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Unveiling the effect of paper matrix on the electrochemical response of diffusive redox probes

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ABSTRACT

This study evaluates the influence of paper substrate selection on the electrochemical response of an electrochemical paper-based analytical device (ePAD). Various paper substrates commonly employed in this type of devices such as cellulose, nitrocellulose and glass-fibre based medical grade materials are evaluated using chronoamperometric measurements. Both theoretical modelling and experimental analyses are conducted to understand the diffusion behaviour of widely employed two redox probes, $[Fe(CN)_6]^{3-}$ and FcMeOH. Findings show that glass fibre substrates show similar performance to liquid drop conditions while nitrocellulose cause a decrease in current after a short measurement period, mainly due to a thin-layer effect. Cellulose-based substrates decrease the diffusivity of redox species, especially for charged species, indicating potential limitations in their use for chronoamperometric measurements. The study offers valuable insights into the electrochemical behaviour of paper substrates in ePADs, laying the groundwork for future research in this area.

1. Introduction

Paper-based analytical devices (PADs) have been employed for various biomedical and environmental applications for decades. In the early years, they were employed for purposes such as metal ion detection, separation of amino acids or detecting glucose in urine [1]. A significant breakthrough occurred in the 1970s with the introduction of lateral flow assays, which transformed Enzyme-Linked ImmunoSorbent Assays (ELISA) into streamlined, one or two-step paper strip formats [2]. Tests utilizing this technology are now available for a wide range of analytes (virus, drugs, proteins...) and have proven to play a crucial role during the COVID-19 pandemic for point-of-care detection of the SARS-CoV-2 at large scales. In 2007, Whitesides and co-workers introduced the idea of fabricating microfluidic channels on paper and stablish multi-directional flows for multiplex analyte colorimetric detection [3]. Since then, many new areas of fabrication and exploration have opened up. PADs that rely on capillarity to perform chromatographic separation of mixtures, sequential mixing of reactants or filtering - to name some examples - proved to be affordable approaches for fluid handling.

The majority of PADs rely on colorimetry to produce an analytical response by inducing color changes through chemical or enzymatic reactions [4]. Nonetheless, these tests possess certain limitations. Visual interpretation can be influenced by user acuity and environmental conditions, and the response tends to be qualitative or semi-quantitative, thus limiting precise range detection. To overcome these challenges, the diagnostics field has directed resources toward signal digitization [5]. Various strategies for signal quantification in PADs, including techniques like SERS, photothermal, or chemiluminescent methods have been developed [6-8]. However, these strategies often require sophisticated equipment with associated costs that hinder the massive deployment as point-of-care solutions. In 2009, Professor Henry's team reported the advantages of combining paper and electrochemical techniques. The resulting devices - labelled as ePADS - gave rise to a promising pathway towards the digitalization of PADs [9]. The main advantage of using electrochemical methods lays in the fact the voltage/current signal readout can be implemented using cost-effective electronics [10-12]. ePADs are composed of a paper-based substrate and a miniaturized compact setup of two or three electrodes. The

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classification of ePADs by architecture can be divided in two categories: (i) electrodes printed directly onto paper [13,14] and (ii) electrodes fabricated separately, such as Screen-Printed Electrodes (SPE) and subsequently attached to a paper substrate to form a complete device [15,16]. Case (i) achieves compact electrode miniaturization, yet encounters challenges such as the optimization of ink embedding during the screen-printing process [17]. Case (ii) circumvents fabrication variability and capitalizes on the extensively studied physicochemical properties of both paper and SPEs separately.

Concerning paper-based substrates, extensive studies have examined properties affecting their characteristics and functionality within PADs; modification of paper-based substrates with diverse functional groups to enhance PAD responses, enabling of biomolecule anchoring or capillarybased fluidics are extensively explored aspects [4]. Besides, SPE fabrication procedures as well as their electrochemical responses on micro and macro scales have been widely reported [18]. As an example, the effects of working electrode thickness and roughness on electrochemical response [19]. required chemical and electrochemical pretreatments for surface activation [20], and the characterization of various conductive inks from different commercial brands have been studied in depth [21].

Surprisingly, although ePADs have been extensively reported in the last decade, no systematic study has examined how paper type choice influences electrochemical measurements. Research primarily focuses on device architecture, assay type, or quantification technique, often overlooking the combined SPE-paper interaction [17,22].

