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# Ion-exchange voltammetry of dopamine at Nafion-coated glassy carbon electrodes: Quantitative features of ion-exchange partition and reassessment on the oxidation mechanism of dopamine in the presence of excess ascorbic acid

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**Abstract**

The incorporation/exclusion features of dopamine (DA), ascorbic acid (AA) and uric acid (UA) are evaluated for Nafion (NA)-coated glassy carbon electrodes (GCE) of different thicknesses.

The ion-exchange partition of $DA^+$ between the NA film and the sodium phosphate electrolyte is evaluated by determining the partition coefficient ($k_D$) and the apparent diffusion coefficient ($D_{app}$) in thick NA films which were 401 and $1.5\times10^{-9}$ cm$^2$ s$^{-1}$, respectively. The solution diffusion coefficient was found to be $6.0\times10^{-6}$ cm$^2$ s$^{-1}$.

Also, the effect of NA loading and of the voltammetric timescale on DA voltammetry in the presence of excess AA is assessed, at physiologic like conditions. It is demonstrated that, although AA is excluded at the NA coating, a catalytic regeneration of DA, induced by AA, occurs at the interface NA film/electrolyte resulting from the diffusion of the *o*-quinone product of DA oxidation from the electrode surface to that interface. The interference of AA in the voltammetric signal of DA is eliminated using 18 μg mm$^{-2}$ NA films and $v\geq0.5$ V s$^{-1}$. Therefore, fast, selective and sensitive voltammetric analysis of DA at concentrations $<100$ μM in the presence of excess AA, e.g., 1 mM is achieved.


*Keywords:* Ion-exchange voltammetry; Nafion; Dopamine oxidation; Partition coefficient; Ascorbic acid; Uric acid

## 1. Introduction

The analytical determination of catecholamines, namely dopamine (DA), in biological fluids is still getting considerable attention and voltammetric determination methods using modified electrodes can provide reliable, sensitive and fast analytical procedures. Several approaches have been tested ranging from clay-modified electrodes [1,2], self-assembled monolayers of transition metal–macrocyclic complexes [3] carbon nanotube films, [4], biosensors [5] and polymer films [2,6–9]. Modification with the cation-exchanger polymer Nafion (NA) affords coatings that are chemically stable, present adequate antifouling ability towards surfactants and, at physiologic pH, effectively incorporate dopamine (DA), excluding anionic interfering species like ascorbate (AA) or urate anions (UA) [6,8,10]. The common preparation procedure of NA films at electrode surfaces involves solvent evaporation techniques, either single drop evaporation [2,6] or spin coating [1,8].

Ascorbate is a major component of the extracellular fluid of the brain tissue and is present at the $10^{-4}$ to $10^{-3}$ M concentration level, highly above that of DA, usually within the micromolar concentration range [11,12]. The presence of AA complicates the determination of DA at carbon based electrodes because their oxidation signals are usually superimposed due to the relatively low displacement of the corresponding peak potentials. In fact, the electrochemical oxidation of AA to dehydroascorbate is an irreversible process and usually proceeds at potentials more positive than the expected formal potential ($E'=-0.016$ V vs. Ag/AgCl sat. [13]. In homogeneous solution, AA can reduce the DA oxidation product, an *o*-quinone species, back to DA resulting in a homogeneous catalytic process that is easily monitored via cyclic

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voltammetry [14]. Therefore, the occurrence of this process precludes a proper analytical quantification of DA by voltammetry. The use of very fast scan rates (e.g., 300 V/s) prevents the occurrence of the AA mediated catalytic cycle but this approach is feasible only for voltammetry at carbon fibre microelectrodes [12].

The use of anionic ionomer-coated electrodes provides a way to increase the selectivity in the detection of DA, due to discrimination towards negatively charged species, such as the anionic forms of ascorbic acid or uric acid. NA-coated glassy carbon electrodes are an example of such modified electrodes that have been tested either with conventional sized electrodes [6,8] or with carbon fibre micro-electrodes for in vivo determinations [10,11,15,16]. Nevertheless, the occurrence of enhanced voltammetric signals for DA (for concentrations ca. 100 μM) in the presence of 5 fold excess AA at NA-coated carbon fibre electrodes, observable at scan rates of 0.05 V $s^{-1}$ has been reported, showing that the AA mediated catalytic cycle is taking place [4]. On the other hand, the use of conventional sized glassy carbon electrodes in amperometric/voltammetric detection of DA and other neurotransmitters for flowing streams is still engaging attention. For glassy carbon coated with relatively thick films of NA, the above mentioned catalytic cycle was not reported to occur [6,8]. However, a clear evaluation of the effect of the NA film thickness was not advanced. Additionally, a marked decrease in the sensitivity of the DA determination, compared to un-coated electrodes was reported [8]. Therefore, for applications where conventional size carbon electrodes are used, the evaluation of the simultaneous effect of the loading (and thickness) of the NA coatings and of the voltammetric timescale on the incorporation and on the voltammetric response of DA in the presence of excess AA has not been reported. Additionally, data on the ion-exchange incorporation features of DA into NA films are scarce.

The present study aims at giving a contribution to the understanding of the effect of the loading of NA coatings on conventional sized glassy carbon electrodes and, simultaneously, of the scan rate used for linear scan voltammetric determination of DA in the presence of AA, at physiologic like conditions (i.e., at pH 7.4 and usual DA and AA concentration levels). Additional information regarding the exclusion of uric acid anion is also provided. Further, data on the incorporation, partition and transport of dopamine within the present NA films is provided.

