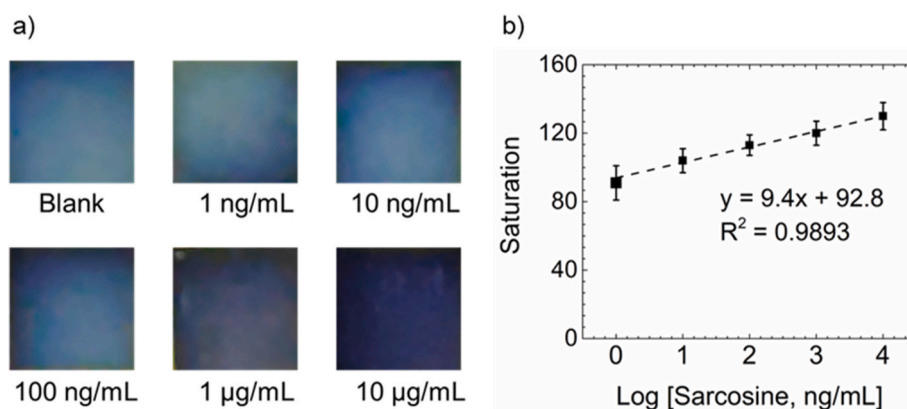


Fig. 6. Color behavior of the self-signaling device (a), composed of the electrochromic cell interfaced in the external circuit of the hybrid DSSC/biosensor device, and the respective calibration (b), determined by the saturation coordinate of the HSL color system vs. $\log(C_{\text{sarcosine}})$. (To check the color changes in this figure, the reader is referred to the Web version of this article.)

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

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.talanta.2023.124340>.

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