Electrochemical Behavior and Analytical Utility of a Controlled Porosity Cellulose Acetate Film Bearing 2,6-Dichlorophenolindophenol

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Abstract
Glassy carbon electrodes coated with a cellulose acetate film incorporating 2,6-dichlorophenolindophenol (CA/DCPI) were developed. At this multifunctional coating DCPI serves as a mediator for the electron transfer kinetics and in conjunction with the cellulose acetate's size exclusion properties it results in a chemical sensor with great selectivity and stability. Access to the surface can be manipulated via controlled hydrolysis of the film in KOH or ZnCl₂ solutions. Different permeabilities are obtainable by hydrolyzing the film over different time periods. Diffusion coefficients $D_{ac}$ of these films for analytes of different molecular sizes were determined with double step chronocoulometry. The electrochemical characteristics of the immobilized DCPI were explored using cyclic voltammetry. The formal potentials of the immobilized DCPI coating hydrolyzed in KOH, ZnCl₂ and with ZnCl₂ in the casting solution were found to be 70, 75, and 79 mV (vs. Ag/AgCl/3M KCl), respectively, at pH 6.5. The dissociation constants of the DCPI redox couple were found to be $5.2 \pm 0.1$ (pKₐ) and $7.4 \pm 0.05$ (pKₒ). The electrochemical rate constant $k$ of the of DCPI redox couple within the film was also evaluated. The behavior of the sensor towards different reducing compounds was investigated. The sensors showed good operational and storage stability.

Keywords: Controlled porosity cellulose acetate film, 2,6-Dichlorophenolindophenol, Permselectivity studies, Electrochemical study

1. Introduction

Electrochemical techniques employing sensitive and selective amperometric sensors are particularly suited for analytical applications in clinical, food and environmental chemistry and thus electrochemical detectors have achieved wide acceptance for the effective monitoring in both batch and flow conditions [1–3]. Flow electrochemical detectors in connection with liquid chromatography (LC-EC) and voltammetric detectors in connection with flow injection analysis (FIA) offer a powerful tool for the detection of a wide range of analytes [4–6].

Prolonged use of electrochemical detectors, especially in biological matrix and undiluted samples, results in fouling of the surface of the solid electrodes commonly used as sensing probes [7]. Fouling is subject to a gradual loss of activity and is primarily due to adsorption of proteins and surfactants or accumulation of highly reactive intermediates (e.g., free radicals) produced in the course of oxidation of many phenolic and aromatic compounds or cofactors (e.g., NADH). Apart from fouling, another drawback is the high activation overpotentials required for the oxidation or reduction of the analytes of interest at the surfaces of many sensors [8]. This is a main problem in electrochemistry as specificity is inversely related to the magnitude of the applied potential. Furthermore under these conditions formation of highly reactive intermediates is favored giving rise to enhanced fouling phenomena.

The use of mediators has been proved as an effective approach to restrict or to eliminate these problems. A lot of work has been done on this direction and the serious advantages from the use of mediators have been documented in comprehensive reviews [9, 10]. However, some drawbacks are introduced since the mediators may facilitate charge transfer between possible interferants and the electrode, increasing the interference problem and leaching from the system, producing thus a progressively diminishing response to the analyte. The choice of mediator is critical and varies depending on the application. A detailed review dealing with the criteria which should be satisfied by a mediator is reported by Turner [11].

The above mentioned problems could be potentially faced by combining the advantages of working at low redox potential of the mediator and/or the permselective properties of a polymeric membrane employed in the probe. Different transport mechanisms based on size exclusion (e.g., cellulose acetate), charge exclusion (e.g., Naion, polyester sulfonic acid, polyvinylpyridine), polarity (e.g., phosphatidylcholine), mixed control (e.g., cellulose acetate/Nafion) or permselectivity/electrocatalysis (e.g., cellulose acetate/cobalt phthalocyanine, Naion/cobalt phthalocyanine) have been extensively reviewed [12–13]. Chemical modifications of several polymeric membranes, such as cellulose acetate with non-anionic surfactants [14] or organosilanes [15], polyvinylchloride with surfactants [16] or different plasticizers [16] and finally polycarbonate membranes treated with liquid phase lipid [14] have been reported to provide promising approaches for increasing selectivity of electrochemical sensors.

