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Article

Evaluation of a Nafion Coated Glassy Carbon Electrode for Determination of Paraquat by Differential Pulse Voltammetry

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Este trabalho apresenta uma avaliação de um eletrodo de carbono vítreo recoberto com filme de Nafion para a determinação de paraquat em águas de rio e urina por voltametria de pulso diferencial. O filme foi formado adicionando-se 4 μL de uma solução de Nafion 4% (m/v) na superfície do eletrodo, seguindo-se evaporação do solvente com luz infra-vermelha. A máxima relação sinal/ruído foi obtida em meio de tampão Britton-Robinson 40 mmol L^{-1} com pH 12 como eletrólito suporte. Utilizando-se um tempo de acumulação de 5 min em circuito aberto, o limite de detecção foi de 0,7 μg L^{-1}, enquanto o limite de quantificação foi de 1,0 μg L^{-1}, com uma faixa de resposta linear até 12 μg L^{-1}. Nestas condições, uma série de dez experimentos revelou um desvio padrão relativo de 2,2% para uma solução de paraquat 10 μg L^{-1}. A formação de par iônico em solução foi o principal fator de erro nas análises. Apesar do filme evitar a adsorção de espécies aniônicas na superfície do eletrodo, o paraquat sofre fortes associações em solução com substâncias húmicas, proteínas, argilas, surfactantes aniônicos, etc. Estas interações diminuem a acumulação do paraquat no filme de Nafion e, em conseqüência, diminuem a magnitude da corrente de pico de redução, requerendo que as quantificações sejam feitas por adição de padrão. Recuperações entre 87 e 106% foram obtidas para amostras de água de rio e urina enriquecidas aos níveis de 1,0 e 3,0 μg L^{-1}.

This paper presents an evaluation of a Nafion coated glassy carbon electrode for determination of paraquat in river water and urine by differential pulse voltammetry. The film was formed applying 4 μL of a 1% (m/v) Nafion solution, which was evaporated under an infrared light. The maximum signal to noise ratio was obtained using a 40 mmol L^{-1} Britton-Robinson buffer at pH 12 as supporting electrolyte. For an accumulation time of 5.0 min in open circuit, the limit of detection was 0.7 μg L^{-1}, while the quantification limit was 1.0 μg L^{-1}, with a linear dynamic range up to 12 μg L^{-1}. In these conditions, a sequence of ten experiments lead to a relative standard deviation of peak current of 2.2% for a 10 μg L^{-1} paraquat solution. Ion pair formation in solution was the major factor of error in analysis. Despite the film to avoid adsorption of anionic species at the electrode surface, paraquat suffers strong association in solution with humic substances, proteins, clays, anionic surfactants, etc. These interactions decrease the accumulation of paraquat in the Nafion film and, as a consequence, the magnitude of the reduction peak, requiring that quantifications have to be made by standard addition. Recoveries between 87 and 106% were obtained for river waters and urine samples spiked with 1.0 and 3.0 μg L^{-1} paraquat.

Keywords: paraquat determination, river water, urine, modified electrode, voltammetry, ion exchange

Introduction

Paraquat (1,1'-dimethyl-4,4'-bypiridilium ion), also known as methyl viologen, is a defoliant and desiccant agent used to control herbal growth in terrestrial and aquatic environments.[1] It is considered toxic for algae, fishes and other organisms,[2] including humans.[3] The adverse effects caused by chronically and acute expositions to this herbicide are well known and cases of death by casual ingestion or poisoning are documented in the literature.[4,5]

Due to the widespread use of paraquat, as well as its long residence time and elevated toxicity, even at low concentrations, regulatory agencies established maximum acceptable concentration limits in environmental samples. The Environmental Protective Agency (EPA) established a maximum concentration of 3 μg L^{-1} in natural waters,[2] while the European Community established 0.1 μg L^{-1} for the same kind of sample.[6]

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A large variety of analytical techniques have been proposed for determination of paraquat, such as molecular absorption spectrophotometry,[7,8] liquid chromatography with UV detection,[9,10] liquid and gas chromatography coupled to mass spectrometry detection,[11,12] gas chromatography with specific detector for nitrogen and phosphorus (NPD),[13] ELISA[14] as well as potentiometric and amperometric sensors.[15, 16] More recently, capillary electrophoresis with ultraviolet (CE-UV)[17] or mass spectrometry (CE-MS)[18] detection was proposed. These techniques suffer with the instability of reagents, interferences, high detection limits, extensive sample treatment and high cost per analysis.

