Redox active films of salicylic acid-based molecules as pH and ion sensors for monitoring ionophore activity in supported lipid deposits

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Redox active films of salicylic acid-based molecules as pH and ion sensors for monitoring ionophore activity in supported lipid deposits

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Abstract:

5-aminosalicylic acid and salicylic acid have been used to form redox active films onto glassy carbon electrodes through recurrent cyclic voltammetry. The variation of the formal potential of the film obtained from 5-aminosalicylic acid as a function of pH is linear over the entire pH range studied (pH 2 to 10) with a slope of -80 mV per pH unit. Salicylic acid-based redox active films permit the detection of sodium and potassium ions (with a slope of -10 mV per 1 mM of cation) and chloride (with a slope of +11 mV per 1 mM of chloride). A lipid deposit of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) onto these modified electrodes allowed the integration of ionophores (valinomycin and nigericin) and the monitoring of the pH and potassium ion concentration variation at the modified electrode / lipid deposit interface.

1 Introduction:

The living cell membrane acts as a natural barrier to ion diffusion while specific membrane transporters like proteins (*e.g.*: ion channels, pumps) or non-proteins (*e.g.*: ionophores) have the ability to move ions across it [1-3]. Malfunctioning of these proteins can induce diseases and conditions known as channelopathies [4]. Ionophores are used as antimicrobial compounds because they can destabilize the ionic traffic through a biological membrane [5-9]. Physiologically relevant models of the cell membrane can be made with layers or deposits of pure or mixtures of synthetic or natural phospholipids. The most common systems include lipid mono- bi- or multi-layers, lipid vesicles and lipid deposits supported on solids [10] and electrodes [11-17]. The ability to measure analyte (e.g.: H^+, K^+) concentrations at the electrode/lipid membrane interface is essential for the study of biochemical processes

involving membrane transporters [1-9]. By electrically coupling the biological transporters in a lipid deposit mimicking a cellular membrane to redox active sensing systems onto electrode surface, the activity of these transporters can be monitored and the interfacial target analyte concentration at the electrode/lipid interface determined. In order to detect the pH changes at the glassy carbon electrode / lipid layer interface induced by these biological ion transporters, electrodes were modified by pH responsive redox active molecules. In particular, electrodes modified with electrodeposited redox active films are good candidates for replacement of pH glass electrodes [18]. In the literature many examples of pH sensors using electro-polymerization are described like for example electro-polymerization of pyrrole, aniline, thiophene or phenol derivatives [19-23]. In this work, a pH responsive redox film was immobilized onto glassy carbon electrode from 5-aminosalicylic acid (5ASA, Figure 1). Aminosalicylic acid oxidation pathway is complex and has been previously investigated by Eriksson and Nyholm [24]. This oxidation pathway involves the formation of benzoquinone imine which mainly undergoes hydrolysis to yield the corresponding quinone [24,25]. In the present paper we have used this electrodeposited redox active film as a robust pH sensor to monitor pH changes induced by biological ion transporters at an electrode / lipid layer interface.

To test the influence of the amino group on the formation of a pH-sensitive redox active film, salicylic acid (SA, Figure 1) and 5-aminosalicyclic acid (5ASA, Figure 1) were investigated. With SA, the modified electrode proved to be sensitive not to the pH but to the variation of monovalent ions such as sodium, potassium or chloride. While the redox fate of SA upon oxidation has been studied [26,27] this paper reports for the first time the use of SA-based redox film as a sensor for monovalent ions.

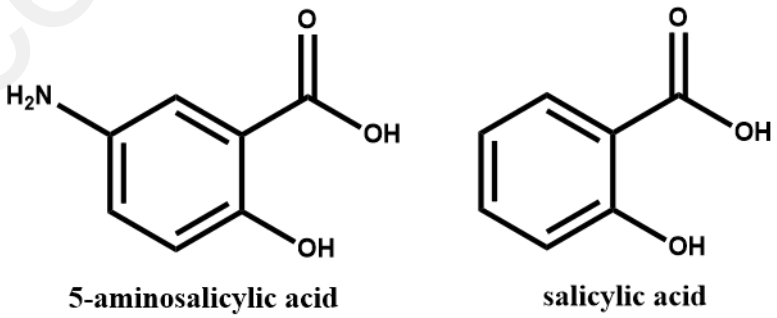

Figure 1: Molecular structure of 5-aminosalicylic acid (5ASA) and salicylic acid (SA).

5ASA-based redox active films at electrodes can be used to monitor pH and SA-based redox active films can be used to detect sodium, potassium and chloride concentration changes in

solution. These modified electrodes with redox active films can also be applied to the monitoring of pH or ions concentration induced by ionic transporters in supported lipid deposits. As an illustration we consider here the case of two ionophores, namely valinomycin and nigericin, integrated in lipid deposits supported onto a modified electrode. Valinomycin is a ionophore transporting ions through lipid membranes and has been reported to be highly potassium ion selective [8,9]. Nigericin act as an antiporter and exchanges one potassium ion for one proton [6,7].

In this paper, we first discuss the process of film formation at different electrodes (bare or modified glassy carbon, graphite, gold, Indium Tin Oxide (ITO)) and the electrochemical characterization of 5ASA-based redox active films as pH sensor and that of SA-based films as monovalent ion sensor. Then, these electrodes with pH- or ion-responsive redox active films are used to monitor and quantify the activities of valinomycin and nigericin in supported lipid deposits onto the modified electrodes.

2 Experimental

2.1. Electrochemical measurements

A three-electrode cell was used. A glassy carbon or a gold disk electrode (3 mm diameter, 0.071 cm^2, IJ Cambria, UK), graphite or ITO were used as working electrodes. The graphite rod electrodes were obtained from Morgan Carbon (France) and their surface were partially covered by Teflon tape in order to obtain a surface area similar to that of the glassy carbon or gold electrodes. A platinum wire was used as the counter electrode, and an Ag/AgCl sat. KCl reference electrode from BASI (USA) were used to perform all electrochemical measurements. The working electrode was mirror-polished under a flux of ultrapure water on a fine grid silicon carbide paper from Struers (4000-grid SiC for glassy carbon or gold and 2400-grid for graphite electrode) mounted on a DAP-V Struers polishing equipment rotating at 3.0 x 100 rpm then rinsed in ultrapure water before each experiment. Electrochemical experiments were carried out at room temperature (21 ± 3°C) with an Autolab AUT83857 potentiostat/galvanostat (Eco Chemie B.V., Netherlands) using Nova 2.1.1 as electrochemical software (Metrohm). Electrochemical impedance spectroscopy (EIS) measurements were performed at open circuit potential in the frequency range from 100 kHz down to 50 mHz with a signal amplitude of 10 mV in 10 mM phosphate buffer at pH 7 for 5-aminosalicylic acid and ionophores tests and in 10 mM ammonium acetate at pH 5 for salicylic acid. Before each measurement, all solutions were deaerated by bubbling argon for at least 3 minutes.