This work aims to assess the influence on the electrochemical performance of different porous membranes used to hold the liquid analyte over a screen-printed electrode. Four of the most typically used materials in academic research and diagnostics industry have been considered for this study. Experimental characterization and finite element simulations have been combined to understand the signal variations between paper substrates and to derive the concentration gradient profiles over time in each substrate. The results from this work provide insightful guidance for selecting appropriate materials during design and optimization of electrochemical paper-based analytical devices.

2. Experimental

2.1. Reagents and electrodes

Ferrocenemethanol (FcMeOH, General Reagent, 97 %), potassium hexacyanoferrate (III) (BioUltra, >99.0 %), from now $[Fe(CN)_6]^{3-}$, and NaCl (BioXtra, >99.0 %) were purchased from Sigma-Aldrich. The final concentrations were 1 mM of FcMeOH or $[Fe(CN)_6]^{3-}$ in 0.1 M NaCl. The screen-printed carbon electrodes (SPE, Model 110, DropSens, Spain) that were used consisted of a 4 mm diameter carbon working electrode (WE), Ag/AgCl paste as pseudo-reference electrode (RE) and carbon paste as counter electrode (CE). The papers used were cellulose 238 (CF, Cotton linter fiber C238, Ahlstrom Munksjö, Helsinki, Finland), glass fiber (GF, Whatman Standard 14, Cytiva, Marlborough USA), nitrocellulose (NC, FF80HP, Cytiva Marlborough, USA) and chromatographic paper Whatman #1 (W1, Cytiva, Marlborough USA).

2.2. Finite element analysis

The Electroanalysis physics interface of COMSOL Multiphysics software was used to numerically solve the partial differential equations (PDEs) of the mass transport equation. Simulations were performed with an Intel® i5, Xeon® E5–1620 processors (SI.70 GHz) using the software package COMSOL Multiphysics® version 5.6. The COMSOL optimization module was employed to obtain values through iterations. Further information regarding Optimization Module can be found in the reference [23].

2.3. Electrochemical measurements

Chronoamperometric (CA) measurements were carried out using an Autolab PGstat 302 N potentiostat/galvanostat equipped with Nova 2.1 software. The CA measurements were recorded for 60 s in FcMeOH and $[Fe(CN)_6]^{3-}$ at 0.3 V and -0.2 V, respectively. The paper-based membranes were cut in circles with a radius of 5.0 mm using a CO₂ laser (Mini 24, Epilog Laser, Golden, Co, USA). The cutting parameters were set as follows: power 8, velocity 30 for GF; power 7, velocity 30 for W1; and power 20, velocity 50 for CF. Since the NC cannot be cut by the CO₂ laser due to its flammability, the cuts were made manually using a 5 mm diameter die cut.

For the experiments where paper was used, the procedure consisted on placing the piece of paper on top of the electrodes and then, with the help of a micropipette, adding the amount of solution required to saturate the paper according to the water absorption capacity provided by the manufacturer. In the case of measurements without paper, an 80 μ L drop of solution was added directly on top of the three electrodes. All measurements were conducted (repeated) 3 times (n = 3), with error bars in the plots representing standard deviation. Prior to use, each SPE was electrochemically activated by alternating 1.5 V and -1.5 V vs screen-printed Ag pseudo-reference electrode for 30 s at each potential. Next, the SPE was rinsed with distilled water and dried under nitrogen flow.

The electrochemical impedance spectroscopy (EIS) measurements were conducted in a 1 mM solution of both the ferro/ferricyanide redox pairs and 0.1 M NaCl. Measurements were taken at the open circuit potential (OCP), with a perturbation of 5 mV to ensure pseudo-linearity, at frequencies ranging from 10^{5} Hz to 0.1 Hz.

2.4. SEM

Morphological inspection of paper samples was performed using an Auriga 40 Field Emission Scanning Electron Microscopy from Carl Zeiss (Germany).