For membrane-based electrochemical devices, various characteristics of great analytical interest such as, the retention of the mediators, cofactors or activators, the permeability of the membrane to the analyte, the extension of the linearity over the environmental concentration range, and the protection of fragile biomaterial from low pH inactivation, could be achieved [17]. However, under the diffusion limitations of these configurations sensitivity of probes is not sufficient. Parameters regarding the membrane thickness, the extent of pore size modification and the concentration of the analyte of interest for a specific application should be taken into account in order to achieve an acceptable response and short time of analysis [13]. Further to the above requirements, the incorporation of the mediator in a polymeric backbone results a charge propagation in the film that may contribute to the rate-limiting kinetics during the kinetic cycle of a chemically modified electrode. In this case extra criteria such as the rate constant of the chemical reaction between the mediator...
and the analyte of interest, the apparent electron-transfer rate of the immobilized redox molecule and the diffusion coefficient of the analyte in the polymer film should be taken into account for the choice of mediator and the membrane coating [18–19].

2. Experimental

2.1. Reagents

2,6-Dichlorophenolindophenol sodium salt dihydrate (C12H8Cl2N2NaO2·2H2O, Cat. No 103028) and potassium hydroxide (pro analysis) were obtained from Merck (Darmstadt, Germany) and used without further purification. Sodium sulfide, ampicillin (sodium salt), cyclohexanone (99%+) and acetone (99%+) were supplied by Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Ascorbic acid, NADH (disodium salt), potassium hexacyanoferrate(III) and aluminum oxide (particle size 0.3 μm) were obtained from BDH (Poole, UK). Zinc chloride was supplied by Sigma-Aldrich Chemie GmbH (%). Sodium sulfide, hydroxide (pro analysis) were obtained from Merck (Darmstadt, Germany) and used without further purification. Sodium sulfide, hydroxide (pro analysis) were obtained from Merck (Darmstadt, Germany) and used without further purification.

Obtained from Riedel-de Haen (Seelze, Germany). All other chemicals were of analytical grade from Merck and Sigma.

2.2. Apparatus

All electrochemical experiments were conducted with a computer controlled potentiostat, the Autolab electrochemical analyzer (Eco Chemie, Utrecht, The Netherlands). The flow injection experiments were carried out using an in-house fully automated flow injection manifold. A detailed description of the FI manifold and the resident program, employed for the full control of the FI components, is given elsewhere [20]. The working electrode was a glassy carbon electrode (MF-2070, 3 mm diameter, BAS, West Lafayette, IN, USA) mounted in a wall-jet type flow through detector (Metrohm 656, volume < 1 μL, Herisau, Switzerland).

Cyclic voltammetry, double step chronocoulometry and batch experiments were performed with a voltammetry cell (VC2, BAS) using glassy carbon as the working electrode, an Ag/AgCl/3 M KCl reference electrode (BAS) and a Pt wire as auxiliary electrode with a gold connecting pin (BAS). The total effective surface area A of the working electrode was determined by performing chronocoulometric measurements on hexacyanoferrate(III) (diffusion coefficient, \( D^* = 7.6 \times 10^{-6} \text{cm}^2 \text{s}^{-1} \)) and was found equal to that determined from its geometrical dimensions. All experiments were carried out at 25°C.

For casting cellulose acetate membranes a wet film applicator (5 inches, 1–8 mils, URAI, Milan, Italy) was used.

2.3. Procedure

The applied potential for the flow injection measurements was +100 mV (vs. Ag/AgCl/3 M KCl). After its application to the modified electrode, the background current was allowed to reach a constant value under continuous flow (1–2 nA within 30–45 min). The carrier (0.05 M phosphate buffer in 0.05 M KCl, pH 6.5) was continuously pumped at a flow rate 0.32 mL min⁻¹. Standard or sample solutions were injected with a 130 μL injection loop. The current peak height was taken as a measure of the analyte concentration.

2.4. Preparation of the Modified Electrodes

Before use, electrodes were polished by a cotton cloth with aluminum oxide slurry, washed thoroughly with distilled water and finally sonicated for 10 min in distilled water to ensure complete removal of aluminum particles. The electrodes were then scanned in the working buffer solution at a short potential window (0 to 600 mV) in order to obtain a low background current.

