https://dx.doi.org/10.21577/0103-5053.20230053

J. Braz. Chem. Soc., Vol. 34, No. 10, 1410-1419, 2023
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Article

A Simple Method for Determination of Chloroquine Based on Electrogenerated Chemiluminescence

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Chloroquine is a drug belonging to the aminoquinoline family that is widely used for treating diseases such as lupus, cancer, and malaria. In this sense, the present work describes the development of a simple method based on electrogenerated chemiluminescence (ECL) and a screen-printed carbon electrode (SPE) modified with carbon black for chloroquine determination. The carbon black modified SPE was characterized by scanning electron microscopy, Raman spectroscopy, cyclic voltammetry and linear sweep voltammetry to obtain the ECL-potential curves. The ECL method is based on the chemiluminescence resulting from the interaction between chloroquine and the tris(2'2-bipyridyl) ruthenium(II) complex. Under optimized experimental conditions, the method showed a wide linear working range between 0.5 and 500 μmol L^{-1}. The method presented good precision and accuracy in drug samples used in the treatment of malaria and artificial urine sample, showing recovery values from 100 to 103% and 99 to 103%, respectively.

Keywords: electrochemiluminescence, chloroquine, ruthenium complex

Introduction

Chloroquine belongs to the group of aminoquinolines, and it was first synthesized in 1934.[1] This drug has been widely prescribed for the prevention and treatment of malaria, cancer, as well as for the treatment of autoimmune diseases such as arthritis and systemic lupus erythematosus.[1,2] However, there has been related some adverse effects associated to the use of this drug, including impairment of renal function, insomnia and other rare disorders, such as cardiac failure.[3-5] In addition, it has been reported chloroquine poisoning of a child after the infant ingested only one tablet of 300 mg resulting in a lethal dose/blood level for children of few μmol *per* liter.[6] On the other hand, it has been reported levels of chloroquine in urine samples tested by days after treatment of adult patients varying from few micrograms *per* liter up to few milligrams *per* liter.[7]

In this sense, the development of novel analytical methods that present some advantages over the existing methods is of high interest nowadays. The most used methods for detecting chloroquine are the chromatographic, spectrometric and colorimetric methods.[8-12] Although the previously mentioned methods become robust, reliable, reproducible, sensitive, and precise, they can show some disadvantages, including high-cost instrumentation, high amount of reagents, exigence of skilled operators, troublesome sample preparation steps, and relatively long analysis time.

Electrochemical methods are a low-cost, low-analysis time, sensitive, and simple alternatives for identifying and quantifying several electroactive compounds (ionic species or molecules).[13,14] These methods are widely used due to their interesting features such as good selectivity, rapid response, the requirement of low volume of solutions and easy usage. In addition, the electrochemical methods deliver reliable results and excellent quality. These methods may involve the use of chemically modified electrodes to control the physical-chemical properties of the electrode-

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Editor handled this article: Rodrigo A. A. Muñoz (Associate)

solution interface and allow the determination of species with high sensitivity.

The electrochemical methods are commonly based on redox processes of interest molecules, where applying a potential difference in the system can induce the oxidation or reduction processes, generating a measurable electrical signal. In addition, the electrochemical methods can be exploited to generate chemiluminescence in some systems, which can be controlled by alterations of the applied potential, allowing high control over the initiation, rate and course of the reactions.

The electrogenerated chemiluminescence (ECL) methods use the advantages of electrochemistry and chemiluminescence. These methods operate based on the emission of light from a compound that has its electrons excited through a reaction initiated by an electrochemical stimulus.[15,16] As ECL-based methods use the advantages of electrochemical and chemiluminescent methods, require simple instrumentation, and may have high sensitivity and a short response time.[16]

In the ECL analysis, some elements can affect the method's performance, such as luminophores and co-reagents, which correspond to the analyte of interest.[17] The most commonly used luminophores in ECL are the ruthenium(II) and luminol complex. ECL based on the tris(2,2'-bipyridyl)ruthenium(II) chloride hexahydrate complex ($Ru(bpy)_3^{2+}$) complex has been widely used in analytical science due to its inherent advantages, such as extreme stability, good water solubility, high sensitivity, wide dynamic range, and a wide variety of analytes.[18]