The reversible redox properties of paraquat are well known, being used in the development of voltammetric methods based on its reduction or oxidation. Some of these methods use techniques such as differential pulse,[19] square wave,[20, 21] and cathodic stripping voltammetry.[22] Paraquat is a di-cation that can be easily accumulated on the surface of electrodes modified with cation exchanger materials, such as Amberlite XAD-2,[23] clays[24] and some surfactants.[25] Although some of these methodologies have excellent detection limits, serious interferences of surfactants and humic substances were reported. Nafion is a polymeric resin with cation exchanger properties and high mechanical resistance that can act as an effective barrier against surfactants, proteins and humic substances. Due to these characteristics, Nafion has been extensively used in the preparation of modified electrodes.[26, 27] Nafion coated electrodes have been used for determination of heavy metal cations in natural waters[28] and some organic compounds, including pesticides such as the insecticide parathion[29] and the herbicide amitrole.[30] Lu and Su[31] developed an electrocatalytic method for determination of paraquat in river and tap waters using a glassy carbon electrode covered with a Nafion film. After the accumulation step, the electrode was transferred to another cell containing permanganate ions and perchlorate in pH 8.2, where the determination is performed by cathodic differential pulse voltammetry.

The purpose of the present paper was the electroanalytical determination of paraquat without the need for sample treatment, derivative chemical reactions and medium exchange, using differential pulse voltammetry with a Nafion Coated Glassy Carbon Electrode (NCGCE). A systematic study of the influence of heavy metal species, humic substances, pesticides, surfactants and clays was performed. The method was applied to a river water and urine.

Experimental

Apparatus and reagents

All experiments were performed with a 263A potentiostat from EG&G Instruments Inc. (Princeton, NJ, USA) controlled by a Pentium 100 computer using the model 270/250 Research Electrochemistry Software 4.30, also from EG&G. The three electrodes cell consisted of the NCGCE as the working electrode (3 mm diameter), an Ag/AgCl (KCl saturated) reference electrode and a Pt wire as auxiliary electrode.

The 5% (m/v) Nafion solution solved in a mixture of aliphatic alcohols and 10% (v/v) water was purchased from Sigma. The dichloride salt of paraquat was purchased from Promochem GmBh. Standard 1000 mg L^{-1} solutions of the heavy metal cations Cu(II), Pb(II), Cd(II), and Zn(II) were obtained from Carlo Erba in ampoules and properly diluted. The surfactants Triton X-100 (non-ionic), Cetiltrimethylammonium bromide (CTAB, cationic), and sodium dodecilsulfate (SDS, anionic) were obtained from Sigma.

The supporting electrolytes in all experiments were Britton-Robinson (BR) buffers prepared in a concentration of 40 mmol L^{-1} of each component (phosphoric acid, acetic acid and boric acid), having the pH adjusted in the interval between 2 and 12 adding appropriate amounts of NaOH. To minimize the interference of heavy metal cation in real samples, the supporting electrolytes were prepared in 2.0 mmol L^{-1} EDTA.

The paraquat working solutions were prepared with de-ionized water by appropriate dilution of a 1000 mg L^{-1} stock solution.

Preparing the Nafion Coated Glassy Carbon Electrode (NCGCE)

The bare glassy carbon electrode was polished to a mirror-like surface with 0.50 μm alumina in aqueous slurry, and then exhaustively washed with de-ionized water. The electrode was immersed in a beaker containing 50% (v/v) HNO_3 and treated in an ultrasonic cleaner for 5 min. This treatment was repeated using de-ionized water instead the HNO_3 solution. The electrode was washed with methanol and dried under a flow of ultra-pure nitrogen. Next, 4 μL of a 1% (m/v) Nafion solution prepared in ethanol were applied on the surface of the glassy carbon. The solvent was evaporated with the aid of an infrared lamp, located a few centimeters from the electrode.