2.4.1. Casting of Cellulose Acetate Bearing DCPI

This membrane was prepared by dissolving 1.00 g of cellulose acetate and 0.013 g of DCPI (0.4 mM) in a mixture of 55 mL of acetone and 45 mL of cyclohexanone. The mixture was agitated for 1 h and 2 mL of the clear polymeric solution were pipetted onto a smooth glass surface and spread along it with the aid of a wet film applicator leaving a uniform film of 200 μm thickness. The film was left to air dry overnight and the membrane (20 μm thickness) was pulled out by immersing the glass surface in a water bath. After drying, the membrane sheet was cut in 1 × 1 cm² portions and kept inside parafilm in a cool amber place.

An alternative of this method was the direct casting of the CA/DCPI membrane onto the surface of the probe by droplet evaporation of 10 μL of the casting solution: CA/DCPI-CME (0.4 mM DCPI in 1% cellulose acetate solution) or CA/DCPI/Zn-CME (0.4 mM DCPI plus an appropriate amount of ZnCl₂ in 1% cellulose acetate solution).

2.4.2. Membrane Modification and Electrode Assembly

CA/DCPI (indirect casting) and CA/DCPI-CME membranes were hydrolyzed in a 0.07 M KOH or in a 3.2 M ZnCl₂ solution. For this, the electrode or the membrane portion were immersed in a stirred base or inorganic salt solution for the desired time. Then they were washed thoroughly with double distilled water and finally immersed in a stirred solution of the supporting electrolyte for 10 min to wash out the residual solvent. Resulting probes (in the case of indirect casting, the membrane was placed over the electrode surface with the aid of an O-ring) were mounted in the electrochemical flow cell, which was equilibrated with the working buffer under the working potential.

3. Results and Discussion

3.1. Casting of Membranes and Swelling Process

Penetration of the crystalline regions of the cellulose structure by liquids or vapors with controlled increase in volume (swelling) can be achieved utilizing a number of swelling agents, such as alkali solutions, certain salt solutions, inorganic or mineral acids, etc. According to the swelling capacity of the agent used accessible regions are hydrolyzed in order to control the porosity of the cellulose membrane [21]. Different approaches to increase the porosity of the cellulose films were tested including treatment with an alkali (potassium hydroxide) or an inorganic salt solution (zinc chloride). In order to develop a procedure for controlling the size exclusion properties of the polymeric film, particular attention was paid to conserve the excellent electrochemical properties of incorporated DCPI [22]. As is shown in Table 1, DCPI exhibits a clear electrochemistry. Formal potentials \( (E^0) \) of 70, 75 and 79 mV (vs. Ag/AgCl/3 M KCl) at pH 6.5 were calculated, as the mean of the cathodic and anodic potential,
Table 1. Electrochemical characteristics of immobilized DCPI in cellulose acetate films treated with KOH or ZnCl₂. Buffer: 0.25 M phosphate in 0.05 M KCl, pH 6.5. Scan rate: 50 mV s⁻¹.

<table>
<thead>
<tr>
<th>DCPI [a] [mM]</th>
<th>ZnCl₂ [a] [mM]</th>
<th>( t_{\text{hydrolysis}} ) [min]</th>
<th>( \Delta E_p ) [mV]</th>
<th>( I_s/I_c )</th>
<th>( \Gamma ) [b] [nmol cm⁻²]</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA/DCPI-CME hydrolyzed in 0.07 M KOH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.4</td>
<td>–</td>
<td>20</td>
<td>32</td>
<td>0.97</td>
<td>0.04</td>
</tr>
<tr>
<td>1.0</td>
<td>–</td>
<td>20</td>
<td>45</td>
<td>0.95</td>
<td>0.12</td>
</tr>
<tr>
<td>1.2</td>
<td>–</td>
<td>20</td>
<td>57</td>
<td>1.15</td>
<td>0.17</td>
</tr>
<tr>
<td>1.5</td>
<td>–</td>
<td>20</td>
<td>60</td>
<td>1.22</td>
<td>0.18</td>
</tr>
<tr>
<td>CA/DCPI-CME hydrolyzed in 3.2 M ZnCl₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.4</td>
<td>–</td>
<td>16</td>
<td>38</td>
<td>0.90</td>
<td>0.14</td>
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<tr>
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<td>–</td>
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<td>0.86</td>
<td>0.33</td>
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<tr>
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<td>–</td>
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<td>62</td>
<td>0.64</td>
<td>0.35</td>
</tr>
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<td></td>
</tr>
<tr>
<td>0.4</td>
<td>3</td>
<td>–</td>
<td>46</td>
<td>0.91</td>
<td>0.10</td>
</tr>
<tr>
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<td>10</td>
<td>–</td>
<td>45</td>
<td>1.11</td>
<td>0.09</td>
</tr>
<tr>
<td>0.4</td>
<td>15</td>
<td>–</td>
<td>45</td>
<td>0.89</td>
<td>0.11</td>
</tr>
<tr>
<td>0.4</td>
<td>20</td>
<td>–</td>
<td>52</td>
<td>0.72</td>
<td>0.09</td>
</tr>
<tr>
<td>0.4</td>
<td>30</td>
<td>–</td>
<td>55</td>
<td>1.09</td>
<td>0.16</td>
</tr>
</tbody>
</table>