Screen-printed electrodes (SPEs) are used as electrochemical sensors due to their high sensitivity, wide linear working range and fast results.[19] The electrochemiluminescent methods allied to the use of SPE become an excellent alternative for carrying out ECL analyses since the SPE-ECL system requires a low volume of samples being an ecologically correct and portable method allowing good performance for analysis without analyte derivatization, avoiding sample pre-treatment, minimizing waste generation and requiring small sample volumes (volume less than 100 μL).[20]

In the last decade, unmodified SPEs have largely been replaced by SPEs modified with nanomaterials such as graphene and carbon nanotubes.[21-23] In this context, another nanomaterial that has stood out immensely is carbon black (CB) due to its excellent cost-effectiveness and excellent conductive property when compared to other carbon-based materials.[21] Thus, the present work aims to develop a simple ECL-based method using SPE modified with CB to determine chloroquine. To the best of our knowledge, this is the first ECL method that explores the use of a CB-modified SPE for the anodic detection of chloroquine from the interaction with the $Ru(bpy)_3^{2+}$ luminophore employing a simple printed electrode modified with carbon black, a low cost material.

Experimental

Reagents and solutions

Chloroquine diphosphate (CQ), oflaxicin and ruthenium(II) tris-2,2'-bipyridyldichloro hexahydrate ($Ru(bpy)_3^{2+}$) were purchased from Sigma-Aldrich (São Paulo, Brazil). Monopotassium phosphate, disodium phosphate, sodium chloride, calcium chloride, creatinine, urea, citric acid, ammonium sulfate and sodium sulfate were obtained from Isofar-Indústria de Comércios e Produtos Químicos Ltda (Duque de Caxias, Rio de Janeiro, Brazil). All reagents used in the present work were of analytical grade and all solutions were prepared using deionized water. CQ stock solutions were prepared daily in a 0.1 mol L^{-1} phosphate buffer solution, pH 7.0. The $Ru(bpy)_3^{2+}$ solution was prepared in deionized water. Macllvaine buffer was prepared using 0.1 mol L^{-1} of disodium phosphate adjusted with 0.1 mol L^{-1} of citric acid. Carbon black (Black Pearls 2000) was acquired from Cabot Corporation (Boston, USA). The CB dispersions were prepared in deionized water.

Carbon black characterization by scanning electron microscopy and Raman spectroscopy

The morphology of the CB-modified SPE was characterized by scanning electron microscopy (SEM). The SEM image was obtained using Zeiss microscope, model EVO HD (Oberkoche, Germany). Raman measurements were performed with a Horiba-Jobin-Yvon triple spectrometer, model T64000 (Kyoto, Japan), operating in a single mode. The samples were excited with a laser (LAS-532-100 HREV) of a wavenumber of 532.0 nm with a spectral resolution of 2 cm^{-1}.

Electrochemical and electrogenerated chemiluminescence measurements

The electrochemical and electrogenerated chemiluminescence measurements were performed with a DropSens portable μStat electrochemiluminescence (ECL) Instrument from DropSens (Oviedo, Spain) coupled for a computer containing the DropView software to data acquisition. A screen-printed carbon electrode (SPE) purchased from DropSens (Oviedo, Spain) was used as a platform for the ECL cell. The SPE contained three electrodes screen

printed on the same planar ceramic platform, consisting of a carbon disk shaped (4 mm diameter) working electrode, a carbon counter electrode and a silver (Ag) pseudo-reference electrode. A mass of 5 mg of CB was dispersed in 500 μL of deionized water for modification of SPE. The suspension was manually stirred and presented good dispersion. The SPE was modified using 10 μL of the CB dispersion and allowed to dry at room temperature before ECL measurements. The active surface area of the electrode after modification with the CB was determined by the Randles Sevick equation[24] using 5 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ as redox probe in 0.1 mol L^{-1} KCl. The effective surface area of CB/SPE was 0.32 cm^2.

Electrochemical and electrochemiluminescence measurements were obtained in a potential range of 0.1 to 1.6 V at a scan rate of 0.1 V s^{-1} using cyclic voltammetry (CV) and linear sweep voltammetry to obtain the ECL-potential curves. The volume of support electrolyte used consisted of a mixture of 20 μL of the CQ solution and 20 μL of the $Ru(bpy)_3^{2+}$ solution.