During electrochemical measurements an argon flux was kept in the electrochemical cell above the solution. For each measurement discussed in this article, at least 3 independent tests were performed.

2.2. Chemicals and solutions

Milli-Q water (18.2 MΩ cm) was used to prepare all solutions. 5-aminosalicylic acid (5ASA), 4-aminosalicylic acid (4ASA), 3-aminosalicylic (3ASA), salicylic acid (SA), 4-decylaniline, 4-bromoaniline, 4-aminobenzenesulfonic acid, ammonium acetate, sodium nitrite, valinomycin, nigericin and hydrochloric acid solution were obtained from Sigma Aldrich (USA). Ammonia solution is obtained from VWR/ Prolabo (USA). 5ASA and SA were used without further purification. Sodium hydroxide, acetic acid and ammonium acetate were obtained from Alfa Aesar (USA). Commercial buffer solutions were purchased from Hamilton (China). 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) lipids were purchased as a powder from Avanti Polar Lipids (USA) and the powder was stored in a freezer.

Unbuffered solutions are made with various concentrations of hydrochloric acid and / or sodium hydroxide aqueous solutions containing 10 mM of sodium chloride (from Sigma Aldrich, USA) in Milli-Q water. 10 mM phosphate buffer solution (pH 7) was made by dissolving 1.548 g of potassium phosphate dibasic from VWR (USA) and 0.583 g of potassium phosphate monobasic from VWR (USA) in 800 mL of Milli-Q water. 10 mM of ammonium acetate solution at pH 5 was obtained after dissolving 0.007 g of ammonium acetate into 10 mL of Milli-Q water. In order to modulate the pH of the 10 mM ammonium acetate solution, various concentrations of acetic acid and ammonia solutions were used. The pH of the solution was checked before use with a pH meter (Hanna Instruments, USA). The lipid solution was prepared by dissolving 3.300 g of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) in 1 mL of ethanol and was used immediately or stored in the fridge if needed for less than 1 hour before use.

2.3. Surface modifications

For the formation of the redox active film from 5-aminosalicylic acid, a 5ASA solution (typically 5 mM) was prepared just before use by dissolving 5-ASA in 10 mM hydrochloric acid (pH 2) following the procedure described by Eriksson and Nyholm [24]. For SA-based film, the solution (typically 1 mM) was prepared just before use by dissolving salicylic acid in

10 mM of phosphate buffer solution at pH 7. For both molecules, recurrent cyclic voltammetry (usually 8 to 20 cycles) were recorded between -0.8 and +0.8 V at 100 mV/s.

In order to electrodeposit approximatively the same amount of material on the electrode, the total charge passed during electrodeposition of the 5ASA or SA is set at 3.5 mC. Once this target charge is reached, the electrodeposition procedure stops at the end of the cycle.

Glassy carbon electrode surface modifications (to evaluate redox films formation onto different hydrophobic/hydrophilic conductive surfaces, see section 3.1) were performed by the cathodic electroreduction of aryldiazonium salts generated in-situ from the corresponding arylamines, namely 4-decylaniline, 4-bromoaniline and 4-aminobenzenesulfonic acid. To a 1 mM arylamine acidic solution (10 mM HCl solution, pH 2) was added 3 mM of $NaNO_2$ and the cell was then immediately chilled in an ice bath. After 20 minutes, electrografting experiments were realized in an ice bath by cyclic voltammetry (two cycles at 100 mV/s between +0.40 and -0.60 V *vs*. Ag/AgCl sat. KCl, under argon). After electrografting the modified electrode was abundantly rinsed with ethanol and deionized water to remove residual adsorbates and was subsequently dried under an argon flow.

2.4. Supported lipid deposits and immobilization of ionophores onto modified electrodes

The DMPC lipid deposit film was obtained by solvent evaporation on glassy carbon disk electrodes. 1 µL of DMPC in ethanol solution (5 mM) was deposited on the electrode surface with a micropipette. Ethanol was then evaporated under argon. After the complete evaporation of ethanol, the dry electrode was immediately dipped into the aqueous electrolyte.

Ionophores (valinomycin or nigericin) were each incorporated into a supported DMPC lipid deposit by incubation in a fresh electrolyte of 10 mM of phosphate buffer at pH 7 containing 5 mM of ionophore, for 1 hour at room temperature and under argon.

After incubation, the modified electrodes with the supported lipid deposit containing the ionophore are dipped into aqueous electrolyte (10 mM of ammonium acetate at pH 7) under argon and characterized by electrochemical measurements. The pH of the solution was changed and adjusted by addition of solutions of various concentrations of acetic acid or ammonia. In order to activate the ionophores immobilized in the lipid deposit, the electrode was dipped in a fresh ammonium acetate electrolyte containing 5 mM of potassium acetate.

After 5 minutes, electrochemical measurements were carried out to test the effect of potassium ions.

3 Results and Discussion

This section focuses on the characterization of the redox active films resulting from the oxidation of 5ASA or SA and their use as a pH or monovalent ion sensors to detect ionophore activity at a modified electrode / lipid deposit interface.

3.1. Redox active film from 5-aminosalicylic acid onto bare and modified electrodes as pH sensors

The active redox film from 5-aminosalicylic acid (5ASA, Figure 1) onto glassy carbon is formed according to the previous work of Eriksson and Nyholm [24]. Figure 2 shows consecutive cyclic voltammograms recorded in 5 mM 5ASA and 10 mM HCl aqueous solution (pH 2). A chemically reversible oxidation peak is detected at the formal potential ($E^{0'}$) $E^{0'}{}_1$ = +0.10 V *vs*. Ag/AgCl sat. KCl with a broad oxidative chemically irreversible system (oxidative peak potential, Ep_{ox}) at $Ep_{ox\ 2}$ = +0.60 V. Upon recurrent cycles, the irreversible oxidation peak current at $Ep_{ox}{}'_2$ decreases while that of the redox system at $E^{0'}{}_1$ increases. This observation is in agreement with the work reported by Eriksson and Nyholm [24]. The irreversible oxidation peak located at +0.60 V ($Ep_{ox\ 2}$) is assigned to the oxidation of the 5ASA monomer and the reversible redox signal observed at +0.10 V ($E^{0'}{}_1$) evidences the formation of a redox active film onto the glassy carbon electrode surface.