3. Theoretical background used in our finite element method model

Models used to determine the concentration gradients generated in a liquid by the consumption of a redox specie in a macroelectrode system, usually consider that concentration changes take place only in the direction perpendicular to the electrode surface. However, for disk electrodes with relatively small diameter (d = 4 mm), such as the SPEs used in this work, radial diffusion can have non-negligible effects at long times [24]. This results in a two-dimensional simulation space, r and z, parallel and perpendicular to the electrode respectively. In the theoretical model presented in this work, the WE of the SPE with a radius of 2 mm (r_e) is assumed to be flat and inlaid into the insulator planar surface. All simulations performed in this paper assumed the following electrochemical reaction:

$$A \rightleftharpoons B + e^{-} \tag{1}$$

in which "A" is the reduced form of an electrochemical species, which at the beginning of the experiment (t = 0) is the only one present in the solution in a concentration (C_A) of 1.0 mM:

$$t = 0, \ r \ge 0, \ z \ge 0: \ C_A = C_A^* = 1.0$$
 (2)

In CA, the potential applied to the electrode is stepped from a value where no electron transfer occurs to a potential in which the electrochemical reaction rate is governed by the mass transport of the reactant from the bulk to the surface of the electrode. Diffusion is the only effective mass transport mode for the experiments under study here. Thus, migration is neglected because experiments were conducted using a high enough concentration of supporting electrolyte (0.1 M of NaCl). Moreover, natural convection influence on the electrochemical response of porous media is negligible in the time scale of the experiment. Under these conditions, the temporal and spatial variation of the concentration of species A is governed by Fick's second law:

$$\frac{\partial C_A}{\partial t} = D_A \left(\frac{\partial^2 C_A}{\partial z^2} + \frac{1}{r} \frac{\partial C_A}{\partial r} + \frac{\partial^2 C_A}{\partial r^2} \right)$$
(3)

where D_A is the diffusion coefficient of the electroactive species. However, through a porous medium, such as paper matrix, the effective coefficient diffusion ($D_{A,eff}$) becomes different due to the distance that a molecule must travel between two points. $D_{A,eff}$ can be expressed as [25]:

$$D_{A,eff} = \frac{\varepsilon}{\tau^2} D_A \tag{4}$$

where ε and τ are porosity and tortuosity respectively. Not only these values are difficult to derive experimentally in a real system, but contradictory approaches in their determination are often found in the literature [26]. Therefore, we will use $D_{A,eff}$, instead of D_A , as the parameter that accounts for the combined effect of porosity and tortuosity on the diffusivity of the redox specie in the paper matrix.

As described in the literature, semi-infinite diffusion conditions will be considered to hold in the *r*-direction so that the far end of the simulation space away from the electrode is notably larger than the thickness of the diffusion layer [27]; therefore, the corresponding maximum distance can be set as:

$$r_{max} = r_e + 6\sqrt{D_A t_{max}}$$
 : $C_A = C_A^* = 1.0$ (5)

As common values of D_A are between 10^{-9} and 10^{-10} m² s⁻¹, r_{max} is between 2.5 and 3.5 mm when t_{max} is 60 s, smaller than the width of the paper size used in the experiments. In the *z*-direction, the limit of the diffusion domain is determined either by the height of the paper matrix, h_{paper} :

$$z_{max} = h_{paper} : \left(\frac{\partial C_A}{\partial z}\right) = 0 \tag{6}$$

Or, in the semi-infinite systems, the limit in z axis is:

$$z_{max} = 6\sqrt{D_A t_{max}}$$
 : $C_A = C_A^* = 1.0$ (7)

When the electrode potential is stepped (t > 0) to a high enough potential, the boundary condition at the surface of the electrode becomes:

$$t > 0, \ 0 \le r \le r_e, \ z = 0 \ : \ C_A = 0$$
 (8)

According to all described parameters, the Fick's first law and Faraday's law, the current response, *I*, is:

$$i = F j_{A,0} \tag{9}$$

where $j_{A,0}$ is the flux of electroactive reactant at the surface of the electrode:

$$j_{A,0} = -2\pi D_A \int_0^{r_e} \left(\frac{\partial C_A}{\partial z}\right)_{z=0} r dr$$
(10)

The Electroanalysis physics interface of COMSOL Multiphysics® software was employed to solve numerically the above boundary value problem of partial differential equations (PDEs). The model was drawn using a 2D axisymmetric model. This allows avoiding 3D geometry calculation and accurately describe the diffusion field.