## 2. Experimental

### 2.1. Apparatus and reagents

Nafion perfluorinated ion-exchange resin, NA (5 wt.% solution in a mixture of lower aliphatic alcohols and water) was purchased from Sigma-Aldrich and used as received. All chemicals were of analytical reagent grade and all solutions were prepared with ultra-pure water (18.2 MΩ cm, Milli-Q systems, Millipore-waters). Stock solutions of NA (11.1 or 39.7 mM in monomer units) were prepared in ethanol and stored at 4 °C. Phosphate buffer solution (0.022 M $NaH_2PO_4$/0.041 M $Na_2HPO_4$, pH 7.4, ionic strength $0.12_4$ M) was used as the supporting electrolyte for voltammetry. Dopamine standard solutions (approximately 0.5000 or 5.000 mM), L(+)-ascorbic acid and uric acid (both ca. 1.000 mM) were prepared in phosphate buffer. Biohit Proline pipettes equipped with disposable tips were used for appropriate dilutions. All voltammetric measurements were performed with a BAS 100B/W electrochemical analyser (Bioanalytical Systems) connected to a Cell Stand BAS-C2. The working electrode was a glassy carbon disc (BAS, MF-2012; 3 mm diameter), the auxiliary electrode was a Pt wire and the reference electrode was Ag/AgCl (sat. KCl). A combined glass electrode (Orion 9104SC) connected to a pH meter (Cole Parmer, Model 05669-20) was used for pH measurements.

### 2.2. Electrode preparation

Prior to coating, the glassy carbon electrode (GCE) was conditioned following a reported polishing/cleaning procedure [17]. The NA coatings on the GCE were obtained by the droplet evaporation method, by application of 3.0 μL of the NA polymeric solution directly on the electrode surface and evaporating the solvent under a low flow warm air stream (ca. 60 °C). The estimated thickness of the NA films was 2.6 and 9.2 μm, corresponding to mass loadings of 5.1 and 18 μg $mm^{-2}$. The film thickness was estimated using the recast density of 1.98 g $cm^{-3}$ [18]. The electrochemically active surface area of the glassy carbon electrode, (7.192±0.065) $mm^2$, was measured by chronoamperometry (in $9.854\times10^{-4}$ M ferricyanide/1.0 M KCl solution; 3 polishing experiments, each one with 4 replicate determinations).

### 2.3. Voltammetric procedures

Linear scan voltammetry (LSV) was used for the examination of the analytical performance of the NA-coated GCE towards DA determination. All peak currents quoted are mean values of 3 replicate measurements. All solutions were purged with nitrogen for 5 min prior to the voltammetric experiments. Cyclic voltammetry was also used for the examination of the incorporation features of DA into the NA-coated GCE. All voltammetric experiments were carried out in 10 mL of pH 7.4 sodium phosphate buffer solution.

The determination of DA concentration incorporated in the NA film was done by measuring the electric charge due to the oxidation of DA by exhaustive electrolysis experiments conducted at a constant potential of +0.6 V, for ca. 1 h. (i.e., until the electrolysis current reached 0.05% of its initial value). The NA films were initially equilibrated in an unstirred DA solution (in pH 7.4 phosphate buffer, ionic strength $0.12_4$ M) for an equilibration time ranging from 25 min for DA solution concentrations above $10^{-5}$ M and for 24 h for the lower concentrations. Then, the electrode was removed from the equilibration solution, briefly rinsed with pure water and placed in the electrochemical cell containing merely the supporting electrolyte (a diluted pH 7.4 phosphate buffer, ionic strength 0.025 M, no added DA). The charges for DA were all corrected from blank values obtained by exhaustive electrolysis experiments conducted at the same

experimental conditions. The same NA film was re-used for successive equilibration experiments with DA solutions of different concentrations.

All measurements were carried out at room temperature (18–20 °C).

## 3. Results and discussion

### *3.1. Voltammetry of dopamine, uric acid and ascorbic acid on NA-coated GCE of different NA thickness*

At the working pH of 7.4, L(+)-ascorbic acid ($pK_a$=4.10) and uric acid ($pK_a$=5.4) are both anionic, and dopamine ($pK_b$=8.87) is cationic. All species are electroactive at the glassy carbon electrode within the potential window [−0.2 V; +1 V]. Fig. 1 shows typical cyclic voltammograms of these species at the GCE and at a NA-modified GCE. The NA-coated electrodes are charge selective, regardless of the mass loading used (5.1 and 18 μg mm$^{-2}$), i.e., ascorbate and urate anions are totally excluded and dopamine cation is incorporated into the negatively charged NA film via ion-exchange with the polymer $Na^+$ counter-ions. In fact, for both NA modified electrodes the oxidation peak current for DA had an increase of 4 to 6 fold relative to the bare GCE (depending on scan rate and DA concentration). This data shows that an effective incorporation of DA exists irrespectively of the NA film thickness used. In fact, it could be expected that a higher content of sulfonate cation-exchange groups (i.e., for the 18 μg mm$^{-2}$ NA electrode, thickness 9.2 μm) would lead to an improvement of the incorporation of the positively charged DA. It must be highlighted that Nafion is an ionomer with a rather low content of ion-exchange groups [19–21]. This confines the usable thickness of NA films, i.e., in order to have suitable ion-exchange features, the molar loading (and thickness) has to be rather high to assure a proper content of sulfonate groups. Obviously, this may cause mass transport limitations, depending mainly on the size of the analyte. In the case of dopamine, the mass transport limitations eventually occurring for thicker films, associated to its low positive charge, can lead to a decreased sensitivity in the voltammetric determinations. Though, for DA, these disadvantages may be counterbalanced by the hydrophobic nature of this species and by the known affinity of NA for hydrophobic cations [21,22]. These aspects will be evaluated in the present work.

The key question in the utilization of NA-coated GCE as an analytical tool for the determination of DA is how to obtain good selectivity towards negatively charged interfering species, keeping an adequate sensitivity. Most applications use relatively thick NA coatings which lead to sensitivity decreases, compared to a bare GCE, due to difficult mass transport of DA throughout the thick layer.