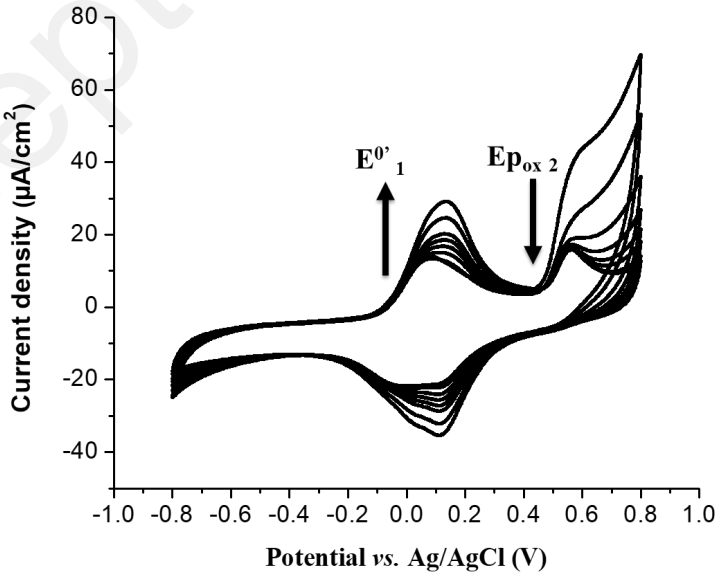

Figure 2: Consecutive cyclic voltammograms (8 scans) showing the electrodeposition of a

5ASA-based redox active film from 5 mM 5ASA and 10 mM HCl aqueous solution (pH 2) onto glassy carbon electrode. Scan rate: 100 mV/s.

5ASA possesses three pKa of 3, 6 and 13.9 respectively for the carboxylic acid, amine and hydroxyl groups. [28] As shown in Table S1 the amount and stability of the film obtained by oxidation is dependent on the pH conditions and hence on the nature of the acid/base groups in 5ASA. The film deposition is best performed at pH 2 where all functional groups are in the acidic form. At this pH the amount of film deposited is maximal (as evidenced by the estimated electrode surface coverage and the increase of the total impedance at low frequency) while displaying the highest stability upon consecutive cyclic voltammetry at pH 7 (See Table S1).

Following the same procedure, the formation of 5ASA redox film was studied on gold and ITO electrode surfaces (with the same geometrical surface area) and these more hydrophilic surfaces do not permit the electrodeposition of 5ASA-based films (Supporting Information, Figures S1 and S2). In contrast, the formation of 5ASA-based film can be efficiently carried out onto glassy carbon (Figure 2) and graphite electrodes (Supporting Information, Figure S3). It is well known that gold and indium doped tin oxide surfaces are more hydrophilic than carbon surfaces [29-31] and this property plays a crucial role in the formation of 5ASA-based redox films.

In order to confirm that the formation of 5ASA-based redox film is only possible onto hydrophobic electrodes, gold was modified with hydrophobic alkyl chains and glassy carbon with hydrophilic moieties. When a gold electrode is modified by hydrophobic species through the cathodic reduction of aryldiazonium generated in-situ from the corresponding aryl amine (4-decylaniline [32] or 4-bromoaniline [33]), the formation of a 5ASA-based film onto modified gold surface becomes possible (Supporting Information, Figures S4 and S5). In addition, a glassy carbon electrode modified by hydrophilic sulfonate functionalities resulting from the electrochemical reduction of aryldiazonium generated in-situ from 4-aminobenzenesulfonic acid [34] does not permit the electrodeposition of 5ASA on its surface (Supporting Information, Figure S6). These results demonstrate that a hydrophobic conductive surface is necessary for the efficient electrodeposition of 5ASA-based redox film onto electrode.

After the electrodeposition of 5ASA-based film (Figure 2), the modified glassy carbon electrode was transferred in a fresh potassium phosphate buffer aqueous solution at pH 7

(Figure 3) to evaluate the electrochemical properties of the immobilized redox active film by cyclic voltammetry.

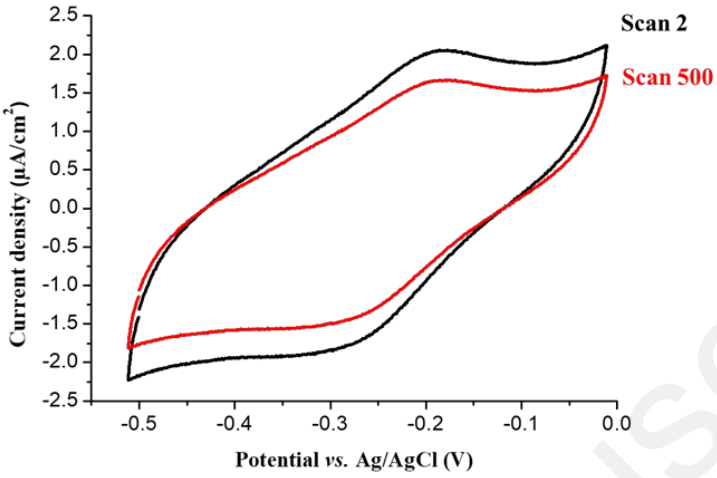

Figure 3: Cyclic voltammograms of 5ASA-based redox active film immobilized onto glassy carbon electrode after 2 (black) and 500 (red) consecutive scans in potassium phosphate buffer at pH 7. Scan rate: 5 mV/s.

In Figure 3, a chemically reversible redox system is observed at $E^{0'}$ = -0.23 V *vs*. Ag/AgCl corresponding to the immobilized 5ASA-based redox active film electrochemical response at pH 7. For 3 independent experiments and after 500 consecutive cycles recorded between -0.50 V and +0.00 V at 5 mV/s in 10 mM potassium phosphate buffer at pH 7 under argon and at room temperature (25°C), the faradic current of the immobilized redox system decreases only by about 20 ± 5% (Figure 3). This relatively low decrease of the faradic current of the 5ASA-based film after 500 cycles indicates that the modified electrode is stable enough to be used as a pH sensor as discussed next.

The formal potential of 5ASA-based film is pH sensitive (Figure 4A) and the modified electrode is an efficient pH sensor on a wide pH range (Figure 4B).