Experimental parameters, analytical characteristics and application in drug and artificial urine samples

The effects of the luminophore and CB concentration were evaluated by varying the concentration of the luminophore at 1.5, 2.5, 5.0, 15.0 and 25.00 mmol L^{-1} (while maintaining at 40 μL the volume of the mixture containing the $Ru(bpy)_3^{2+}$ and CQ) and CB at 1.0, 3.0, 5.0, 7.0 and 10.0 mg mL^{-1} (for 20 μL of CB in dispersion). The effect of the pH of the solution was evaluated in 0.1 mol L^{-1} of phosphate buffer solution at pH 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0. Finally, in the study of the type of buffer solution to be exploited in the determination of CQ, the effect of HEPES (4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid), PIPES (1,4-piperazinediethanesulfonic acid), Macllvaine and phosphate buffers at a concentration of 0.1 mol L^{-1} was evaluated on the response of the system. For the construction of the analytical curve, a volume of 20 μL of the luminophore were mixed with 20 μL of different concentrations of CQ. The mixture was added in an ECL cell on the surface of the carbon SPE modified with CB, and the ECL signal was measured for each CQ concentration.

In order to prepare the samples, four tablet samples containing 150 mg of CQ were weighed, macerated, divided into four portions and mixed with phosphate buffer, pH 7. Each solution was transferred to a 100 mL volumetric flask and completed with phosphate buffer until measuring the meniscus. 10 μL of each solution were mixed with 10 μL of the buffer and 20 μL of the $Ru(bpy)_3^{2+}$ solution. This mixture was placed directly on the surface of the CB/SPE for ECL analysis. For the CQ addition and recovery studies, 10 μL of each drug solution were mixed with 10 μL of a solution of known concentration of the standard CQ solution and 20 μL of the $Ru(bpy)_3^{2+}$ solution in order to obtain different concentrations of CQ on the samples for further ECL analysis.

The artificial urine sample was prepared using a procedure from the literature.[25] The pH of the sample solutions was approximately 6.6 (± 0.1). 1 mL of artificial urine sample was added to deionized water to give 10 mL of diluted sample. In an Eppendorf tube, 10 μL of a CQ solution were added to 50 μL of diluted urine, and then 20 μL of this solution were mixed with 20 μL of the ruthenium complex and analyzed on the CB/SPE.

Results and Discussion

Characterization of CB by scanning electron microscopy and Raman spectroscopy

Figures 1a and 1b show the SEM images of the unmodified SPE and CB/SPE, respectively, at magnitude of 10 μm. According to Figure 1a, it is observed that the surface of SPE not modified with CB was less rough than the surface of the CB/SPE. As can be seen in Figure 1b, the CB particles can form aggregates well distributed over the surface of SPE.

The Raman spectrum of the CB (Figure 1c) shows two well-defined bands at 1325 and 1577 cm^{-1}, which are associated, respectively, with the D and G bands of CB.[26-28] The D band is associated with carbon atoms characterized by sp^3 hybridization, which is related to structural defects and disorders in the carbon material.

On the other hand, the G band corresponds to the sp^2 carbon atoms of graphite in a two-dimensional hexagonal network, that is, the tensile mode of the C=C bond in the typical graphite. The strength of D and G peaks depends on the type of carbon material and can reflect the degree of graphitization of the material. The integral I_D/I_G ratio obtained from the Raman spectrum indicates the graphitization degree of carbon materials. In this sense, an integral I_D/I_G ratio obtained for the CB of about 1.75 indicates that the material presents an elevated degree of graphitization which can contribute to the exposition of edge planes of CB particles, while the bare electrode presented an I_D/I_G ratio of about 1.23. The Raman spectrum presents two other bands at about 1200 and 1500 cm^{-1}, which are associated with different defects on the CB structure. While the first band is attributed to hydrocarbon components or aliphatic moieties grafted on the basic structural units, the latter band is related to a mixture of sp^3