Differential pulse voltammograms

The electrochemical cell was assembled containing the three electrodes and 10.0 mL of the supporting electrolyte (pH 12). An appropriate volume of standard stock paraquat solution is added to the cell providing analyte concentrations in a range between 1.0 and 12 μg L^{-1}. The solution was de-aerated for 5 min with ultra-pure N_2. After an equilibration time of 30 s, the potential scan from

–0.2 to –1.2 V *vs*. Ag/AgCl was made by differential pulse voltammetry using a pulse amplitude of 25 mV and pulse width of 2 ms. For the standard addition experiments, the de-aeration time was maintained at 5 min despite the small volume of standard added to the cell. This time of 5 min works also as the accumulation time, which is long enough to allow the ionic exchange process between paraquat and the Nafion membrane to reach the equilibrium. During this time the electric circuit is open, so that no electron transfer occur in the working and auxiliary electrodes.

Samples

The river water was sampled in the Taiaçupeba-Mirim River, which is located in the Taiaçupeba River basin in the Mogi das Cruzes County, an agricultural area of São Paulo state, near the Metropolitan Area of São Paulo city. The water was filtered through a 0.45 μm cellulose acetate membrane and stored in a polyethylene bottle at 4 °C. For analyses, 4.00 mL of the river water were transferred to a 5.0 mL volumetric flask, and the volume was completed with 0.20 mol L^{-1} BR buffer solution at pH 12. An aliquot of 4.00 mL was transferred to the electrochemical cell, and the paraquat was determined by the standard addition method using 5 min of accumulation time.

In the case of urine, 3.00 mL of sample were transferred to a 5.00 mL volumetric flask to which 500 μL of 10% (m/v) trichloroacetic acid were added. The volume was completed with 0.20 mol L^{-1} BR buffer at pH 12. The mixture was transferred to a test tube, stirred in a vortex for 2 min, and centrifuged at 3000 rpm for 15 min. Four milliliters of the supernatant solution were transferred to the electrochemical cell for quantification of paraquat by standard addition.

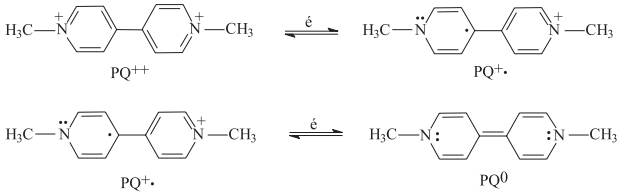
Results and Discussion

The results of a systematic study of the influence of pH on the magnitude of peak currents obtained with NCGCE in a 1.0 mg L^{-1} paraquat solution are presented in Table 1.

Table 1. Peak potential and peak currents obtained for a 1.0 mg L^{-1} paraquat solution in medium of 40 mmol L^{-1} BR buffer in a pH range between 2 and 12 obtained by Differential Pulse Voltammetry using the Nafion Coated Glassy Carbon Electrode

pH	Ep_1 (mV)	Ep_2 (mV)	i_{p1} (μA)	i_{p2} (μA)
2.0	- 666	-968	6.7	2.2
4.0	-660	-1030	5.2	0.5
6.0	-642	-1066	3.9	1.7
8.0	-628	-1078	3.1	2.2
10	-612	-1082	2.4	2.1
12	-600	-1098	53	24

Two reduction waves were observed for paraquat, one between – 0.60 to –0.66 V and the other between –0.98 to –1.10 V *vs*. Ag/AgCl. The reduction of paraquat may be attributed to the following processes:[32]



The paraquat cation, PQ^{++}, suffers a fast and reversible reduction to the radical $PQ^{\bullet+}$ at approximately –0.6 V. This radical cation adsorbs to the electrode surface and, upon scanning the potential to more negative values, is reversibly reduced to the neutral species PQ^0 at –1.0 V.[31]

The best signal to noise ratio was observed at pH 12. Despite the fact that protons do not participate of the electrochemical reduction, the influence of pH may be attributed to the accumulation process, since at low pH the hydrogen ions compete with paraquat for exchange at the $-SO_3^-$ sites of the Nafion film. A comparison between the differential pulse voltammogram obtained with NCGCE and the bare glassy carbon electrode is shown in Figure 1 for a 1.0 mg L^{-1} paraquat solution in 40 mmol L^{-1} BR buffer at pH 12, evidencing the significant