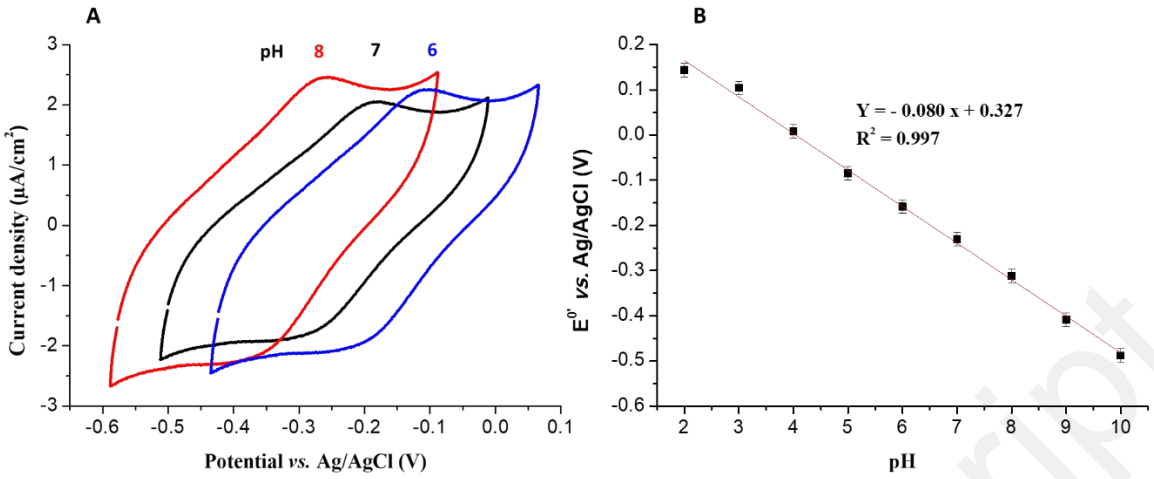

*Figure 4: **A**. Cyclic voltammograms of 5ASA-based film onto glassy carbon electrode in 10 mM KCl solution at pH 8 (red), 7 (black) and 6 (blue). Scan rate: 5 mV/s. **B**. Influence of pH on the formal potential of the 5ASA-based film in commercial buffered solutions.*

The variation of the formal potential of 5ASA-based film is linearly proportional to the pH of the solution (-80 mV per increase of pH unit) in the pH 2 to 10 range in buffered solutions (Figure 4B). Outside this pH range no redox signal is detected.

The experimental variation of 80 mV per pH unit is about 20 mV higher than the theoretical variation of 59 mV/pH unit for an even number of electron and protons exchanged [35-38]. This different proportion of protons and electrons can be explained by a mixture of different active quinoid sites in the film resulting from the oxidation of 5ASA.

The electrodeposited 5ASA-based redox active film is sensitive to pH changes, with variation of -80 mV per pH unit in the studied pH range (pH 2 to pH 10). The detection of various ions gradients by the formation of a redox active film from salicylic acid (SA) is discussed in the next section.

3.2. Redox active film from salicylic acid onto bare and modified electrodes as monovalent ions sensors

The electrochemical behavior of salicylic acid (SA, molecular structure in Figure 1) at a glassy carbon electrode was reported by Evans *et al.* [26] and Torreiro *et al.* [27]. Here we describe that a redox active film obtained from salicylic acid (SA) is a robust sensor of small monovalent ions.

Cyclic voltammograms presented in Figure 5 and recorded in phosphate buffer at pH 7 in the presence of salicylic acid, show an anodic redox signal at $Ep_{ox\ 1}$ = +0.26 V, an irreversible oxidation peak located at $Ep_{ox\ 2}$ = +0.70 V *vs*. Ag/AgCl sat. KCl and a cathodic redox signal at $Ep_{red\ 3}$ = -0.35 V. The irreversible oxidation peak current ($Ep_{ox\ 2}$) decreases while the redox signals at $Ep_{ox\ 1}$ and $Ep_{red\ 3}$ increases during consecutive cyclic voltammograms. Consistent with previous reports, [26-27] the system at $Ep_{ox\ 2}$ (+0.70 V) corresponds to the oxidation of the SA monomer and that at $Ep_{ox\ 1}$ (-0.26 V) is assigned to the formation of the electroactive film onto the electrode surface.

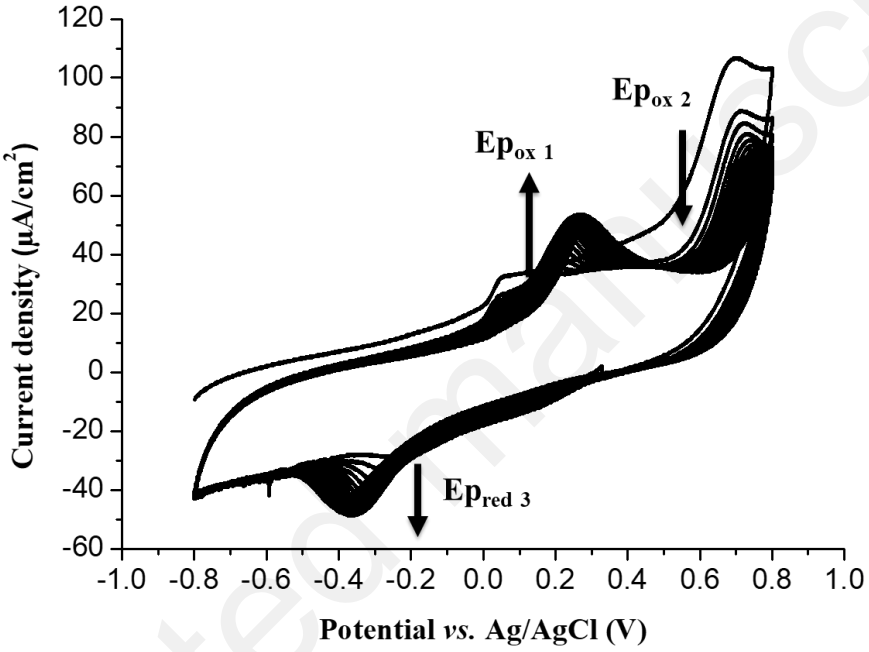

Figure 5: Consecutive cyclic voltammograms (20 scans) showing the formation of a SA-based redox active film from 1 mM SA in 10 mM phosphate buffer at pH 7 onto glassy carbon electrode. Scan rate: 100 mV/s.