For the present NA coatings, simply prepared by droplet evaporation (no added casting solvents), the selectivity is very good (cf. Fig. 1). The sensitivity towards DA was evaluated for the thicker (9.2 μm) NA-coated electrode from the calibration curve data of the direct LSV determination of DA at $v$=0.2 V s$^{-1}$. Within the DA concentration range 10.0 to 100 μM the slope was 0.158±0.007 A mol$^{-1}$ dm$^3$ ($r$=0.999, $N$=6). The

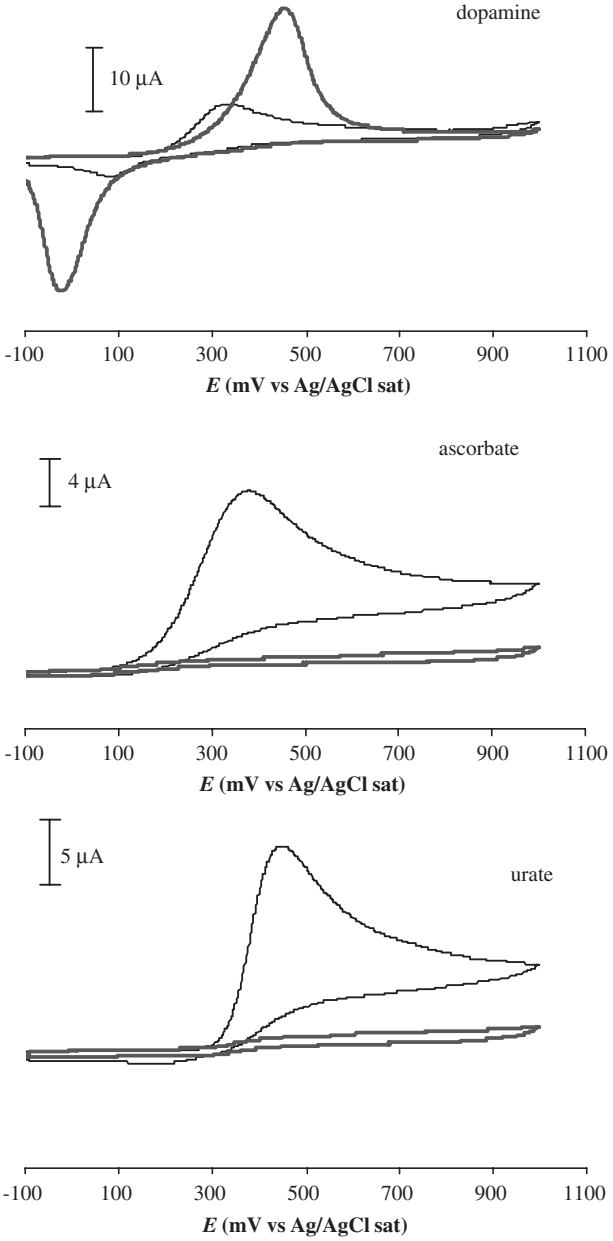



Fig. 1. Cyclic voltammograms illustrating the incorporation of dopamine and the exclusion of ascorbate and of urate anions at the 5.1 μg mm$^{-2}$ (2.6 μm) NA-coated GCE (thick lines) compared to the bare GCE (thin lines). Concentration of species (mM): dopamine, 0.4870; ascorbate, 1.050 and urate, 1.050. Supporting electrolyte: pH 7.4 sodium phosphate buffer. Scan rate 0.05 V s$^{-1}$.

limit of detection was 3 μM (3 $\sigma$). For the same experimental conditions, the calibration curve data for DA at the bare GCE was: slope 0.029±0.002 A mol$^{-1}$ dm$^3$ ($r$=0.999, $N$=6 and limit of detection 5 μM (3 $\sigma$). These data show that the 9.2 μm NA-coating allows an effective incorporation and a rather uncomplicated mass transport of DA, resulting in a 5 fold increase of the sensitivity of its analytical determination, compared to the un-coated GCE. The limit of detection obtained for the NA-coated GCE did not reflect the increase in sensitivity and this might be related to noise problems related to the complex NA matrix. The RSD values varied between 1% and 5% for determinations with both electrodes. Hence, it may be concluded

that a single NA-coated electrode can be consecutively used for the DA detection with a good performance, i.e., with good repeatability, expressed by relatively low RSD values.

The calibration experiments were performed also in the presence of excess amounts of urate and ascorbate anions, under the same experimental conditions. The calibration curves for DA in the presence of 1 mM UA revealed no statistically noticeable differences compared to those in the absence of urate. In fact, in a mixed solution of DA and UA, the urate anion is totally excluded by the NA-coated electrode and Fig. 2 gives a typical example of that feature. In fact, at the bare GCE (Fig. 2A) there was a marked interference of UA in the DA oxidation peak (which will get worse in physiological conditions where the concentration of DA is usually lower that that used in Fig. 2) whereas at the NA-coated GCE (2.6 μm) the DA oxidation peak current remained unaltered in the presence of UA (Fig. 2B).

However, when AA was present in the voltammetric cell (with a concentration 0.554 mM), major alterations were noticed. Fig. 3A shows that for 50 μM DA and $v=0.05$ V $s^{-1}$ a significant increment (ca. +66%) in the DA oxidation signal occurred comparing with that of DA alone associated to a pronounced decay of the corresponding reduction peak (ca. 70%). Diminishing the scan rate to 0.025 V $s^{-1}$ leads to a slight increase of the DA oxidation current and to a large decrease (ca. 8×) in the current of the reduction of the *o*-quinone species (Fig. 3B). These features are indicative of the occurrence of a regenerative process, certainly induced by AA, where the reactant of the electrochemical reaction is produced at the expense of the chemically consumption of the product of that reaction (*o*-quinone species, in the present case) which, obviously cannot give a reduction signal. For the initial timescale ($v=0.05$ V $s^{-1}$) the decrease of the DA concentration to 10 μM originated a voltammogram typical of a catalytic process where the DA oxidation current changes to a wave-shape profile (Fig. 3C). In this case there was a two fold increase in the oxidation current compared to the situation in the absence of AA. Interestingly, keeping all other experimental conditions constant, the regenative process induced by AA is more effective for lower concentrations of DA in solution, i.e., for higher excess concentrations of ascorbate relative to DA (cf. curves b vs. a in Fig. 3A and C). This is indicative of the marked dependence of the catalytic current on the actual excess concentration of ascorbate at the polymer/solution interface. Nevertheless, the overall process will also depend on the mass transport/ionic exchange features of the intervenient species within the NA layer. One must have in mind that while the chemical regenerative process occurs at the NA/electrolyte solution interface, the voltammetric signals are due to the heterogeneous reaction at the GCE surface. Obviously, the catalytic reaction of ascorbate with the dopamine-*o*-quinone product will occur whenever the *o*-quinone product has time to diffuse from the electrode surface to that interface. This will be the case at the present experimental conditions (i.e., scan rate ≤ 0.050 V $s^{-1}$ and relatively high excess of AA).