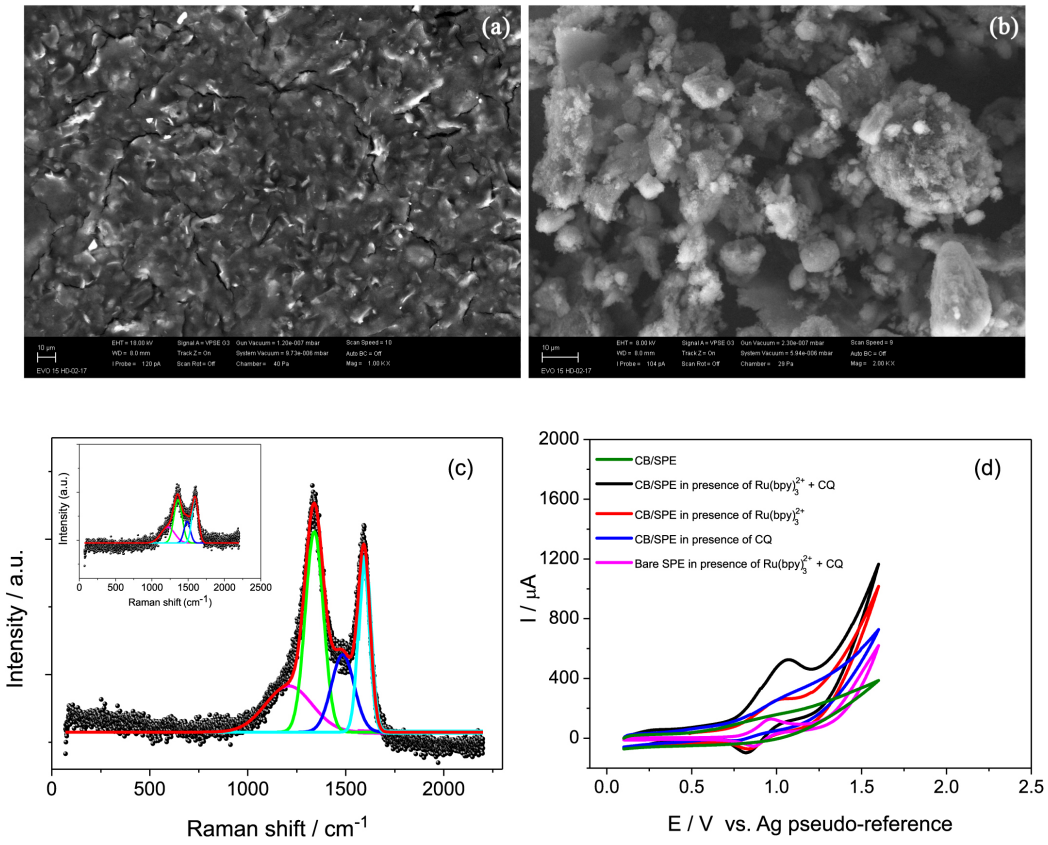


Figure 1. SEM images of surface of: (a) unmodified SPE and (b) CB/SPE; (c) Raman spectrum of carbon black. The inset of (c) is the Raman spectrum of unmodified SPE. (d) Green CV referring to the response of CB/SPE in absence de CQ and with $Ru(bpy)_3^{2+}$; blue CV referring to the redox processes of CQ on the CB/SPE; pink CV referring to the interaction of CQ with $Ru(bpy)_3^{2+}$ on the unmodified SPE, red CV referring to the redox processes of $Ru(bpy)_3^{2+}$ on the CB/SPE and the black CV referring to the interaction of CQ with $Ru(bpy)_3^{2+}$ on the SPE modified with CB. [CB]: 5 mg mL^{-1}; [$Ru(bpy)_3^{2+}$]: 5 mmol L^{-1}; [CQ]: 300 µmol L^{-1}. Scan rate: 0.1 V s^{-1}.

carbon between sp^2 carbon rings. The amorphous degree of the CB sample can also be evaluated by the intensity ratio between this band and the G band.[26]

Study of the voltammetric behavior of CQ and mixture of CQ with $Ru(bpy)_3^{2+}$ on SPE modified CB and SPE no modified