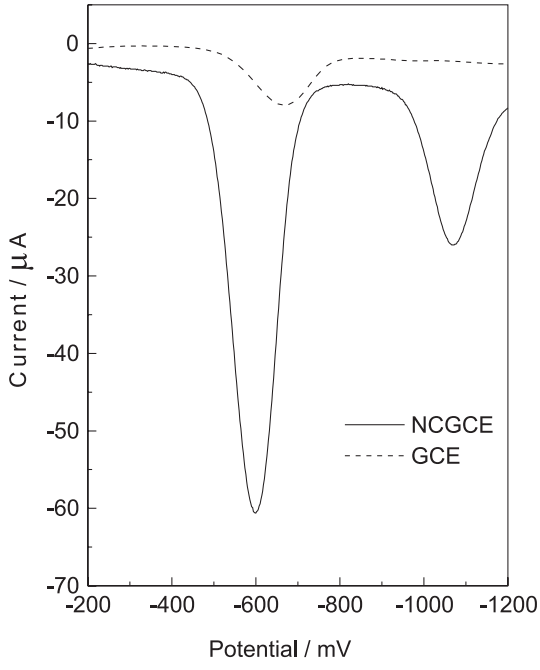


Figure 1 – Comparison of differential pulse voltammograms of a 1.0 mg L^{-1} paraquat solution in 40 mmol L^{-1} BR buffer at pH 12 using the Nafion Coated Glassy Carbon Electrode (NCGCE) and the bare Glassy Carbon Electrode (GCE).

enhancement of the first and second peak currents (i_{p1} and i_{p2}, respectively) obtained with the modified electrode. The second reduction peak in the bare glassy carbon electrode almost does not appear in Figure 1. The strong effect of pH 12 on peak current is not clear at this time and should be subject of new studies. It is known from the literature that OH^- ions attack the carbon of one the methyl groups, giving methanol and mono-demethylated paraquat.[32] In alkaline solution methanol reduces paraquat to its radical and is itself oxidized to formaldehyde, resulting a deep blue solution. Paraquat solutions in presence of the 40 mmol L^{-1} BR buffer at pH 12 did not show color changes during the experiments. But, on the other hand, one can speculate that the significant increase of peak current in this medium is related with the above mentioned reactions, which may facilitate the heterogeneous electron transfer that govern the electrolytic reduction of paraquat.

The effect of the Nafion concentration and the volume of the Nafion solution applied on the electrode surface were studied with a 10 μg L^{-1} paraquat solution in 40 mmol L^{-1} BR buffer at pH 12 (Figure 2). The exchange and accumulation capacities of the film depend on the concentration of Nafion and on the film thickness. The increase of the Nafion concentration leads to an increase of the ion-exchange capacity of the film up to certain limit, which is determined by the nature of the analyte and its diffusion through the film. Too thick films can make difficult the diffusion of paraquat toward the electrode surface, where the electrochemical reaction occurs, decreasing the sensitivity of the peak current measurements. The best signal (peak current) to noise ratio was obtained applying 4 μL of a 1% (v/v) Nafion solution on the bare glassy carbon surface.

Using the best condition found to form the Nafion film, a kinetic curve showing the analyte accumulation was obtained for paraquat concentrations 3.8, 7.4 and 11.0 μg L^{-1} (Figure 3). For accumulation times longer than 150 s, the peak currents approach a steady value, indicating that the ion exchange process between the PQ^{2+} species and the Nafion film is reaching the equilibrium. For an accumulation time of 5 min, the repeatability of the peak currents was determined for a 10 μg L^{-1} paraquat solution in a medium of 40 mmol L^{-1} BR buffer at pH 12. The average peak current was 4.6±0.1 μA for 10 measurements, which corresponds to a relative standard deviation of 2.2%. These voltammograms were obtained in sequence using the same electrode immersed in the same solution. The excellent repeatabilility indicates that after 5 min of accumulation the system electrode-solution reaches the equilibrium, with no more net accumulation of paraquat on the Nafion film.

Using 5 min of accumulation and de-aeration time, an analytical curve for paraquat was obtained in a concentration range between 1.0 and 12 μg L^{-1} (Figure 4), fitting the linear equation $i_p = (0.514 \pm 0.007)[PQ^{2+}] - (0.07 \pm 0.05)$, where i_p is the peak current (in μA) and [*PQ*] the paraquat concentration in μg L^{-1}. The limit of detection (*LD*) was computed using the equation: $LD = 3S_b/m$, where