Additionally, Fig. 3D shows that an increase of the thickness of the Nafion coating from 2.6 to 9.2 μm induces a dramatic change in the voltammogram of DA in the presence of AA. Now, the catalytic cycle is occurring at a lesser extent: the oxidation peak current of DA decayed 75% and the reduction peak of the *o*-quinone increased and is closer to the value obtained in the absence of AA (in fact, the relative difference is ca. −15%). Therefore, although a thicker NA coating contains more ion-exchange groups, the prevailing effect was the actual increase of the length for the mass transport of the intervenient species in the regeneration cycle, especially of the *o*-quinone produced at the electrode surface towards the NA/electrolyte solution interface. If no *o*-quinone arrives at the NA/electrolyte interface within the experimental timescale then no catalytic

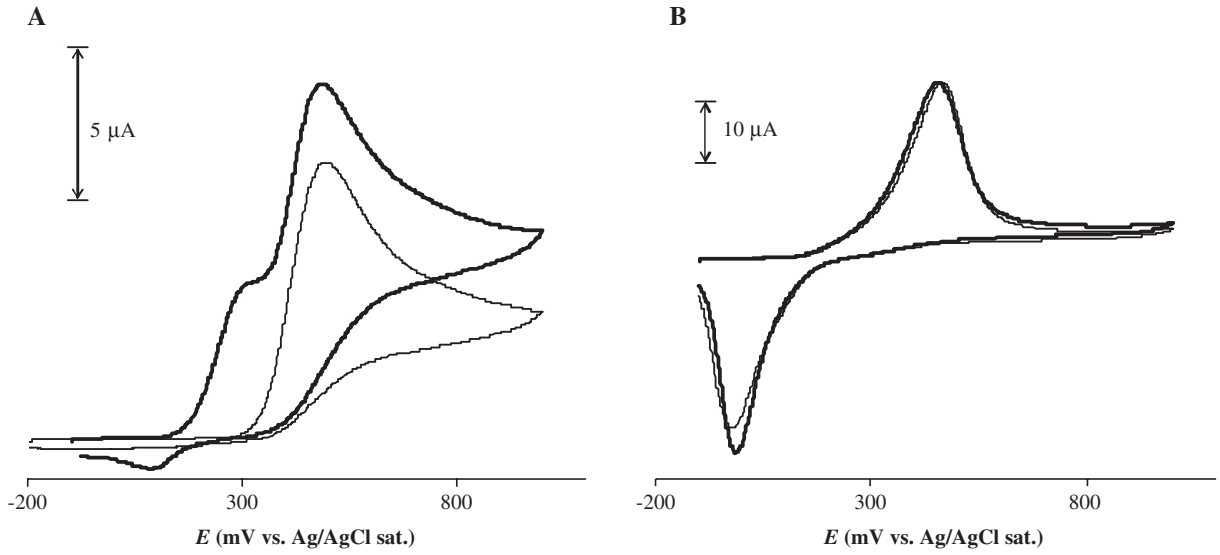



Fig. 2. Cyclic voltammograms illustrating the effect of the urate anion (1.000 mM), on the voltammetric features of the dopamine. (A) Bare GCE where the thin line corresponds to UA alone and the thick line to the mixture of UA+DA; [DA]=500 μM. (B) NA-coated GCE electrode (thickness 2.6 μm) GCE where the thin line corresponds to DA alone and the thick line to the mixture of UA+DA; [DA]=490 μM. Supporting electrolyte: pH 7.4 sodium phosphate buffer. Scan rate 0.05 V $s^{-1}$.