Whatever the pH of the electrodeposition solution, an increase in the total impedance (Z) at low frequency (0.05 Hz, Table S2) is observed. This increase of Z is assigned to a deposit onto the electrode surface. Nevertheless, only solutions with pH in the range 4 to 7 lead to a redox active film. According to cyclic voltammetry and EIS of the electrodeposited SA-based redox active film, the maximal amount of deposited film is obtained at pH 4. This can be rationalized by the poor stability of SA in aqueous solutions, especially at pH above 5 [39-40] contrary to the greater stability of 5ASA at pH 2 to10 [25]. The most stable redox active film is however that obtained at pH 7 as shown by the relative retaining of the faradaic current after 500 consecutive cyclic voltammetry (Table S2). SA possesses two pKa of 2.97 and 13.4

respectively for the carboxylic acid and the hydroxyl groups [41]. Table S2 shows that the amount and stability of the film obtained by oxidation is dependent on the pH conditions and hence on the protonation of its acid/base functionalities. The film deposition is best performed at pH 4-7 where the carboxylic acid is in the carboxylate form. Although the mechanism for forming the active redox film onto electrode surface is not known, this illustrate the effect of the amino group in the respective structure and properties of the redox active films obtained from 5ASA and SA.

Like the formation of the 5ASA-based film, the SA-based redox active film can be formed only onto hydrophobic surfaces (Supporting Information, Figures S7 to S9).

After electrodeposition of a redox active film (Figure 5), the modified glassy carbon electrode was transferred into a fresh 10 mM ammonium acetate aqueous solution at pH 5 (Figure 6) to assess the electrochemical properties of the immobilized redox active film by cyclic voltammetry.

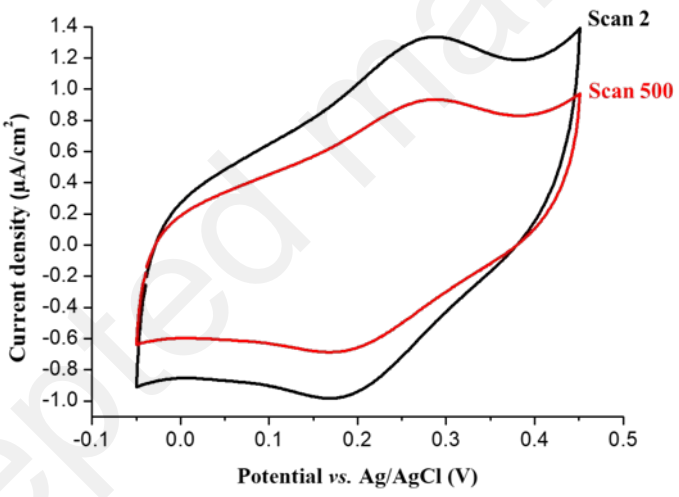

Figure 6: Cyclic voltammograms of SA-based redox active film immobilized onto glassy carbon electrode after 2 (black) and 500 (red) consecutive scans recorded in ammonium acetate at pH 5. Scan rate: 5 mV/s.

For 3 independent experiments and after 500 consecutive cyclic voltammograms recorded between -0.05 V and +0.45 V at 5 mV/s in 10 mM ammonium acetate at pH 5, under argon and at room temperature (25°C), the faradic current of the immobilized redox system decreases only about 30 ± 5% (Figure 6). This confirms the relative stability of the electroactivity of the SA-based electrode deposit and indicates that the functionalized electrode can be further used on longer time scale to measure small ions concentration (see

below).

In the cyclic voltammograms presented in Figure 6, the chemically reversible redox system located at the formal potential $E^{0'}$ = +0.23 V *vs*. Ag/AgCl corresponds to the immobilized SA-based redox active film electrochemical response at pH 5, in the absence of any monovalent ions in solution (*e.g.*: Na^+, K^+ or Cl^-). As shown in Table 1 and Figure 7, the formal potential of the SA-based redox active film shifts only in the presence of small monovalent ions (Na^+, K^+ or Cl^-). Divalent ions like magnesium (Mg^{2+}) or sulfate (SO_4^{2-}) have no effect on the formal potential of the film and, likewise, larger monovalent ions like acetate (CH_3COO^-) or ammonium (NH_4^+) do not induce any potential variation. Hence, ammonium and acetate were used as innocent ions in the electrolyte in subsequent experiments.

Salts	**ΔE (V) after 10 mM salt addition**
$K^+ Cl^-$	+0.01
$Na^+ Cl^-$	+0.01
$NH_4^+ Cl^-$	+0.11
$Na^+ NO_3^-$	-0.10
$K^+ NO_3^-$	-0.10
$Mg^{2+} SO_4^{2-}$	0.00
NH_4^+ CH_3COO^-	0.00
$K^+ CH_3COO^-$	-0.10
$Na^+ CH_3COO^-$	-0.10

Table 1: Influence of 10 mM salts on the formal potential of SA-based redox active film in unbuffered solution of 10 mM ammonium acetate at pH 5, under argon onto glassy carbon electrode (0.071cm²) at 25°C. The formal potential of SA-based redox active film in 10 mM ammonium acetate without additional salts is +0.23 V vs. Ag/AgCl.