4. Results and discussion

4.1. Structure analysis

Scanning Electron Microscopy (SEM) was used to study the morphological structure of the selected paper-based materials. Whatman paper grade 1 (W1, Fig. 1a) has been extensively used as chromatographic paper and it was the material used in the seminal work on paper microfluidics by Professor Whitesides' group [3]. Nitrocellulose (NC, Fig. 1b) has been selected because it is used as a substrate for antibody immobilization in lateral flow tests. Cotton fiber (CF, Fig. 1c) is used as an absorbent in lateral flow tests to drain the liquid facilitating a continuous fluid flow. Finally, Standard 14 glass fiber (GF, Fig. 1d) is used to define the sample and/or conjugation pad in lateral flow tests, storing antibodies labeled with gold or latex nanoparticles. It has been selected because it presents low chemical interaction with analytes and biomolecules and has a large porosity, which allows to hold larger liquid volumes per paper surface.

As depicted in Fig. 1, these materials exhibit varied structural characteristics. W1 and CF have a similar structure that reveal an irregular fiber mesh characterized by large variation in pore sizes. In contrast, NC features a uniform sponge-like porous arrangement with pores of a few microns, whereas GF consists of a network of fine and tubular fibers that confer the material with a high porosity.

The significant variation in both pore structure and size across the SEM images of these materials anticipates that diffusion of electroactive species through these substrates ($D_{A,eff}$) may present significant differences.

4.2. Chronoamperometry in the presence vs the absence of a porous paper matrix

To compare the (effective) diffusion coefficient of an electroactive species both in the presence and in the absence of a porous material, a chronoamperometry (CA) experiment was conducted at a potential where the response was diffusion-controlled. Therefore, the CA responses resulting from the reduction of 1.0 mM $[Fe(CN)_6]^{3-}$ were compared both in the absence and presence of a paper substrate. In the first case, an 80 µL drop of $[Fe(CN)_6]^{3-}$ solution was deposited onto the SPE, ensuring coverage of all three electrodes. In the second case, a circular piece of W1 with a radius of 5 mm, large enough to cover the electrodes, was positioned on top of the SPE and impregnated with the $[Fe(CN)_6]^{3-}$ solution. The decision to use W1 is based on it being the most widely used paper in both PADs and ePADs since Whitesides' group published its seminal work on paper-based microfluidics [3].

The obtained results are depicted in Fig. 2a, clearly illustrating discernible differences in the recorded currents. Notably, the measurement conducted in the presence of the paper substrate yields a lower current value compared to that recorded without the presence of the paper substrate. The decrease in current could be originated from the decrease of the effective surface area of the electrode surface into contact with the solution due to the presence of the paper may have change due to the tortuosity, also contributing to the observed current decrease. It is possible that a combination of both effects caused such a decrease. To unravel the impact of each of these plausible contributions, the CA was represented in a double logarithmic form.

In the log-log representation, a slope of 0.5 corresponds to the existence of a Cottrelian regime, in which diffusion of species to the electrode takes place in a semi-infinite homogeneous domain. Fig. 2b illustrates the obtained currents on a double logarithmic scale. The CA response obtained with a liquid drop confirms Cottrellian dependence with a single slope of approximately 0.5. In contrast, the presence of paper substrate W1 reveals two response regions with different slopes. In the first seconds, the obtained intensity values align with those recorded in the drop, thus eliminating the hypothesis of current decrease due to a



Fig. 1. SEM images of the most commonly used papers in ePADs (a) W1, (b) NC, (c) CF, and (d) GF.



Fig. 2. Chronoamperometric response of 1.0 mM [Fe(CN)₆]³⁻ at -0.20 V vs Ag/AgCl in 0.1 M KCl on an SPE measured in a drop (black) or on W1 paper impregnated with the solution (red). a) linear scale representation; b) log-log scale representation. Error bars correspond to the standard deviation of three repeats.

partial blocking of the active area of the electrodes by the paper. However, after this initial period, the logarithmic response shifts its slope and transitions towards lower currents, indicating a diminution of the effective diffusivity of $[Fe(CN)_6]^{3-}$ due to the presence of the paper matrix.