[a] Initial concentration in the casting solution. [b] Calculated from the integrated anodic peak of the cyclic voltammograms in the range –200 to +300 mV, at 50 mV s⁻¹ and corrected for the background current.

when the polymer coatings were hydrolyzed in 0.07 M KOH, 3.2 M ZnCl₂, and with 15 mM ZnCl₂ included in the casting solution, respectively. Comparison of these values with the one taken with soluble redox compound under the same conditions (\( E_{\text{w}} \) = 49 mV) shows, that the hydrolyzing procedure does not strongly perturb the electronic structures of the immobilized compound. However, this shift (16–30 mV) is higher than that of the 1–5 mV observed, when DCPI was physically adsorbed or chemically bound onto spectroscopic graphite electrodes [22]. It is important to denote the discrepancies between the applied mechanisms when basic (in the case of KOH) or acidic (in the case of ZnCl₂, pH 3–4) hydrolysis occurs. The acidic environment in the cellulose film (1 % w/v cellulose acetate, pH 4) may also contribute to the positive shift of the formal potentials.

Different concentrations of cellulose acetate solutions (1–5 % w/v) and DCPI (0.4–1.5 mM) were tried for the construction of CA/DCPI-CMEs. Values of 1 % w/v of cellulose acetate, 0.4–1 mM DCPI (1 mM DCPI was employed for analytical applications), 0.07 M KOH and 3.2 M ZnCl₂ were chosen, since the resulting membranes demonstrated good characteristics in terms of membrane thickness, mechanical strength, electrocatalytic properties of the immobilized mediator, retention of mediator, reproducibility of the hydrolyzing process and short times of hydrolysis.

Membranes cast from a 5 % (w/v) solution of cellulose acetate [23] require extended time of hydrolysis (e.g., 40 min for a compound has a molecular weight of about 140). Under these conditions the preparation of the membrane is time consuming and the prolonged exposure to the alkaline solution may cause the inactivation of the mediator.

Under these conditions porosity of the films could be manipulated and controlled in a reproducible way to the desired extent within 16 min (3.2 M ZnCl₂) or 20 min (0.07 M KOH). This porosity corresponds to a polymeric film with a molecular weight cut-off of about 150–170 Da and ensures sufficient diffusion to molecules with lower molecular weight and retention of the mediator (mol. weight 290) in the polymer structure. Hydrolysis times longer than 30 min result in higher coating permeabilities. However, at this extent analytical utility of the probes is limited as many solutes can penetrate the film and leaching of the mediator may be initiated. Up to about 30 min (3.2 M ZnCl₂) or 35 min (0.07 M KOH) of hydrolyzing process the membranes exhibit good mechanical characteristics and are suitable for use, even in flow conditions. Longer hydrolyzing times caused membranes to become brittle and easily damaged.

Apart from molecular weight (which is considered proportional to the size of the molecule, especially in the case of organic compounds), other factors as the net electric charge, the conformation or the shape of the molecules also contribute to the mass transport resistance [24].

In the case of the CA/DCPI/Zn-CMEs permeability around a molecular weight of 200 was observed at concentrations of the hydrolyzing agent (ZnCl₂) higher than 20 mM. This study was performed by recording the catalytic current which is due to the oxidation of ascorbic acid at +0.1 V using CA/DCPI/Zn-CME prepared with a concentration of ZnCl₂ ranging between 3–150 mM (data not shown). Concentrations higher than 100 mM of ZnCl₂ result in unstable coating with the film falling apart from the supporting electrolyte. As can be seen in Table 2 a loss of about 30 % of the initial activity was observed, for the latter case as the swelling agent acts in the film for extended time.