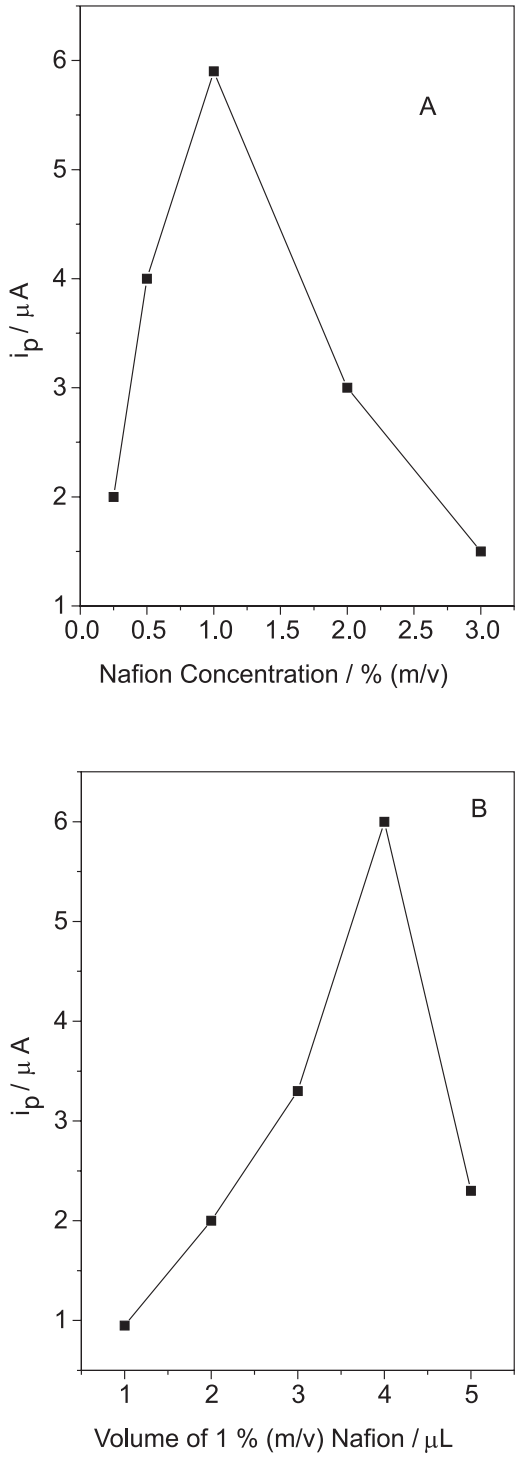


Figure 2 – Variation of the reduction peak current of a 10 mg L^{-1} paraquat solution in medium of 40 mmol L^{-1} BR buffer at pH 12 as a function of the Nafion concentration used to form Nafion film (A) and the volume of the 1.0% (m/v) Nafion solution (B).

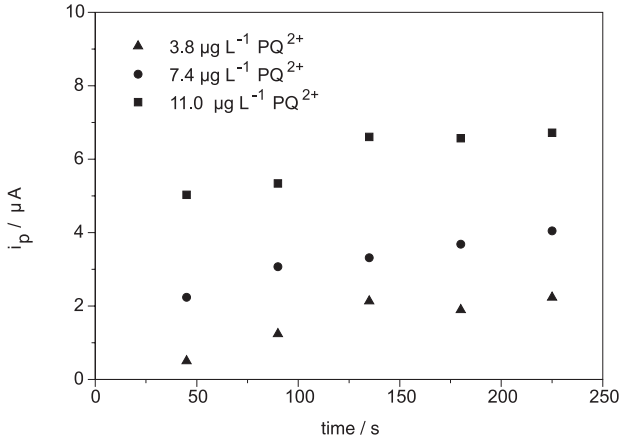


Figure 3 – Accumulation kinetic of three paraquat concentrations on the NCGCE in medium of 40 mmol L^{-1} BR buffer at pH 12. The film was formed applying 4 μL of a 1% (m/v) Nafion solution on the glassy carbon electrode.