Figure 1d shows CVs of the SPE obtained under different conditions at a scan rate of 0.1 V s^{-1}. The green CV is referent to the response of the CB/SPE at buffer solution in the absence of CQ and $Ru(bpy)_3^{2+}$. The CV obtained for the CB/SPE (blue) in the presence of CQ solution presented an anodic wave between 0.9 and 1.2 V, referring to the oxidation of CQ. This CV presented an anodic current of about 20 µA. The CV obtained for the CB/SPE (red) in the presence of the $Ru(bpy)_3^{2+}$ molecule presented a redox couple referring to the oxidation and reduction of the ruthenium complex at potentials of 0.97 and 0.87 V, respectively. The peak currents obtained for the oxidation and reduction of $Ru(bpy)_3^{2+}$ on the CB/SPE platform were about 62 and –64 µA, respectively, resulting in an anodic peak current-to-cathodic peak current ratio of about 0.97. The CV of the CB/SPE in the presence of the $Ru(bpy)_3^{2+}$/CQ mixture (black) resulted in a redox couple at about 1.06 and 0.83 V, with anodic and cathodic peak currents about of 180 and –45 µA, respectively. In addition, it was performed a CV for the unmodified SPE in the presence of the $Ru(bpy)_3^{2+}$/CQ mixture (pink CV) for comparison purposes. This CV shows a redox pair referring to the oxidation and reduction of the ruthenium complex after interacting with the CQ at similar potentials to those observed with the CB/SPE. However, the peak currents for the $Ru(bpy)_3^{2+}$/CQ mixture obtained in the absence of CB were about 80 µA for the oxidation and around –35 µA for the reduction, which were much lower than the currents measured with the CB/SPE in the presence of the $Ru(bpy)_3^{2+}$/CQ mixture (black CV). This result suggests that CB improves the reaction between the co-reagent and $Ru(bpy)_3^{2+}$ luminophore, thus favoring the detection of CQ with greater sensitivity.

In this context, the comparison between the black and pink CVs suggests also that this increase in the anodic and cathodic peak currents observed for the CB modified SPE may be related to the fact that the ruthenium complex and

chloroquine present more intense electron transfer on the CB/SPE surface. In addition, as can be seen in black CV, the interaction between $Ru(bpy)_3^{2+}$ and CQ on the CB/SPE results in an anodic peak current about 9 times higher than the current observed for the CQ in the absence of the ruthenium complex on the CB/SPE (blue CV). Finally, it can be seen that the CB and the $Ru(bpy)_3^{2+}$ luminophore presented a fundamental role in the chloroquine detection process, thus allowing an increase in the sensitivity of the system.

Electrochemiluminescent behavior of the chloroquine, ruthenium complex and the ruthenium complex/chloroquine mixture

Figure 2 shows the ECL-potential curves obtained for: CB/SPE in CQ solution (Figure 2a); CB/SPE in $Ru(bpy)_3^{2+}$ solution (Figure 2b); CB/SPE in $Ru(bpy)_3^{2+}$/CQ mixture (Figure 2c); and unmodified SPE in $Ru(bpy)_3^{2+}$/CQ (Figure 2d). ECL-potential curves (a) and (b) show the ECL signals obtained for CB/SPE in CQ and ruthenium complex solutions, respectively. The magnitude of the ECL signal obtained for the CB/SPE in CQ and ruthenium solution were only 2 and 8 a.u., respectively.

On the other hand, the ECL signal obtained for the CB/SPE in a ruthenium complex/CQ mixture was about 295 a.u. at a potential of 1.3 V, while the same mixture showed an ECL signal of 164 a.u. at a potential of 1.13 V over the unmodified SPE. This result suggests that the presence of CB is essential for the efficient generation of an ECL signal suitable for the sensitive detection of CQ. The SPE modified with CB provides a greater intensity of electrochemiluminescent signal, which can be associated with an improvement in the electron transfer process of the electrode after modification with the carbon material and, consequently, a higher efficiency of the reaction between

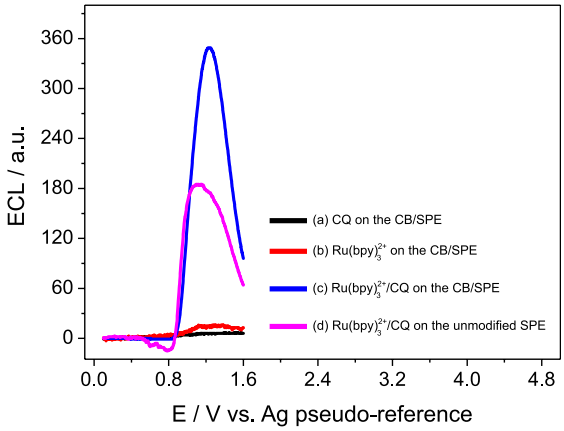


Figure 2. ECL-potential curves obtained for: (a) CB/SPE in CQ solution; (b) CB/SPE in $Ru(bpy)_3^{2+}$ solution; (c) CB/SPE in $Ru(bpy)_3^{2+}$/CQ mixture; and (d) unmodified SPE in $Ru(bpy)_3^{2+}$/CQ. Scan rate: 0.1 V s^{-1}.