3.2. Electrochemistry of the Immobilized DCPI

The electrochemical characteristics of the immobilized DCPI were explored applying cyclic voltammetry on a 0.25 M phosphate buffer in 0.5 M KCl at pH 6.5. Values of peak potential separation (\( \Delta E_p \)), ratios of anodic and cathodic current intensities (\( I_s/I_c \)) and surface concentrations of the immobilized mediator (\( \Gamma \)) are illustrated in Table 1. It is obvious that the electrochemical behavior of DCPI is governed by mass transport within the cellulose film and deviations from the theoretical values of \( \Delta E_p \), \( I_s/I_c \) are a function of the surface coverage of the immobilized mediator. Increasing the scan rate from 10 to 200 mV s⁻¹ the \( \Delta E_p \) values moderate between 30–58 mV; while for scan rates 200–1400 mV s⁻¹ the \( \Delta E_p \) varies between 60 to 91 mV. The \( I_s/I_c \) ratio presents in general only small deviations from the theoretical value. The voltammograms (especially at high coverages and fast scan rates) possess shapes which resemble classical diffusional controlled behavior. The observed nonideal electrochemical behavior of the immobilized mediator was expected because of the strong interactions between immobilized molecules, the surface structural heterogeneity of the film, the electron transfer progress through pinholes and channels in the film, the diffusion of the molecules within the film and the penetration of electrolyte through the pores [18, 25].

Table 2. Stability of DCPI/CA-CMEs measured with the estimated amount of DCPI attached in the cellulosic membrane. Buffer: 0.25 M phosphate in 0.5 M KCl, pH 6.5. Scan rate: 50 mV s⁻¹.

<table>
<thead>
<tr>
<th>Sensor</th>
<th>15 min</th>
<th>60 min</th>
<th>200 min</th>
<th>300 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCPI/CA-CME hydrolyzed 20 min in 0.07 M KOH</td>
<td>97</td>
<td>96</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>DCPI/CA-CME hydrolyzed 16 min in 3.2 M ZnCl₂</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>DCPI/CA/Zn-CME</td>
<td>90</td>
<td>79</td>
<td>74</td>
<td>72</td>
</tr>
</tbody>
</table>

Electroanalysis 2000, 12, No. 5
A plot of \( I_p \) vs. scan rate \( v \) for CA/DCPI-CME hydrolyzed in 0.07 M KOH for 20 min shows a linear relation up to 200 mV s\(^{-1}\) (Fig. 1a) suggesting facile charge transfer kinetics. Above 200 mV s\(^{-1}\) the relationship becomes linear only when plotted versus the square root of \( v \) (Fig. 1b) which provides additional evidence for the validity of the above mentioned charge transfer mechanism. Similar behavior was observed when CA/DCPI-CME was hydrolyzed in 3.2 M ZnCl\(_2\) for 16 min. Since both equilibrium (low coverage, slow scan rate) and kinetically controlled behavior occur for the same charge transfer center (DCPI), the kinetic phenomena must be ascribed only to the polymer environment in which the DCPI sites are imbedded and not to the intrinsic properties of DCPI [22].

The number of the participating electrons in the redox reaction was evaluated from the slope of the linear part of Figure 1a as 1.22 ± 0.02 (KOH) and 1.30 ± 0.03 (ZnCl\(_2\)) (data not shown). The \( n \)-value was also calculated from the equation of the peak current \( I_p = (n^2F^2/4RT)FvA \) for an immobilized compound, where terms have their usual meaning. Values of 1.80 ± 0.02 at 10 mV s\(^{-1}\) (KOH) and 1.71 ± 0.05 at 20 mV s\(^{-1}\) (ZnCl\(_2\)) were calculated. All the above deviations from the theoretical value can be attributed to interactions occurring among the immobilized molecules of DCPI in the membrane. It is therefore reasonable to assume that two electrons participate in the redox reaction of DCPI.