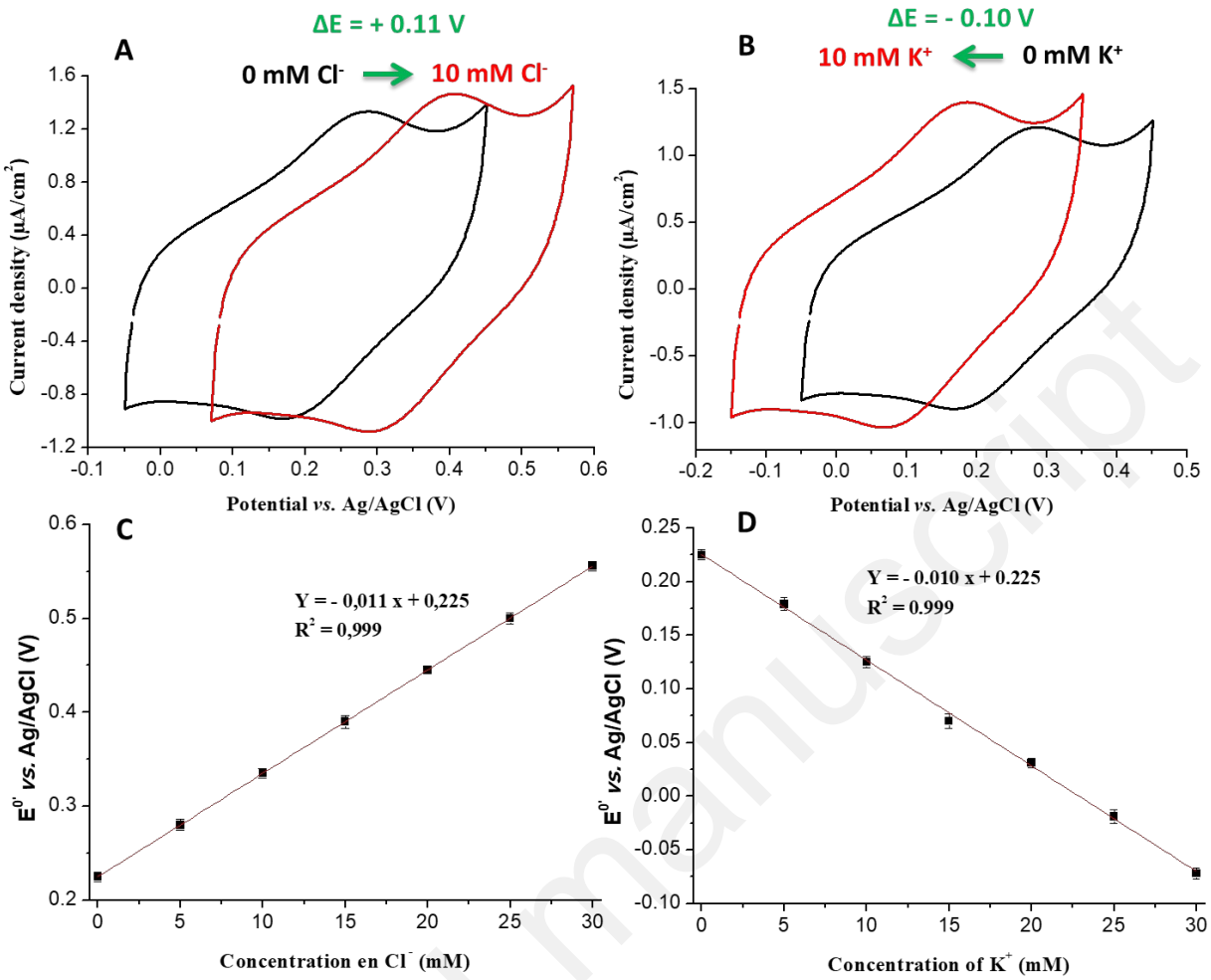

*Figure 7: **A and B**. Cyclic voltammograms of SA redox active film onto glassy carbon electrode before (black) and after adding 10 mM of monovalent ions (red) recorded in 10 mM ammonium acetate at pH 5. Scan rate: 5 mV/s. **C and D**. Influence of chloride (**C**) or potassium (**D**) ions concentration on the formal potential of the SA-based redox film in 10 mM ammonium acetate recorded at 100 mV/s onto glassy carbon electrode. 3 independent tests were used to obtain the standard deviations. Counter-ions were NH_4^+ for Cl^-, CH_3COO^- for K^+.*

The formal potential of the SA-based redox active film varies only with the presence of monovalent ions such as sodium, chloride and potassium. The potential shifts towards more positive values in the presence of an anion (*e.g.*: Cl^-) and to more negative values in the presence of a cation (*e.g.*: K^+). The variation of potential induced by a mixture of ions and cations in the same proportions is the sum of the variations induced by each ion, as is the case with sodium chloride and potassium chloride (Table 1). Note also that the SA-based redox film is insensitive to pH (Supporting Information, Figure S10).

The variation of the formal potential of the immobilized SA redox active film is linearly proportional to the ions concentration in the solution over the studied concentration range (from 0 to 30 mM). For chloride ions, the slope is 11 mV per 1 mM of chloride (Figure 7D), for potassium ions and sodium ions, the slope is -10 mV per 1 mM of cations (Figure 7C and Supporting Information, Figure S11). Outside this range, no potential change is detected. Although the structure of the SA-based film is not yet established, the linearity of the potential shift with small monovalent ions only can be ascribed hypothetically to a combination of a relatively weak interaction between the ions and the film [42,43] and to a competitive ion pairing with the larger ions of the electrolyte [44].

The potential variation of redox active films formed from salicylic acid can be used as small monovalent ions sensor. Hence the immobilized salicylic acid redox active film is a good candidate to detect Na^+, K^+ or Cl^- gradients induced by a ionophore incorporated into a supported lipid deposit.

The next section discusses the use of glassy carbon electrodes modified with 5ASA- and SA-based redox active films to detect the ionophore activity incorporated in supported lipid deposits.

3.3. Modified electrodes by 5-aminosalicylic- or salicylic acid-based redox active films to detect ionophores activity through supported lipid deposits

5-aminosalicylic-based redox active films can be used to monitor pH over a wide range (from pH 2 to 10) while salicylic acid-based redox active films can be used to detect sodium, potassium and chloride. These redox active films deposited onto electrodes can be useful to monitor the activity of ionophores integrated into an insulating supported lipid deposit as discussed now with two examples: valinomycin and nigericin.

First, the case of valinomycin is considered. Valinomycin is a selective potassium ion transporter in cell membranes and does not permit the exchange of protons through the ion channel [8,9]. Glassy carbon electrodes were then modified with redox active film as described above and covered with a DMPC lipid deposit (see Experimental Section).

As shown in Figure 8, after deposition of the lipids (DMPC) by solvent evaporation onto the redox film modified electrodes, the redox signals of either 5ASA- or SA-based redox active films (Figures 8.1) are no longer visible in cyclic voltammetry experiments (Figures 8.2).

The loss of the electroactivity of the electrodeposited films in the cyclic voltammograms is

assigned to the insulating property of the lipid deposit.

The incorporation of valinomycin into the supported lipid deposit (see Experimental Section) does not restore the detection of the redox films electroactivity as shown in Figure 8.3. Hence, in the absence of potassium ions, the integration of the ionophore into the supported lipids does not compromise the insulating properties of the deposit. Addition of potassium ion in the electrolyte activates the ion channel and the redox activity of the film is recovered although with slightly less current density (Figure 8.4). The recovering of the redox signal indicates that an ion flux is established across the lipid deposit through active valinomycin ionophores which is fully consistent with previous reports [45-48]. The detection of the redox activity of the films is directly related to both the presence of ionophores into the lipid deposit and to the addition of K^+ in the electrolyte. In the absence of ionophores in the lipid deposit, the addition of K^+ in the electrolyte does not lead to the recovery of the film redox activity (See Figure S12). The electroactivity of the 5ASA-modified electrode (Figure 8A Left and Table 2) is recovered at the same potential since this redox film has been shown to be sensitive to H^+ only and not to K^+. Consistently, the electroactivity of the SA-modified electrode shifts in the presence of K^+ as this redox active film is sensitive to potassium ion (Figure 8B Right and Table 2).