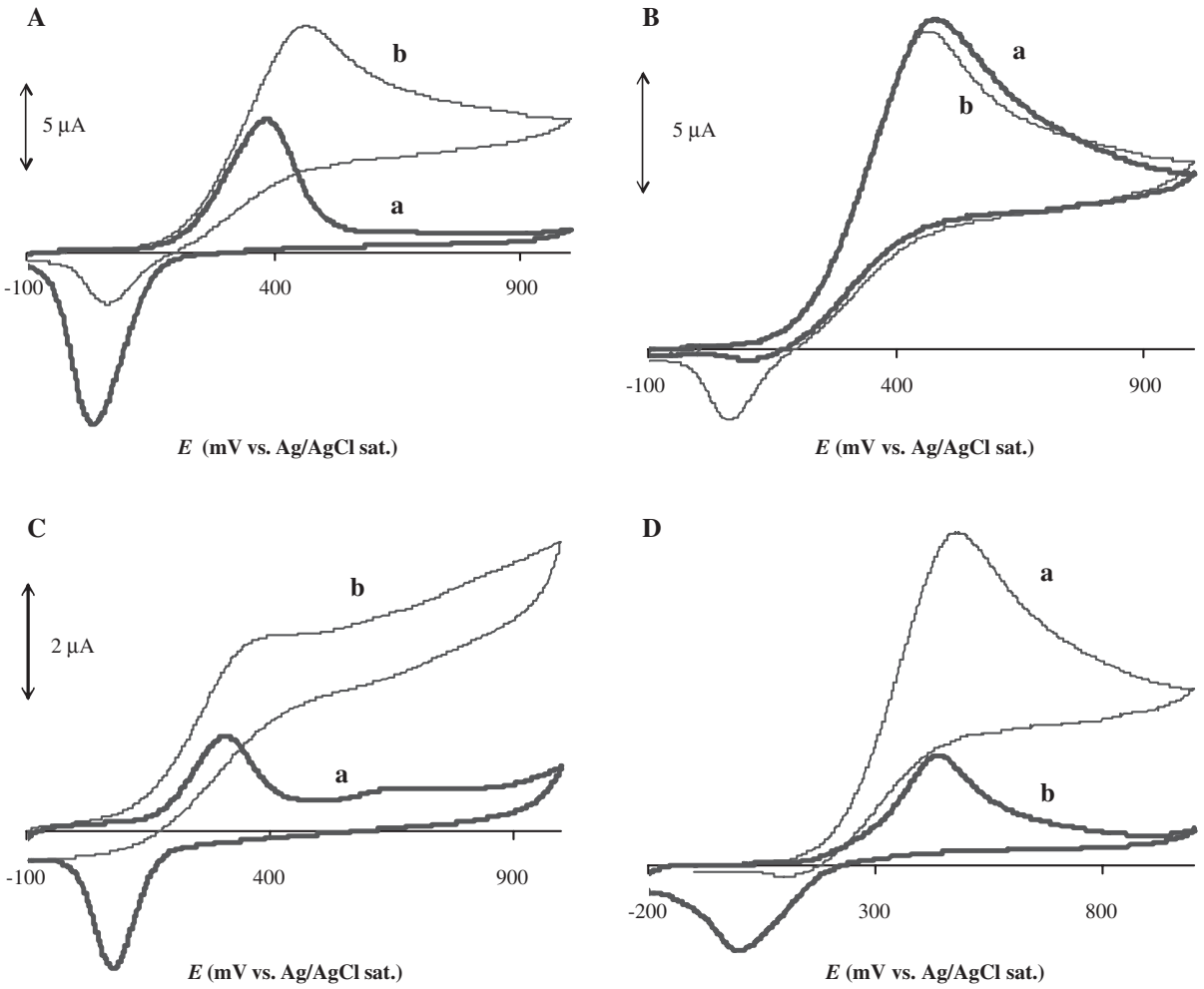



Fig. 3. Cyclic voltammograms illustrating the effect of the ascorbate anion (0.554 mM), on the voltammetric features of dopamine at NA-coated GCE electrodes at different timescales, different DA concentrations and for two NA thicknesses (A, B, C: 2.6 μm and D: 2.6 vs. 9.2 μm). A: [DA]=50.0 μM, $v$=0.05 V $s^{-1}$, in the absence (a) and in the presence (b) of ascorbate. B: [DA]=50.0 μM in the presence of ascorbate, $v$ (V $s^{-1}$)=0.025 (a) and 0.05 (b). C: [DA]=10.0 μM, $v$=0.05 V $s^{-1}$, in the absence (a) and in the presence (b) of ascorbate. D: [DA]=50.0 μM, $v$=0.025 V $s^{-1}$, at NA-coated electrodes of thickness 2.6 μm (a) and 9.2 μm (b). Supporting electrolyte: pH 7.4 sodium phosphate buffer.

process will occur and the voltammogram will be close to the expected for a solution of DA alone.

### 3.2. Overcoming the interference of ascorbic acid

In accordance to the results presented in the previous section, the DA voltammetric features are strongly dependent on the voltammetric timescale, i.e., on the scan rate, as well as on the thickness of the NA coating. Therefore, the effect of the scan rate on the voltammetry of DA was further examined for the GCE modified with the thicker NA film (9.2 μm). Fig. 4 display the overall behaviour in terms of the peak current for DA oxidation, in the scan rate interval 0.015 to 1 V $s^{-1}$, for DA alone and in the presence of an excess of AA ([AA]/[DA]=5). At 0.5 V $s^{-1}$ the voltammograms overlay, meaning that the AA induced catalytic regeneration of DA is now completely hindered. As shown from data in Fig. 3, the effect of AA was a function of the relative excess of ascorbate anion: in fact, if the concentration ratio [AA]/[DA] is made <1, there were no observable effects on the DA voltammograms, regardless of the DA concentration, NA thickness or scan rate.

All these observations are consistent with the reaction pathway displayed in Scheme 1, where the diffusion of the *o*-quinone oxidation product throughout the NA film shall be rate determining. In fact, due to the excess of AA at the NA film/electrolyte solution interface, the regeneration reaction may be assumed as a pseudo-first order reaction (catalytic EC reaction [23]) whose rate shall be dependent on the concentration of the dopamine-*o*-quinone arriving at the interface by diffusion from the electrode surface.

Therefore, for voltammetric determinations of DA (concentrations below 100 μM) in ascorbate containing medium, the use of a NA-coatings on GCE with a mass loading 18 μg $mm^{-2}$ (thickness of 9.2 μm) and LSV scan rates ≥ 0.5 V $s^{-1}$ preclude the occurrence of the AA induced catalytic reaction. Yet, the oxidation peak current for DA at the NA-coated electrodes is still significantly higher (at least with a ca. 5 fold increase) than that observed at the bare GCE, meaning that for these NA films the sensitivity of the

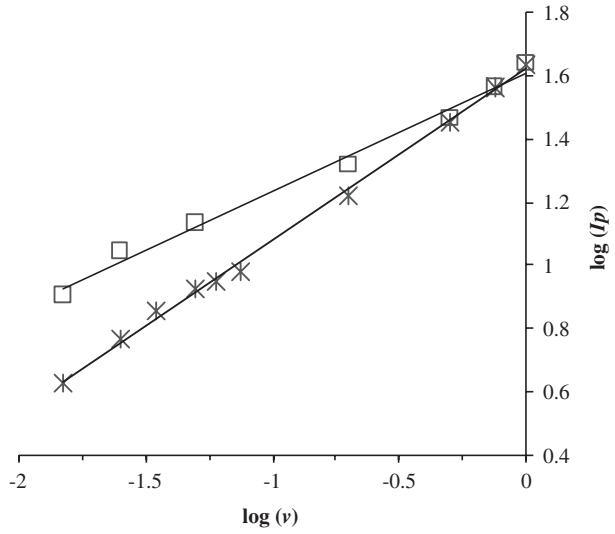



Fig. 4. Plot illustrating the effect of the scan rate on the oxidation peak current of DA (logarithmic representation, $\log(I_p)$ vs. $\log(v)$) in the presence of excess AA at the thick (9.2 μm) NA-coated GCE. Stars: DA alone ([DA]=100 μM) and squares: DA plus AA; [AA]/[DA]=5. DA oxidation peak current in μA and scan rate in $Vs^{-1}$.