**3.2.1. Determination of the Electrochemical Rate Constant and Transfer Coefficients**

The (apparent) electrochemical rate constant \( k_o \) and the transfer coefficients \( z_a \) and \( z_c \) were calculated from Tafel diagrams according to the method described by Laviron [26]. Figure 2 illustrates the procedure used for evaluating \( z_a \), \( k_o \) from the anodic and \( z_c \), \( k_o \) from the cathodic partial reaction. The evaluated values for the coefficients \( z_a \) and \( z_c \) are 0.59 and 0.37 for membranes treated with KOH and 0.48 and 0.55 for membranes treated with ZnCl\(_2\). The sum of transfer coefficients \( z_a \) and \( z_c \) is very close to the theoretical value of 1 [27]. Mean values of \( k_o(KOH) = 6.6 ± 2 \) s\(^{-1}\) and \( k_o(ZnCl_2) = 10.2 ± 3 \) s\(^{-1}\) were evaluated from all the extracted experimental data applying the equation \( k = 2.303 z_a nFv_o/RT \). These values are similar to those reported for well-known mediators immobilized on electrode surfaces [28, 29]. However, they are lower compared to \( k_o \) value reported for the DCPI immobilized on graphite electrodes, suggesting a slower electron kinetics probably due to the polymeric environment of the immobilized DCPI [22].

**3.2.2. Determination of \( pK_a \)**

The dependence of the \( E^0 \) of the immobilized DCPI on the pH was investigated by cyclic voltammetry. Figure 3 shows \( E^0 \) as a function of pH for DCPI immobilized into a cellulose membrane treated with KOH. The \( E^0 \) value was measured at a scan rate 20 mV s\(^{-1}\), where no kinetic effects would adversely distort the peak current [18].

\( pK_a \) values of 5.2 ± 0.1 and 7.4 ± 0.1 for the reduced and oxidized DCPI were graphically calculated. \( pK_a \) was calculated from the intersection of the two straight lines with slopes −63 mV (pH unit)\(^{-1}\) in the acidic region and −43 mV (pH unit)\(^{-1}\) in pH range 5 to 7. \( pK_a \) was calculated from the intersection of the latter line with that in the alkaline region (pH 7 to 8.5) has a slope −89 mV (pH unit)\(^{-1}\). The changes in the slope from −63 to −43 mV (pH unit)\(^{-1}\) and from −43 to −89 mV (pH unit)\(^{-1}\) are attributed at the step by step protonization of the reduced and the oxidized forms of DCPI, respectively. The deviation of the experimental values of the slopes from the theoretical −30 mV (pH unit)\(^{-1}\) (for 1H\(^{+}\) mechanism), −60 mV (pH unit)\(^{-1}\) (for 2H\(^{+}\) mechanism) and −90 mV (pH unit)\(^{-1}\) (for 3H\(^{+}\) mechanism) [30] are probably due to the coexistence of

![Fig. 1. Variation of \( I_p \) with a) the scan rate and b) the square root of the scan rate for the DCPI/CA-CME hydroxylated in 0.07 M KOH for 20 min. Embedding cyclic voltammograms represent the experimental data for scan rates: I) 10, 20, 50 mV s\(^{-1}\). II) 70, 100, 150, 200 mV s\(^{-1}\), and III) 400, 600, 800, 1000, 1200, 1400 mV s\(^{-1}\). Buffer: 0.25 M phosphate in 0.5 M KCl, pH 6.5; surface coverage: 0.07 nmol cm\(^{-2}\).](image1)

![Fig. 2. Dependence of \( E_p \) on log(\( v \)) for the DCPI/CA-CME hydroxylated in 0.07 M KOH for 20 min. Graphical calculation of the critical potential scan rate \( v_o \). Buffer: 0.25 M phosphate in 0.5 M KCl, pH 6.5; surface coverage: 0.07 nmol cm\(^{-2}\).](image2)
The results of this study are in accordance with the reaction scheme:

For \( \text{pH} \leq 5.2 \):
\[
D^- + 2H^+ + 2e^- \rightarrow DH_2^+ \quad (1)
\]

For \( 5.2 \leq \text{pH} \leq 7.4 \):
\[
D^- + H^+ + 2e^- \rightarrow DH_2^+ \quad (2)
\]

For \( 8.5 \geq \text{pH} \geq 7.4 \):
\[
D^- + 3H^+ + 2e^- \rightarrow DH_3^- \quad (3)
\]

where \( D^- \) is the oxidized form of DCPI at high pH.