It is worth highlighting that the presence of two different slopes suggests a transition from a non-tortuous zone (diffusivity equivalent to a free liquid medium) to a zone with tortuosity. Fig. 3a schematically illustrates this situation, in which the liquid is divided in two regions: (1) a thin layer between the electrode surface and the first layer of paper fibers, and (2) the paper matrix itself. Additionally, it is important to highlight that the paper material has a lower thickness (approximately 180 μ m) in comparison to the height of a drop of aqueous solution covering a surface of the same diameter (in the range of 1.0–1.5 mm). The low thickness of the paper may derive in thin-layer type electrochemical responses at long times, in which the diffusion layer of the redox species reaches the thickness of the diffusion domain and the supply of species to the electrode surface decreases, causing a sudden drop in the amperometric response.



Fig. 3. Graphical representation of diffusive regimes within a porous paper. a) Graphic representation; b) Theoretical model.

4.3. Finite-element model of chronoamperometric responses

To interpret and analyze the potential factors influencing electrochemical responses observed in the presence of a paper-based matrix on top of a SPE, a finite-element simulation model has been developed using COMSOL Multiphysics® program. Fig. 3b shows the geometric

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model and applied boundary conditions. The experimental SPE possesses a circular geometry, enabling the creation of a 2D axisymmetric representation model. The equations and boundary conditions are defined in Section 3. The model has been parameterized to enable simulations of different scenario of interest. To achieve this, two distinct zones have been defined, where different diffusion coefficients can be assigned. The first zone is in direct contact with the electrode and has a thickness of a few microns, while the second zone encompasses the remaining physical space of the model. By assigning identical diffusion coefficients to both zones (*i.e.*, $D_{A.eff} = D_A$), a homogeneous system can be simulated. Alternatively, the designation of different diffusion coefficients (*i.e.*, $D_{A.eff} \neq D_A$) allows for the simulation of regions with different diffusivity. To simulate the thin-layer effect, the total height of the model (*h*) can be adjusted until an abrupt drop in intensity is observed.

Table 1 provides a summary of the graphical representation as well as the values used in the different simulated scenarios. Case I simulates a non-porous (free) medium, with a D_A value of $6 \cdot 10^{-10} \text{ m}^2 \text{ s}^{-1}$, which is a typical value for an ion in a saline aqueous solution [28]. Case II represents a porous paper matrix with a $D_{A,\text{eff}}$ value of $1 \cdot 10^{-10} \text{ m}^2 \text{ s}^{-1}$ – six times lower than D_A – and an infinite thickness. Case III illustrates the thin-layer behavior in which a value for h of 150 µm has been set. Finally, Case IV simulates two different diffusive media; a narrow zone *d* of 40 µm and diffusivity D_A of $6 \cdot 10^{-10} \text{ m}^2 \text{ s}^{-1}$ has been set in contact with the electrode whereas $D_{A,\text{eff}}$ of $1 \cdot 10^{-10} \text{ m}^2 \text{ s}^{-1}$ has been defined for the rest of the model, which has an overall thickness h of 300 µm.

The results obtained from the different simulations have been depicted in Figs. 4a and 4b using both linear and double logarithmic scales. Under semi-infinite diffusion (black lines), the log-log representation (Fig. 4b) is linear in the whole-time range with a slope 0.5. Comparing the results obtained for two different values of D_A , $6 \cdot 10^{-10}$ m² s⁻¹ (case I) and $1 \cdot 10^{-10}$ m² s⁻¹ (case II), a significant decrease is observed in the current values when diffusion coefficient of the species

Table 1

Values used in the simulations at different scenarios.





Fig. 4. Results of CA simulations under different diffusive regimes. The geometric model used in the simulations is shown in Fig. 3b, and the values of the different parameters are provided in the legend of the figure. a) Results in linear scale; b) Results in logarithmic scale.

diminishes (Fig. 4a). This also causes a shift of the log-log plot along the y-axis.

A comparison between the CA response of thin-layer system (case III) and that of the semi-infinite system with the same diffusion coefficient (case I) shows that for the first 10 s the amperometric response of both systems is identical. This is because the diffusion layer is still close to the electrode. After approximately 10 s, the diffusion layer reaches the limit of the diffusion domain, resulting in a significant decrease of the current. This effect is more apparent in the log-log representation (Fig. 4b). It should be noted that even at long times, the current does not completely extinguish as one would expect when total species depletion occurs in the vertical axis. However, an axial species contribution is present in small radial systems [24].