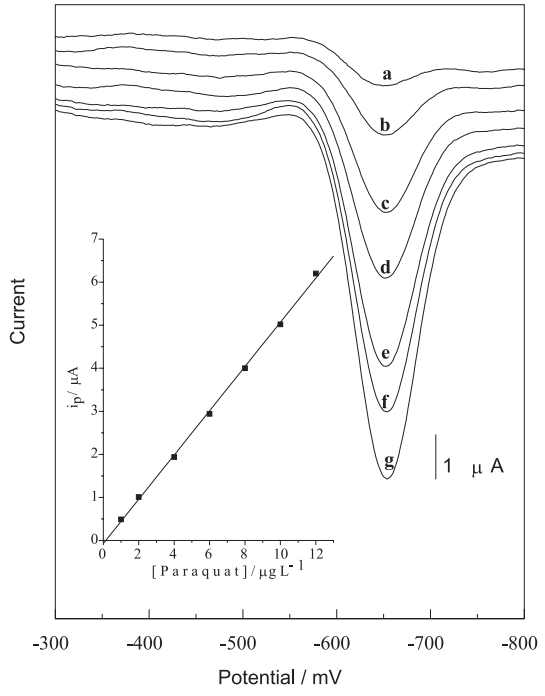


Figure 4 – Differential pulse voltammograms obtained in medium of 40 mmol L^{-1} BR buffer at pH 12 for paraquat solutions at concentrations: (a) 1.0, (b) 2.0, (c) 4.0, (d) 6.0, (e) 8.0, (f) 10.0 and (g) 12.0 μg L^{-1}. The insert shows the analytical curve obtained reading the maximum peak currents as a function of the paraquat concentration.

S_b is the standard deviation of 10 peak current measurements at 660 mV for a 40 mmol L^{-1} BR buffer solution at pH 12 (blank); *m* is the slope of the analytical curve.[33] The limit of quantification (LQ) was computed by the equation: $LQ = 10S_b/m$, for which the terms have the same meaning of the equation for LD. For the proposed method, LD and LQ values were 0.7 and 1.0 μg L^{-1}, respectively.

Study of interferences

The influence of several compounds such as heavy metals, surfactants, humic acid, vermiculite and other herbicides on the peak currents of the paraquat reduction on the NCGCE was studied using a 10 μg L^{-1} paraquat solution. Heavy metals and herbicides are likely to occur in polluted waters, while surfactants and humic substances are representative of natural organic matter occurring in natural waters. Vermiculite is a clay mineral that was chosen as representative of inorganic suspended matter. Table 2 shows the results obtained in this study. The heavy metals Cu(II), Pb(II), Cd(II) and Zn(II) were added at concentration of 10 mg L^{-1}. Under the experimental conditions used, performing the determination in medium of 40 mmol L^{-1} BR buffer at pH 12, none of these four metals showed a significant reduction peak at the NCGCE. Copper and Cd(II) are probably hydrolyzed as inert $Cu(OH)_2$ and $Cd(OH)_2$ species, while the other metallic cations are present as their hydroxo-complexes $Pb(OH)_4^{2-}$ and $Zn(OH)_4^{2-}$. As these complexes are anionic, the lack of electroactivty may be explained by electrostatic repulsion between the Nafion film and the metallic species. Copper and Cd did not present any interference on the reduction peak of paraquat, while Zn(II) and Pb(II) depleted the peak current by 8 and 12%, respectively. An explanation for this interference may be the occurrence of electrostatic interactions between the positively charged paraquat cations and the negatively charged hydroxo-complexes, decreasing the accumulation level of paraquat at the Nafion film. Addition of 2 mmol L^{-1} EDTA minimized the

Table 2- Influence of foreign species on the peak current of a 10 μg L^{-1} paraquat solution in 40 mmol L^{-1} BR buffer at pH 12

Foreign Species	Concentration mg L^{-1} or%[a]	Contribution (%) $i_{p\,[PQ^{2+}]}$ = 100%
Zn(II)	10	-8
Pb(II)	10	-12
Cu(II)	10	0
Cd (II)	10	0
SDS*	0.1	-92
Triton X-100[a]	0.1	-17
CTAB[a]	0.1	-55
Linuron	10	0
Gliphosate	10	-23
Trifluralina	10	0
Atrazine	10	-3
Diquat	10	+30
Humic Acid	1	-54
Humic Acid	5	-73
Humic Acid	10	-85
Vermiculite	1	-68
Vermiculite	5	-81
Vermiculite	10	-100

[a] concentrations in% (m/v).

interference, but did not recover the original peak, a fact that also can be explained by formation of ion pair between the de-protonated EDTA and the paraquat cations, decreasing the accumulation factor at the film. Lu and Sun[31] also verified the depression of the reduction peak in presence of EDTA. These authors used 0.05 mol L^{-1} EDTA concentration as a compromise between masking heavy metals and signal depression. The peak potential of paraquat reduction was not significantly affected in presence of EDTA.