The presence of DCPI in the above forms and their chemical structures have been earlier described [22]. In a study by Ottaway the dissociation constants for DCPI in aqueous solution were found 5.7 (pK\(a_1\)) and 10.1 (pK\(a_2\)) [32]. As it can be seen from the pKa values obtained under the present experimental conditions, where DCPI is in the immobilized state, there is a change in the extent of the dissociation probably due to its incorporation into the cellulose membrane.

**3.2.3. Electrocatalytic Oxidation of Reductive Species**

Figure 4 shows cyclic voltammograms obtained with CA/DCPI modified electrodes in the presence of ascorbic acid and sodium sulfide solution in phosphate buffer of pH 6.5, respectively. There is a significant increase in the current at the applied potential (74–79 mV) where \([\text{DCPI}]_{\text{ox}}\) is formed, compared to the current recorded at the same potential in the absence of the reductive species. This behavior indicates a strong electrocatalytic effect and can be attributed to the diffusion of ascorbic acid or sulfide present in the solution through the cellulose acetate film, and the reduction of the electrochemically produced \([\text{DCPI}]_{\text{ox}}\). The anodic current increases proportionally to the concentration of the reductive species and a shift of the anodic potential is observed as the sweep rate is increased (data not shown) indicating the domination of a kinetic limited regime in the reaction between the DCPI and reductive species. The overall reaction between DCPI and ascorbic acid or sulfide is consistent with a catalytic regeneration mechanism (variation of the EC mechanism) and may be described by Scheme 1 [33]. Experimental data support this mechanism [34].

![Scheme 1](image)

**3.2.4. Diffusion Coefficients**

Diffusion coefficients of sulfide anions (mol. weight 32), ascorbic acid (mol. weight 176), DCPI (mol. weight 290) and NADH (mol. weight 709) were calculated by double step chronocoulometry. Two series of experiments were carried out using first a bare glassy carbon electrodes and then the same electrodes, modified with a 1% cellulose acetate (w/v).
membrane hydrolyzed for 18 min in 0.07 M KOH. The potential was stepped from an initial value $E_i = -0.2 \text{ V} (+0.6 \text{ V in the case of the DCPI})$ up to $E_s = +0.6 \text{ V} (-0.2 \text{ V in the case of the DCPI})$ and finally stepped back to $E_i$. The selection of potentials was based on the cyclic voltammograms of the tested compounds. $E_i$ does not induce any electrochemical reaction, while $E_s$ oxidizes (or reduces the DCPI) the tested compounds present in the solution. Concentrations of 3 mM (0.25 mM in the case of DCPI) in a 0.25 M phosphate buffer of pH 6.5, in 0.5 M KCl were used.

Typical chronocoulometric responses, the plots of charge ($Q$) versus time ($t$) and the Anson plot $Q$ vs. $t^{-1/2}$ are shown in Figure 5. The Anson plot is linear, as expected for a diffusion-limited process according to Equation 4.

$$Q = 2nFAc^*D_{app}^{1/2}/t^{1/2}$$

where $D_{app}$ and $c^*$ are the apparent diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$) and the concentration (M) of the tested compounds in the electrolyte solution [35]. $D_{app}$ is calculated from the slope corresponding to the later part of the charge transient.

As can be seen from the values illustrated in Table 3, $D_{app}$ decreases significantly, as expected, with the molecular weight of the tested compound. These results justify the exclusion size properties of the cellulosic film. The big changes in the tested compound. These results justify the exclusion size properties of the cellulosic film. The big changes in $D_{app}$ suggest that the (irreversible) oxidation of these compounds (except DCPI) is taking place in the interface of the electrode/polymeric film. In the case of DCPI, a reversible redox reaction is probably taking place in the polymeric film/electrolyte interface. This behavior cannot be explained with the data given and must be further elucidated.

### 4. Analytical Utility

The effect of the hydrolyzing procedure on the porosity of the cellulose acetate membrane was recorded on-line utilizing three electroactive species representing a range of molecular weights from 32 to 290. Current intensities, due to the oxidation of the sodium sulfide, uric acid and DCPI, were recorded in respect to the hydrolysis time using untreated glassy carbon electrodes covered with cellulose acetate membrane. This selection of these compounds was based on the molecular weight and their electrochemical stability in strong alkaline conditions (0.07 M KOH, pH 12). The stability of the compounds at pH 12 was checked with cyclic voltammetry. Sulfide (mol. weight 32) gave an oxidation peak at 0.80–0.85 V. Uric acid (mol. weight 168) gave an oxidation peak at 0.6 V very close to the one at pH 6.5 (0.5 V). DCPI (mol. weight 290) gave an oxidation peak at $-0.2 \text{ V}$. According to previous publication, DCPI is unstable above pH 12 [36]. However, cyclic voltammograms were stable within the tested time interval (50 min) and therefore used.