Finally, in the case in which porosity varies in z and r, a simulation was conducted with two diffusive regimes (case IV). In the linear scale, the generated currents were similar, but the log-log scale reveals a clear drop of the current values between the first second of measurement and the tenth. After that, the log-log curve recovers the linear behavior with a 0.5 slope of a Cottrellian system, but at lower intensity values. During the transition period, the diffusion profile crosses the region between the two different diffusive zones, and the system is partially limited in current because the contribution of species from the two layers is different due to the differences in diffusivity in the two media.

4.4. Experimental study of different porous materials

After modeling the different diffusion phenomena and their effects on the generated current, CA measurements were conducted using 1 mM of charged and electrically neutral redox species widely used in electrochemical characterizations due to their good intrinsic activity - [Fe (CN)₆]³⁻ and FcMeOH - in an SPE [29]. Each paper was impregnated with an appropriate amount of solution based on its water absorption capacity and manufacturer specifications. Figs. 5a to 5d show the currents obtained by CA of FcMeOH and $[Fe(CN)_6]^{3-}$ in linear and double logarithmic scales.

First, it can be seen that paper substrate selection has an impact on the current delivered by the system. Cellulose-based materials - W1 and CF - generate the lowest recorded currents. In contrast, the glass fiberbased material – GF - shows the best amperometric response for electroanalytical purposes. In both cases, NC seems to transition from similar currents than the ones obtained with GF paper during the first seconds of measurements to then display a significant drop towards lower intensity values.

The logarithmic representation of the data offers some interesting insights. GF substrates display a purely Cottrellian response throughout the measurement range. In the case of NC, the response is also Cottrellian and very similar to that obtained with GF during the first 10 s. Afterwards, the current decays abruptly. This phenomenon seems attributable to the previously described thin-layer effect, which would occur due to the small thickness of this material compared to the rest (see Table 1). In this case, it is observed that, although the material has a much smaller pore size than GF, the arrangement of fibers does not modify the diffusion of the tested redox species. In contrast, in the cellulose substrates (CF and W1), there is a decrease in the current after the initial second. After a transition period, the log-log slope recovers the Cottrellian behavior, which as previously explained, corresponds to a transition to a material where the studied species diffuses with more difficulty (more tortuous material). Differences in the current values obtained for [Fe(CN)₆]³⁻ in cellulosic CF and W1 substrates were observed, indicating that charged species encounter more difficulty in diffusing through CF. This is likely due to the negatively charged surface of CF, which generates a certain repulsion with the negatively charged $[Fe(CN)_6]^{3-}$ when it passes through its fibers.

Despite the potential influence of the paper positioning on the electrode, we positioned them randomly without observing significant differences between experiments, as supported by the low error bars. Thus, we conclude that for the papers tested in this study, position does not have a substantial impact on the results.

EIS is a powerful technique that can provide extensive information about the physicochemical processes occurring in an electrochemical system. In a semi-infinite and Cottrellian system, the equivalent circuit is the typical Randles circuit (Fig. 6a). Here, R_s represents the solution resistance, R_{ct} represents the charge transfer resistance, and W is the Warburg element, representing the diffusion of the electroactive species. Double-layer capacitance (C_{dl}) is generally non-ideal in electrochemical systems, so it is often represented as a Constant Phase Element (CPE) [30], which not only represents the capacitance but also the non-ideality of the system (see SI for more information).

As expected, the impedance in the absence of paper shows a typical Randles spectrum with the semicircle and the W component with a 45° slope (Fig. 6c). In the presence of GF, we obtained a similar response; the spectrum obtained in this case also follows the Randles behaviour. However, it displays a significantly higher capacitive value (see SI). This difference indicates that the presence of the paper plays an important



Fig. 5. Experimental and simulated CA values obtained for 1.0 mM FcMeOH and 1.0 mM ferricyanide on the different papers: W1, NC, CF, and GF. a) FcMeOH CAs in linear scale; b) FcMeOH CAs in logarithmic scale; c) Ferricyanide CAs in linear scale; d) Ferricyanide CAs in logarithmic scale.

role in the charge formed at the electrode-electrolyte interface, although it does not affect its non-ideality, since in both cases the value of α is close to 0.9. The origin of this effect can be further explored through computational methods, and it is beyond the scope of this work.