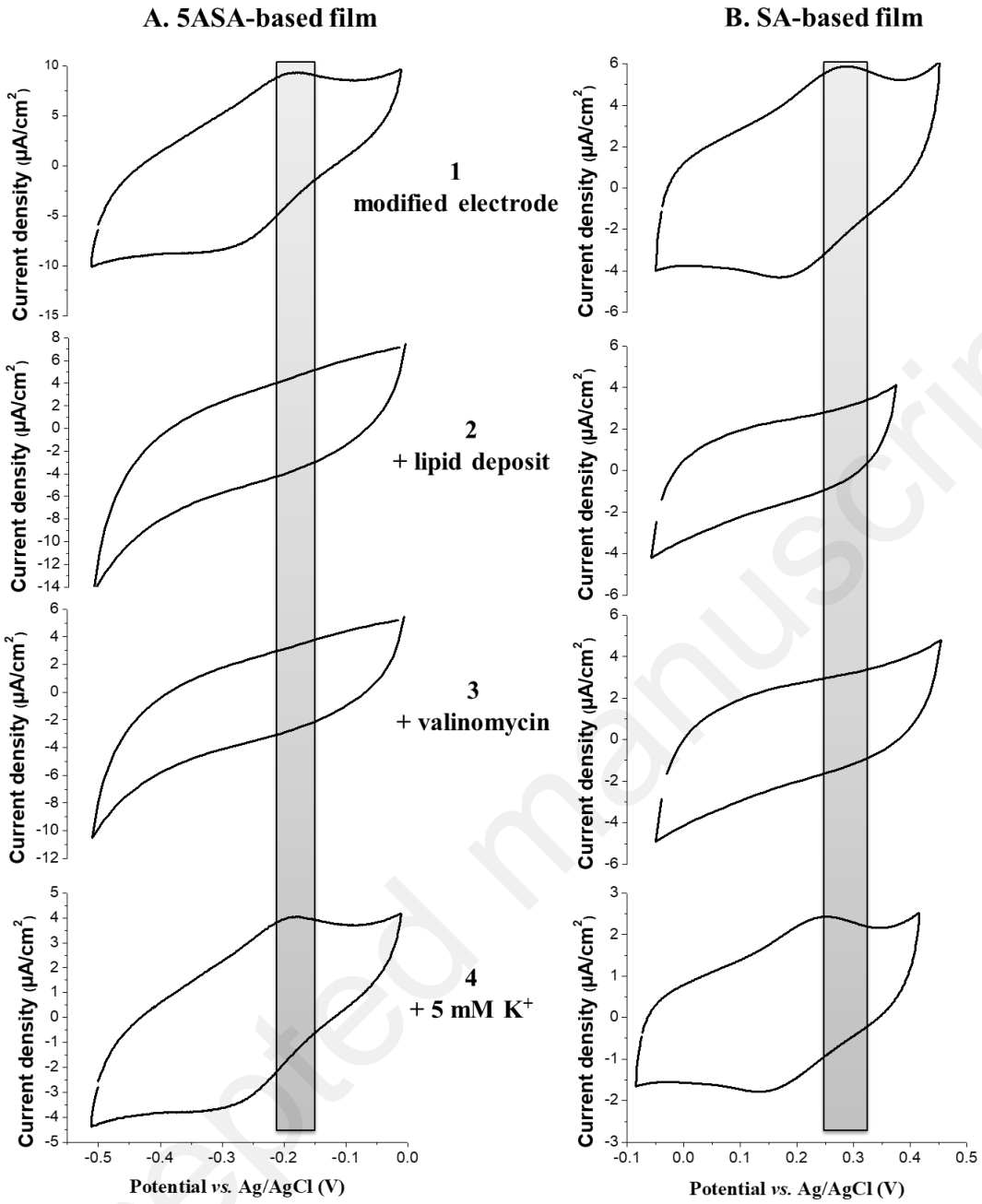

*Figure 8: Experiments showing the study of (**A**) 5ASA- and (**B**) SA-modified electrodes (**1**) in 10 mM ammonium acetate at pH 7 at 100 mV/s under argon and room temperature (20°C). (**2**): After DMPC lipid deposition onto modified electrode. (**3**): After addition of valinomycin in the supported DMPC lipid deposit onto the modified electrode. (**4**): After addition of 5 mM potassium acetate in solution. The grey stripes are indicative of the redox potentials and serve as a guide to the eye.*

Redox active film and conditions	Formal potential	Corresponding pH	Corresponding K^+ concentration
5ASA, 0 mM K^+	-0.23 ± 0.01 V	7.00 ± 0.10	-
5ASA, 5 mM K^+	-0.23 ± 0.01 V	7.00 ± 0.10	-
SA, 0 mM K^+	+0.23 ± 0.00 V	-	0.00 ± 0.00 mM
SA, 5 mM K^+	+0.20 ± 0.01 V	-	3.00 ± 1.00 mM

Table 2: Effect of valinomycin activation on the formal potential of redox active films, pH and potassium ion concentration. All measurements were performed 3 times in 10 mM ammonium acetate at pH 7 at 100 mV/s under argon and room temperature (20°C).

Finally, nigericin, a potassium ion / proton antiporter was also tested through the same procedure with electrodes modified with either of the redox active films [6-7]. In this case, upon potassium ion activation of the nigericin K^+ / H^+ ions antiporter, the increase of the concentration of potassium ion at the electrode surface induces a cathodic shift of the formal potential of the electrodeposited SA-based redox active film. For the 5ASA-based redox active film sensitive to protons, an anodic shift is excepted as the activation of the antiporter by potassium ion will lead to a basification of the electrode/lipid layer interface concurrent to an increase of the surface potassium ion concentration. These expected electrochemical behaviors are illustrated in Figure 9 and Table 3.

Tables 3 and 4 compile the formal potential of the redox active films in the conditions of Figures 8 and 9, and an estimate of the proton or potassium ion surface concentration calculated from the respective calibration curves (Figures 4B and 7D).