DA determination is improved at conditions of high selectivity towards anions of small molecular weight (AA and UA).

### 3.3. Incorporation features of DA in 9.2 μm NA films on GCE

The incorporation of DA (99.6 μM, in pH 7.4 sodium phosphate buffer, ionic strength $0.12_4$ M; sodium ion concentration 0.09 M) into a 9.2 μm NA film is shown in Fig. 5 where the cyclic voltammograms were successively recorded for 5 min time intervals. The peak current for the oxidation of DA increased in time, reaching a plateau for times higher than ca. 20 min, for which the ion exchange equilibrium for DA, given by Eq. (1) was attained (cf. inset in Fig. 5). The corresponding ion-exchange reaction (Eq. (1)) assumes that the incorporation of the protonated DA ($DA^+$ species) results in ejection of $Na^+$ from the film in a 1:1 charge proportion.

$$\begin{aligned} &GC/NA(\sim SO_3^-)(Na^+)^{film} \\ &+ (DA^+)^{soln} \rightleftarrows GC/NA(\sim SO_3^-)(DA^+)^{film} \\ &+ (Na^+)^{soln} \end{aligned} \quad (1)$$

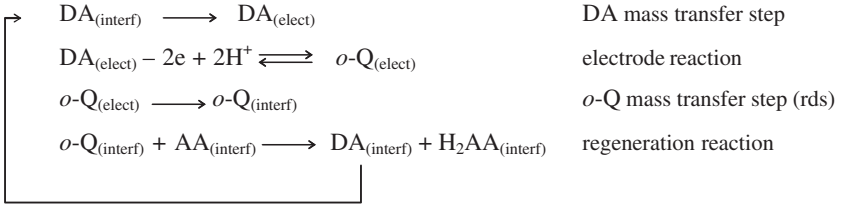
The release of the incorporated DA in the NA film was complete (final peak current < 5% of the initial value) for 3 h contact the sodium phosphate buffer solution of $0.12_4$ M ionic strength ($[Na^+]=0.09$ M), showing that trapping of the monocharged cation DA is not very strong which shall be related to the low charge of DA and to the effect of the competition of the $Na^+$ ions from the supporting electrolyte, reversing reaction (1). In addition, there is an effect of the voltammetric cycling regime upon the ejection of the analyte: if the electrode with incorporated DA is cycled repeatedly, DA is completely excluded from the NA coating after ca. 30 min. This effect has been observed by others for different systems [24].

For both NA-coated electrodes (thicknesses 2.6 and 9.2 μm, the oxidation peak current of DA was proportional to the square root of scan rate in the interval [0.015 to 1 V $s^{-1}$] ($\log I_p$ vs. $\log v$ plot with slopes ranging from 0.505 to 0.541: correlation coefficients 0.998 to 0.999 — cf. Fig. 3. for the 9.2 μm thickness electrode), meaning that, at the present NA films, the DA electrode reaction is diffusion-controlled (no evidences for thin-layer behaviour within the present timescale). This is in agreement with the observed voltammetric peaks having the typical diffusive tail. Further, for the present time scale, the calibration for DA at concentrations lower than ca. 100 μM gave perfect strait lines meaning that the apparent diffusion coefficient for DA in the NA film, $D_{app}$, is constant [20]. In addition, the slope of the $I_p$ vs. $v^{1/2}$ plots for the NA-coated electrodes was higher than for the bare GCE (cf. results in the next section). This accounts for the existence of a much larger concentration of DA incorporated into the NA film compared to that in solution, despite the expected decrease in the apparent diffusion coefficient of DA in the polymeric phase, compared to the value in solution. These aspects will be further evaluated in the next section.

### 3.4. Distribution coefficients and diffusion features for DA in 9.2 μm NA films

The amount of $DA^+$ ions incorporated into the NA film shall be dependent on the charge and size of dopamine cation, on its concentration in the electrolyte solution, on the concentration of competing $Na^+$ ions, as predicted by conventional ion exchange reasoning [20,25]. Also, the DA incorporation will depend on the Nafion film thickness, which determines the amount of sulfonate groups in the film, as well as the DA diffusion in the film.

In order to evaluate the partition of DA between the electrolyte solution and the NA film, the concentration of DA in the




Scheme 1. Suggested catalytic cycle for the regeneration of DA at the interface NA film/supporting electrolyte. DA represents the protonated dopamine, o-Q the dopamine-*o*-quinone the corresponding oxidation product, AA the ascorbate anion and $H_2AA$ the dehydroascorbate species (charges omitted for simplification). The subscripts *elect.* and *interf.* correspond to the species present at the electrode surface and at the NA film/supporting electrolyte interface, respectively. rds means the rate determining step.