In systems with finite domain - such as thin-layer systems - as the frequency decreases the species are consumed, and the diffusion layer reaches the boundaries of the system. In this case, the W component of the circuit does not match to the experimental data and the spectrum evolves towards a capacitive behaviour. In a Nyquist plot, this translates into an evolution of the 45° slope to higher values. This is known as the open Warburg element (Wo) or Finite Space Warburg (FSW) [31,32]. The rest of the components of the equivalent circuit, R_s , CPE_{dl} and R_{ct} , should remain unaltered (Fig. 6b).

The EIS spectrum of the NC reveals that the Warburg element behaves as a finite-length Warburg (FSW) at low frequencies, which is expected for a thin-layer system. For W1, the Nyquist spectrum suggests that it also behaves as an FSW, similar to the NC. This is because, despite not being a thin-layer system, there is a transition from one diffusion coefficient to a smaller one a few micrometers from the electrode, leading to FSW-like behaviour.

In the case of CF, the curve is more complex to interpret, with clearer indications appearing only at the final points of the spectrum. However, considering that CF is a similar system, it is reasonable to expect FSW behaviour as well. Both circuits, Randles and Randles with FSW, were fitted and yielding very similar errors. After studying W1 and considering the physical meaning, the modified circuit was selected.

The presence of low-porosity materials, NC, CF, and W1, also shows changes in R_s towards higher values compared to the absence of paper substrate. This is attributed to the increased movement of charged species in the solution due to the tortuosity of the porous material. Again, in these cases, changes in the CPE_{dl} are also observed, once again evidencing that papers can influence the charge formed at the electrode-electrolyte interface. Finally, and importantly for applications such as impedimetric sensors, the papers have little effect on R_{ct} , meaning they do not affect the charge transfer reaction kinetics between the electroactive species and the electrode surface.

To extract the diffusivity values of the species within the different paper substrates, fitting of experimental data was performed using



Fig. 6. a) Equivalent Randles circuit, used in Liquid drop and GF. b) Equivalent Randles circuit with the finite space Warburg element (FSW) used in CF, W1, and NC. c) Nyquist plot of the experimental results in the absence and presence of GF, CF, W1, and NC.

Table 2
Values of D _A , D _{A,eff} , d, and paper thickness obtained after fitting the results in the
simulation model.

		$D_{\rm A}({ m m}^2{ m s}^{-1})$ x10 ¹⁰	$D_{\rm A,eff}({ m m}^2 s^{-1}) \ { m x10}^{10}$	d thickness (μm)	Paper thickness (µm)
FcMeOH	GF	5.5	D_0	-	355
	CF		1.2	50	340
	W1		1.6	50	> 210
	NC		5.5	-	>150
K ₃ [Fe	GF	7.0	D_0	-	355
(CN) ₆]	CF		1.2	60	340
	W1		2.2	85	>210
	NC		7.0	-	>150

iterations of the axisymmetric model presented in Fig. 3b with COMSOL Multiphysics program. The iterative fitting methodology is described in the bibliography [23]. The model allows to extract the diffusion coefficient values of the species under study in different regions, as well as the thicknesses. The simulated CA response corresponding to the best-fit parameters (Table 2) are plotted as solid lines in Figs. 5a and 5b. It can be observed that the simulated and experimental data are in satisfactory agreement in all cases, pointing out that the model hypotheses can explain the observed behavior. The diffusion coefficient found for FcMeOH in GF and NC was approximately $5.5 \cdot 10^{-10}$ m² s⁻¹, a value similar to that reported for this compound in an aqueous medium [33,

34]. Likewise, the diffusion coefficient obtained for $[Fe(CN)_6]^{3-}$ in these materials also matches the value reported for aqueous medium (7.0 $10^{-10} \text{ m}^2 \text{ s}^{-1}$) [35]. This suggests that the use of these materials in an ePAD does not significantly alter the diffusivity of redox species and therefore its CA response.