Other reducing compounds were rejected for different reasons: i) Sulfite (mol. weight 80) gives an oxidation peak at 1.2 V at pH 6.5 but no oxidation peak at pH 12. ii) Ascorbic acid (mol. weight 176) since it is easily oxidized in alkaline medium. iii) Dopamine (mol. weight 189) was also rejected because it is unstable, although it gives an oxidation peak at 0.6 V (pH 12). A change of the color of its solution from orange yellow to dark brown was observed within 15 minutes. iv) Glutathione (mol. weight 307) gave no oxidation peak.

As it can be seen from Figure 6, at 1200 s (after 20 min of hydrolysis) sulfides have passed through the membrane, while no

<table>
<thead>
<tr>
<th>Substrate (mol. weight)</th>
<th>Bare electrode, $D_b$</th>
<th>Cellulose acetate film, $D_c$</th>
<th>$D_b/D_c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfide (32)</td>
<td>$1.5 \times 10^{-5}$</td>
<td>$3.5 \times 10^{-8}$</td>
<td>429</td>
</tr>
<tr>
<td>Ascorbic acid (176)</td>
<td>$5.2 \times 10^{-6}$</td>
<td>$2.5 \times 10^{-9}$</td>
<td>2080</td>
</tr>
<tr>
<td>DCPI (290)</td>
<td>$4.2 \times 10^{-6}$</td>
<td>$4.0 \times 10^{-6}$</td>
<td>$\approx 1$</td>
</tr>
<tr>
<td>NADH (709)</td>
<td>$2.3 \times 10^{-6}$</td>
<td>$9.5 \times 10^{-10}$</td>
<td>2421</td>
</tr>
</tbody>
</table>

**Fig. 5.** Response for double step chronocoulometric experiments in the presence of a) 3 mM Na$_2$S and b) 3 mM NADH with I) bare glassy carbon electrode and II) glassy carbon covered with 1 % cellulose acetate hydrolyzed for 18 min in 0.07 M KOH. Diagram (c) illustrates the Anson plot for data from trace 5a curve (II). Buffer: 0.25 M phosphate in 0.5 M KCl, pH 6.5; potential steps: $E_i = -0.2 \text{ V}$ and $E_s = +0.6 \text{ V}$.
The operational stability of the sensors was studied by continuous exposure to flow streams using ascorbic acid or sodium sulfide as analytes. As a result the final CA/DCPI-CME activity was 92%, of that at the start of the trial, after 10 h of continuous operation. The sensors displayed good storage stability if stored dry at 4°C, when not in use. The CA/DCPI-CME retained 95% and 85% of its initial activity after 1 week and 2 weeks of storage, respectively.

5. Acknowledgement

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6. References


4.1. Stability of the Sensor

The stability of the CA/DCPI electrodes was verified by monitoring the amount of the active substance after 500 successive sweeps. The amount of DCPI remaining in polymeric film depends on the hydrolysis time and the hydrolysis procedure as illustrated in Table 2. The CA/DCPI electrodes provide a remarkable stability, while at the CA/DCPI/Zn electrodes DCPI gradually decreases probably due to the continuous hydrolysis of the cellulosic film. At CA/DCPI electrodes stability is also depended on the time of hydrolysis (see above).

Fig. 6. Permeability study of the cellulose acetate membrane. Glassy carbon electrodes covered with unmodified cellulose acetate were immersed in a 0.07 M KOH solution (pH 12) in the presence: a) 0.1 mM DCPI at 0.3 V, b) 3 mM uric acid at 0.6 V, and c) 3 mM sodium sulfide at 0.9 V. Inset: Interference effect of 4 mM uric acid (UA) and 4 mM paracetamol (PA) on the flow assay of 0.4 mM ascorbic acid (AA) with a CA/DCPI-CME hydrolyzed for 25 min in 0.07 M KOH. Buffer: 0.05 M phosphate in 0.05 M KCl, pH 6.5; flow rate 0.32 mL min⁻¹; sample volume: 130 µL; applied potential: +0.1 V.