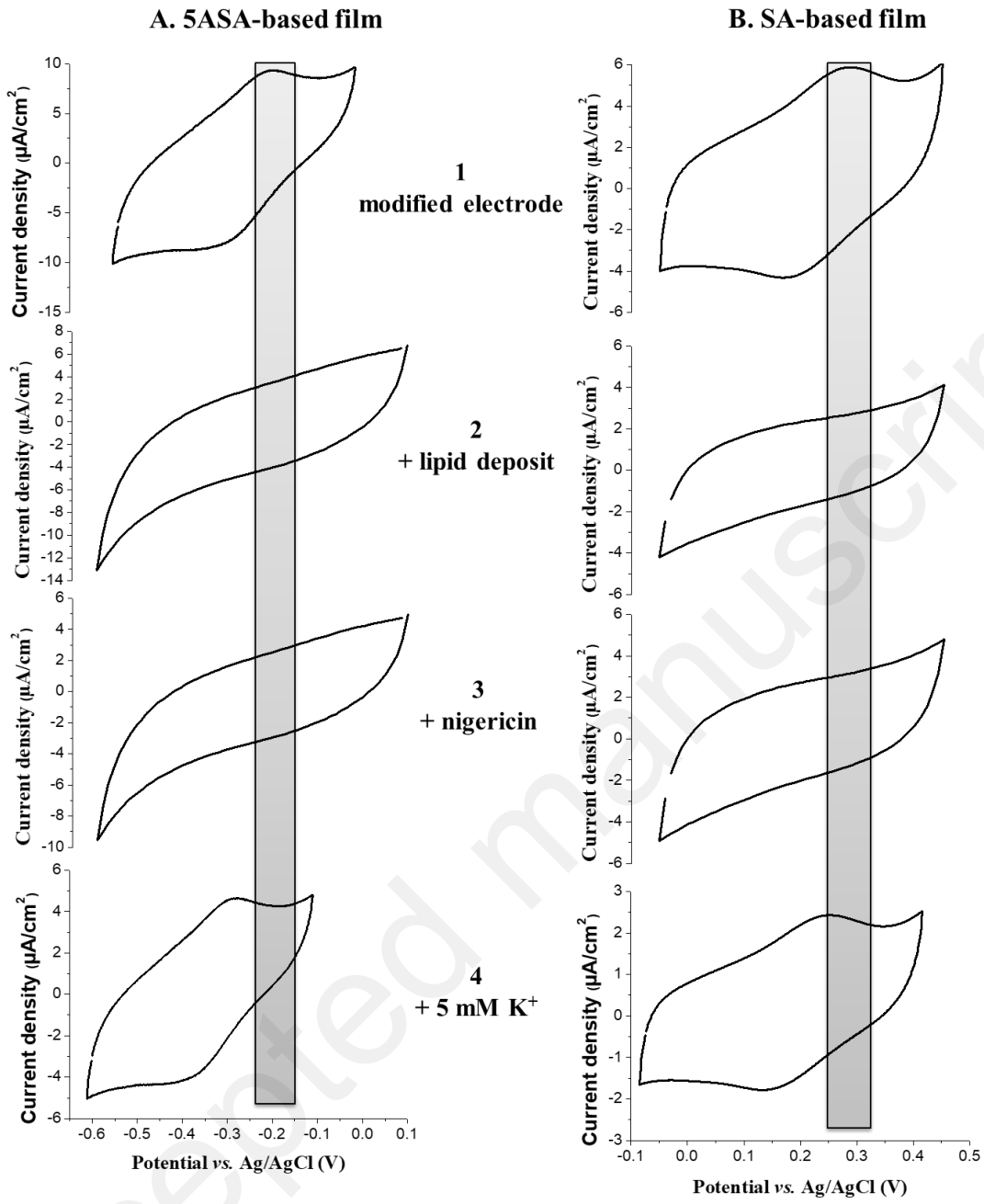

*Figure 9: Experiments showing the study of (**A**) 5ASA- and (**B**) SA-modified electrodes (**1**) in 10 mM ammonium acetate at pH 7 at 100 mV/s under argon and room temperature (20°C). (**2**): After DMPC lipid deposition onto modified electrode. (**3**): After addition of nigericin in the supported DMPC lipid deposit onto the modified electrode. (**4**): After addition of 5 mM potassium acetate in solution. The grey stripes are indicative of the redox potentials and serve as a guide to the eye.*

Redox active film and conditions	Formal potential	Corresponding pH	Corresponding K^+ concentration
5ASA, 0 mM K^+	-0.23 ± 0.01 V	7.00 ± 0.10	-
5ASA, 5 mM K^+	-0.34 ± 0.02 V	8.30 ± 0.20	-
SA, 0 mM K^+	+0.23 ± 0.00 V	-	0.00 ± 0.00 mM
SA, 5 mM K^+	+0.20 ± 0.01 V	-	3.00 ± 1.00 mM

Table 3: Effect of nigericin activation on the formal potential of redox active films, pH and potassium ion concentration. All measurements were performed 3 times independently in 10 mM ammonium acetate at pH 7 at 100 mV/s under argon and room temperature (20°C).

4. Conclusion

The electrodeposition of a redox active film from 5-aminosalicylic acid is possible only onto hydrophobic electrode surfaces and permits the formation of a robust pH-sensitive redox active film. The variation of the formal potential as a function of pH is linear over the entire pH range studied (from pH 2 to pH 10) in buffered and unbuffered solutions, with a slope -80 mV per pH unit.

Likewise, with salicylic acid, the electrodeposition of a robust redox active film is possible only onto hydrophobic electrode surfaces. This film can be used to detect and determine the concentration of monovalent sodium, potassium (with a slope of -10 mV per 1 mM of cations) and chloride ions (with a slope of 11 mV per 1 mM of anions).

Electrodes with these electrodeposited redox active films were modified by a DMPC lipid deposit to incorporate the ionophores valinomycin (K^+ transporter) and nigericin (K^+ / H^+ ions antiporter). The immobilized ionophores were studied in ammonium acetate 10 mM electrolyte at pH 7 and activated by addition of 5 mM potassium acetate. The study demonstrates that the electrodeposited redox active films not only allow the detection of the activation of the ionophores but also permit an estimation of the surface concentration of small ions (here proton and potassium ions). For a more practical implementation of these ion sensors, potentiometry rather than cyclic voltammetry measurements could easily be carried out.

Further studies will be devoted to other ionic transporters like protein antiporters integrated into controlled lipid deposit supported onto electrodes.

The different redox potentials of the two electrodeposited films discussed here (-0.23 V for 5ASA and +0.23 V for SA both in 10 mM of ammonium acetate at pH 7) open the way to a co-deposition on a single electrode with multi-sensing properties.

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6. References

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