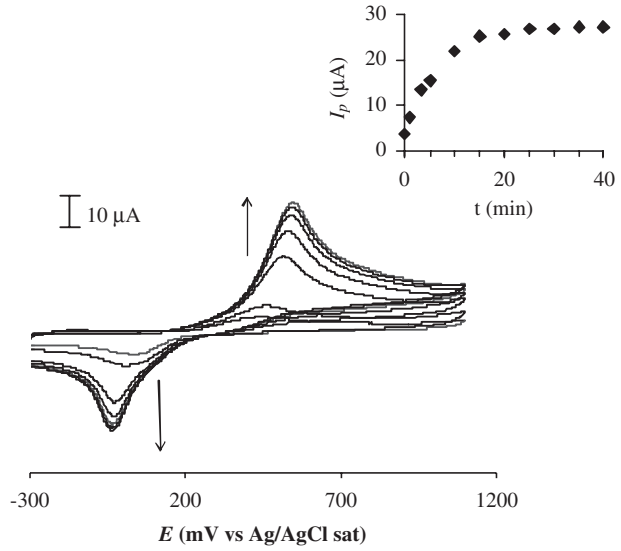



Fig. 5. Incorporation of DA into the 9.2 μm NA-coated electrode: cyclic voltammograms in 99.6 μM DA/pH 7.4 phosphate buffer (ionic strength $0.12_4$ M; $[Na^+]=0.09$ M) recorded in 5 min time intervals. The first voltammogram was taken immediately after transferring the NA-coated electrode to the DA solution. Scan rate: 0.25 V $s^{-1}$. Inset: variation of the DA oxidation peak current with incorporation time.

NA film, $c_{DA}^{film}$, was determined by controlled potential exhaustive electrolysis of DA previously incorporated into the film. The concentration of DA in the NA film (mol $dm^{-3}$) is given by the following equation:

$$c_{DA}^{film} = \frac{Q}{nFAl} \quad (2)$$

where $Q$ is the electric charge (C), $n$ is the number of transferred electrons ($n=2$ for DA), $F$ the Faraday constant (96,485 C $mol^{-1}$), $A$ the electrode area ($dm^2$) and $l$ the NA film thickness (dm). The complete electrolysis of the incorporated DA lasted for ca. 1 h. No DA was detected in the electrolyte solution, meaning that ejection of the incorporated species did not occur in the present time scale. The results were plotted as a partition isotherm i.e., as $c_{DA}^{film}$ vs. $c_{DA}^{soln}$ (Fig. 6A) where $c_{DA}^{soln}$ is the concentration of DA in solution. The plot clearly shows that saturation of the NA exchange sites will occur for the higher values DA solution concentration. The asymptotic value at the plateau corresponds to occupation of all available ion-exchange sites, i.e., can be used to calculate the total concentration of ion-exchange sites. That value i.e., $c_{DA}^{MAX\ (film)}$ was 0.21 M.

The slope of the partition isotherm at the lowest concentration values (Fig. 6B) gives $k_d$, the distribution coefficient of DA partitioning into the NA film. When the exchange analyte is a trace species and solution concentration of the exchanged cation, $Na^+$, is relatively high (in the present case $[Na^+]^{soln}=0.090$ M) the ion-exchange reaction given by Eq. (1), may be regarded as a partition reaction, cf. Eq. (3):

$$DA^{+(soln)} \rightleftarrows DA^{+(film)} \quad (3)$$

and the partition coefficient is given by

$$k_d = \frac{c_{DA}^{film}}{c_{DA}^{soln}} \quad (4)$$

The meaningful value of $k_d$ is that calculated for $c_{DA}^{soln}<0.1$ mM (cf. Fig. 6B) as the slope of the isotherm, i.e., $k_d=401$. This value means that an ion-exchange pre-concentration of DA into the 9.2 μm NA-coated GC electrode will be effective, i.e., the NA film prefers the $DA^+$ counter-cation over $Na^+$. An ion-exchange selectivity coefficient (of $DA^+$ over $Na^+$, corresponding to Eq. (1)) could be calculated once the film volume is known [21]. However, in the present case the film thickness is merely an estimated value based on a recast density which can hold an error depending on the actual swelling of the NA film.

Nevertheless, a pre-concentration occurred at the NA-coated electrodes compared with the bare GCE even for experiments without any deliberated equilibration step (cf. results in Section 3.1.). Therefore, adding a 3 min accumulation step with stirring to the LSV determination scheme for DA (scan rate 0.2 V $s^{-1}$; DA concentration range 2 to $100\times10^{-6}$ M) resulted in a 2 fold improvement of the slope of the calibration curve compared to the value without accumulation (slope, $0.364\pm0.005$ A $mol^{-1}$ $dm^3$; $r=0.999$, $N=6$, cf. data in Section 3.1.). The limit of detection was now 1 μM (3 $\sigma$). This limit of detection is one order of magnitude higher than that obtained by others [22] in similar experimental conditions, except for the concentration of $Na^+$ in the electrolyte solution which was 0.01 M instead of

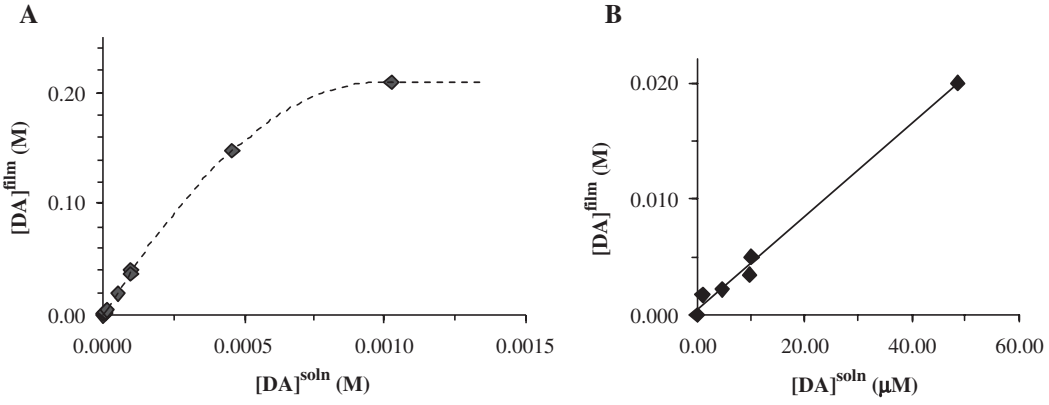



Fig. 6. Concentration of dopamine in the 9.2 μm NA-film $[DA]^{film}$, as a function of $[DA]^{soln}$, the DA concentration in the sodium phosphate electrolyte solution (ionic strength $0.12_4$ M; $[Na^+]=0.09$ M).