The fittings for the cellulose substrates W1 and CF were performed using the D_A obtained from GF. The results indicate that the diffusion coefficient in the layer between the electrode and the porous matrix (labeled as *d* in Fig. 3b) is similar to those in an aqueous medium. The thickness of this layer is around 50 µm for FcMeOH, while the values of $D_{A,eff}$, *i.e.*, the diffusion coefficient within the cellulose matrix, is $1.2 \cdot 10^{-10}$ m² s⁻¹ for cellulose W1 and $1.6 \cdot 10^{-10}$ m² s⁻¹ for CF. Values obtained with [Fe(CN)₆]^{3–} are slightly higher, with significant differences between W1 and CF. Simulations performed with the values of paper thicknesses reported by the manufacturer (not shown) predicted thin layer effects within the measured CA timeframe, which was not observed experimentally, probably due to the expansion of the material after fluid absorption. Simulations yielded thicknesses of at least 150 µm for NC paper and 210 µm for W1.

To depict visually the different phenomena described in this work, Fig. 7 shows the different concentration profiles of $[Fe(CN)_6]^{3-}$ created on different paper substrates at different CA measurement times. The concentration profiles obtained for GF is, as expected, equivalent to those in a system with a homogeneous diffusion coefficient. At 50 s, the diffusion profile is close to the upper limit of the material, although the



Fig. 7. COMSOL simulations of concentration distribution within different porous paper matrices during a CA measurement using 1.0 mM ferricyanide. The white dashed lines represent the H in which there is a change in DA.

current is not yet affected (see Fig. 5d). However, it is expected that at longer times, the current will sharply drop. This effect is evident in the NC substrate, where between the 10th and 50th second, the $[Fe(CN)_6]^{3-}$ concentration decreases drastically due to the consumption of almost all the species located in the vertical direction. In contrast, in the radial direction, the diffusion profile has not yet reached the limit of the domain, which results in an axial contribution of species and explains the residual current observed in CA.

Regarding the materials with two different diffusion domains (the boundary between them identified in Fig. 7 by a dashed line), substrates W1 and CF, it is observed that initially, the concentration depletion propagates rapidly until it reaches the second zone - corresponding to the cellulose porous matrix - and then advances more slowly. This effect is more pronounced in the CF substrate, as indicated by the fittings.

5. Conclusions

The existing research on electrochemical paper-based analytical devices (ePADs) has focused on their various applications and the physical and chemical properties of the paper or electrodes. However, there are no systematically studies that analyse the effect of the porous material selection on the mass transport and, eventually, on the electrochemical response of the system. In this work, electrochemical measurements were performed on different substrates of paper commonly used in the diagnostic industry.

The results showed that the glass-fiber substrate used in the study had a micro-structure with high porosity and large pore size that allowed for nearly identical diffusion to that in the absence of paper. This material was found to be highly recommended for the implementation of ePADs, from an electrochemical standpoint. On the other hand, although the studied nitrocellulose membranes had a similar behavior to glass fiber at short measurement times, the current decreased significantly after the first 10 s due to the diffusion layer reaching the thickness of the material. This material is crucial for immunosensing ePADs as its structure and composition allows for the anchoring of biomolecules used for the recognition of analytes. Therefore, electrochemical measurements in this matrix have to be performed during the first seconds of measurement if sensitivity has to be maximized. Current responses in cellulosic materials were described satisfactorily by assuming the existence of two diffusive zones, one accounting for a film between electrode and paper in which diffusion is similar than the one obtained in a free liquid domain and the other corresponding to a porous zone with reduced diffusion and current generation. Both cellulose materials under study decrease the diffusivity of the species in a significant manner, being this effect more relevant if the species under study are charged. Hence, W1, the most widely employed material in ePADs, may not be the optimal selection for CA measurements.

The study highlights the need to characterize the electrochemical response of paper substrates to avoid the deterioration of sensitivity and detection limits in electrochemical sensing. This work aims to contribute to the understanding of the electrochemical performance of ePADs that leads to progress in the design and optimization of future devices.

Additionally, this work opens the door to multiple studies that will provide a better understanding of the effect of the porous matrix on the electrochemical response. These studies include examining different sizes of redox probes, the effect of adding other components such as antibodies or large organic molecules, the impact of a positive charge depending on the type of porous material, and many others.

CRediT authorship contribution statement

Dmitry Galyamin: Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Eduardo Laborda:** Writing – original draft, Methodology, Investigation, Formal analysis. **Juan Pablo Esquivel:** Writing – review & editing, Writing – original draft,

Supervision. Joaquín González: Writing – original draft, Methodology, Investigation, Formal analysis. Neus Sabaté: Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization.

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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.snr.2024.100224.

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