Electrostatic interaction with formation of ion-pair can also explain the drop of peak currents observed in presence of humic acid, vermiculite, glyphosate and the anionic surfactant (Table 2). At pH 12 all carboxylic groups of the humic acid are de-protonated, as well as the majority of phenolic groups, conferring an anionic character to these macromolecules, which would lead to electrostatic attractions for the paraquat cations, decreasing its affinity for the Nafion film. The same explanation may be valid for vermiculite, a clay mineral that is negatively charged at pH 12, and for the herbicide glyphosate. The later has a phosphate and a carboxylate group in its structure, conferring an anionic character at pH 12.[34]

Studying the influence of surfactants on the reduction peak current of paraquat, the following interference order was observed: anionic (SDS) > cationic (CTAB)> neutral (Triton X-100). At concentration of 0.1% (m/v), SDS almost depleted completely the paraquat signal at the electrode. Formation of ion pair between paraquat and anionic surfactant has been explored for development of sensitive methods for determination of paraquat.[35] Also at concentration of 0.1% (m/v), the cationic surfactant CTAB decreased the peak current of paraquat by 55%, a fact that can be explained by interactions between the Nafion film and the surfactant, decreasing the number of active sites available in the film to interact with paraquat cations. For Triton X-100, a decrease of 17% of the signal was observed. Since this surfactant is neutral, no significant interactions should be expected with both, the Nafion film and the paraquat cations. Thus, the interference observed may be attributed to the high concentration of Triton X-100 used (0.1% m/v), which could affect the solution viscosity and the diffusion coefficient of paraquat in relation to the medium containing only BR buffer.

The herbicides linuron, trifluraline and atrazine, all neutral at pH 12, did not interfere (Table 2). Although these herbicides are electroactive at glassy carbon or mercury drop electrodes, no reduction wave was observed at the NCGCE in medium of 40 mmol L^{-1} BR buffer at pH 12. The only positive interference was observed for the herbicide diquat, a cation with two positive charges, which is reversibly reduced at the NCGCE at potentials between –600 and –650 mV, that is, the same reduction potential interval observed for paraquat. The peak currents for reduction of diquat at the NCGCE is maximum at pH 2, decreasing with the increase of pH, reaching a minimum signal at pH 12. This explains the fact that despite using a diquat concentration of 10 mg L^{-1}, which is 1000 times greater than the paraquat concentration (in mass), the increase in paraquat peak current was only 30% (Table 2).

Samples

The performance of the NCGCE was evaluated determining paraquat in a sample of river water and urine. Since none of the samples presented detectable amounts of the analyte, they were spiked with the paraquat concentrations presented in Table 3. Determinations were made by standard additions. For the river water spiked with 1.0 and 3.0 μg L^{-1} of paraquat, recoveries were 94 and 106%, denoting that no serious interferences occurred in this real matrix.

Table 3- Recovery of paraquat in spiked real samples

Sample	Paraquat Added (μg L^{-1})	Paraquat Recovered (μg L^{-1})	RSD (%)	Recovery (%)
River	1.0	1.06	3.2	106
Water	3.0	2.82	2.0	94
Urine	1.0	0.87	4.8	87
	3.0	2.76	3.5	92

RSD= Relative Standard Deviation for three measurements.

The urine sample was spiked with 1.0 and 3.0 μg L^{-1} of paraquat. After treatment with trichloroacetic acid and buffering with BR buffer at pH 12, recoveries of 87 and 92% were observed. Although one of the applications of Nafion film is to prevent adsorption of proteins on the electrode surface, it seems that the difficulty in applying the method to untreated urine sample is the ion pair formation between the paraquat cation in solution with anionic groups of proteins, preventing its accumulation in the film.

Conclusion

The Nafion Coated Glassy Carbon was a suitable sensor for determination of paraquat at low concentrations using an accumulation time of 5 min. The detection limit of 0.7 μg L^{-1} and the quantification limit of 1.0 μg L^{-1} are adequate to attend some legal acceptable limits, as the 3.0 μg L^{-1} level recommended by the Environmental

Protection Agency (EPA). The Nafion film worked as an efficient material for pre-concentration of paraquat, but did not eliminate the interference of anionic species in solutions. The film prevents adsorption of these species on the electrode surface, but ion pair interactions between the paraquat cation and anionic species, such as humic substances, clays and proteins are very strong, difficulting the analyte accumulation on the Nafion film.

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