0.09 M, used in the present work. That will certainly have an effect on the partition equilibrium of DA, reversing Eq. (1), leading to lesser pre-concentration and thus to a higher limit of detection.

The maximum concentration of incorporated DA, $c_{DA}^{MAX\ (film)}$ which was 0.21 M, gives also the total concentration of ion-exchange sites, corresponding to the total concentration of $Na^+$ counter-ions initially present at the NA film. Considering the estimated thickness of 9.2 μm, that value will give $1.38\times10^{-8}$ mol of ion-exchange groups, $SO_3^-$. If Nafion would have a degree of sulfonation of 100%, the total number of sulfonate exchange sites for the 9.2 μm NA film, estimated from the deposited Nafion mass, would be $1.19\times10^{-7}$ mol. Therefore, the actual degree of sulfonation for NA may be calculated from the ratio of that value to the real concentration of exchange sites. That ratio is 8.5, giving a degree of sulfonation of 13% which compares well with that expected for Nafion 1100, 12.5% [21].

Besides the above mentioned considerations on the ion-exchange features of the present NA films, the overall ion-exchange process depends also on the mass transport of the analyte throughout the coating. Regardless of the actual mechanism of mass transport, the rate of the electrode reaction obeys Fick's laws of diffusion and may be characterized by the apparent diffusion coefficient, $D_{app}$ [20] which in most cases is smaller than the diffusion coefficient, $D$, for the species in solution. The solution value for DA has been determined by flow injection methods in similar conditions as those in the present work (i.e., pH 7.4, 0.1 M phosphate buffer solution). The reported values were $(6.0\pm0.3)\times10^{-6}$ $cm^2\ s^{-1}$ [26] and $(6.05\pm0.25)\times10^{-6}$ $cm^2\ s^{-1}$ [27]. In the present study the diffusion coefficient of DA has been estimated from cyclic voltammetric data with the GCE in ca. $9.70\times10^{-5}$ M DA/pH 7.4, 0.09 M sodium phosphate buffer solutions. For scan rates above 0.1 V $s^{-1}$ the DA electrode reaction at the GCE presented an irreversible behaviour (cathodic-to-anodic peak separations higher than 0.4 V) that could be characterized by the corresponding equation for the oxidation peak current [28]:

$$I_p = 2.99\times10^5 n(\alpha_c n_\alpha)^{1/2} c_{DA} D^{1/2} v^{1/2} \quad (5)$$

where $n_\alpha$ is the number of electrons transferred up to, and including the rate determining step and $\alpha_c$ is the transfer coefficient for the rate determining step.

In these cases the shape factor $|E_p - E_{p/2}|$ is given by [28]:

$$|E_p - E_{p/2}| = 47/(\alpha_c n_\alpha) \text{ in mV at 20 ℃} \quad (6)$$

Therefore, combining the above equations, one can calculate the diffusion coefficient. The plot of the oxidation peak current vs. the square root of the scan rate gave a straight line with a slope $7.01\times10^{-6}$ μA $V^{-1}$ s (mean value of two determinations, $r=0.996$) and the mean $|E_p - E_{p/2}|$ value was 0.102 mV. From these data the diffusion coefficient of DA in the present medium is calculated as $6.1\times10^{-6}$ $cm^2\ s^{-1}$, which is very close to the referenced values. In the case of linear sweep or cyclic voltammetric measurements for an incorporated species at a modified electrode, carried out under diffusion-controlled conditions (which is the case for DA at the present NA electrodes, cf. results in Section 3.3), the comparison of peak currents for the bare GCE and for the NA-coated electrode (with the same surface area $A$), at the same scan rate and dipped in the same DA solution, yield the following relation [20]:

$$\frac{I_p^{film}}{I_p^{soln}} = \left(\frac{D_{app}}{D}\right)^{1/2} k_D \quad (7)$$

where $k_D$ is given by Eq. (4).

In the present case, for $v=0.2$ V $s^{-1}$, the ratio of the cyclic voltammetric oxidation peak currents of DA corresponding to the NA-coated electrode and to the GCE immersed in the same solution of $9.70\times10^{-5}$ M DA/pH 7.4, 0.09 M sodium phosphate buffer, was 6.34. Substituting this value and that of the partition coefficient ($k_D=401$) in Eq. (7), $D_{app}$ is estimated as $1.5\times10^{-9}$ $cm^2\ s^{-1}$, which is, in fact, a much lower value than that in solution. As far as we know there is no referenced value for DA in similar experimental conditions and for NA-coated electrodes. Though, DA diffuses slower than the competing cation $Na^+$ which has an apparent diffusion coefficient of $9.44\times10^{-7}$ $cm^2\ s^{-1}$, in Nafion [21]. This fact, associated to the rather high concentration of sodium cation in solution, may give an explanation for the relatively low pre-concentration of DA (limit of detection ca. 1 μM, for a 3 min accumulation period). However, in spite of this, the present NA-coated electrodes were charge and size selective, excluding low mass anions such as ascorbate and urate, presented a better sensitivity compared to the bare GCE with a fast response time and, above all, can be used under physiological like conditions, in the linear sweep mode at scan rates higher than 0.5 V $s^{-1}$, precluding any interference from the DA regenerative cycle, induced by AA.

## 4. Conclusions

The results reported in the present work demonstrate that (i) relative thick films of NA simply coated by droplet evaporation on glassy carbon electrodes provide sensitive, selective and fast response time voltammetric determinations of DA under physiological like conditions. This indicates that the method can be easily adapted to flow systems; (ii) the use of NA films with a mass loading of 18 μg $mm^{-2}$ (estimated thickness 9.2 μm) and scan rates of at least 0.5 V $s^{-1}$, preclude the occurrence of the DA regenerative catalytic cycle, induced by AA, allowing DA determinations in the presence of large excess of AA and (iii) ion-exchange parameters (apparent diffusion and partition coefficients) indicate that the incorporation features of DA in the present NA thick films could be, eventually, improved namely by diminishing the sodium ion concentration in the supporting electrolyte.

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