the ruthenium complex with the analyte studied in this work. This interaction process is proposed in equations 1-4 and presented in Scheme 1.

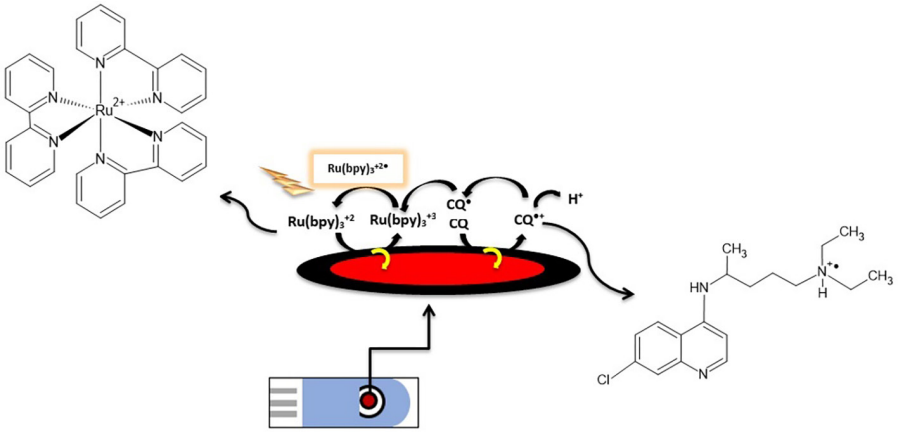
$$Ru(bpy)_3^{2+} - e^- \rightarrow Ru(bpy)_3^{3+} \quad (1)$$

$$CQ - e^- \rightarrow CQ^{\bullet+} \rightarrow CQ^{\bullet} + H^+ \quad (2)$$

$$Ru(bpy)_3^{3+} + CQ^{\bullet} \rightarrow Ru(bpy)_3^{2+\bullet} + \text{products} \quad (3)$$

$$Ru(bpy)_3^{2+\bullet} \rightarrow Ru(bpy)_3^{2+} + h\upsilon \quad (4)$$

This mechanism indicates that the luminophore ($Ru(bpy)_3^{2+}$) and the co-reactant (CQ) will initially be oxidized. Therefore, the oxidized chloroquine will release the H^+ ion forming a reactive species ($CQ^{\bullet}$) that will interact with the luminophore in its oxidized state, leading to the formation of a product and the luminophore in the excited state. This species emits light at a specific wavelength when it returns to its background state. In summary, in order to find an efficient emission of energy by the luminophore, it is necessary that the co-reagent and the luminophore present some affinity. If this does



Scheme 1. Schematic representation of the electrochemiluminescent reaction between chloroquine and the ruthenium complex on the CB-modified SPE surface.

not occur, there will be no generation of the ECL signal, and the technique cannot be utilized for the detection of a specific species.

Effect of the concentration of the luminophore, carbon black, pH and supporting electrolyte

Figure 3a shows the ECL-potential curves referring to the ECL measurements for the SPE modified with different concentrations of $Ru(bpy)_3^{2+}$ (0.5; 1.5; 2.5; 5.0; 15.0 and 25.0 mmol L^{-1}) and a CQ concentration of 300 µmol L^{-1} in a phosphate buffer solution 0.1 mol L^{-1}, pH 7.0 and a scan rate of 0.1 V s^{-1}.

As can be seen from Figure 3a, the plot of the ECL signal as a function of the ruthenium concentration shows that the ECL signal increases with increasing concentration of the ruthenium complex from 0.5 to 5.0 mmol L^{-1} and decreases at higher concentrations. This result suggests that ruthenium concentrations below 5.0 mmol L^{-1} are not enough to reach the maximum amount of light emission from the luminophore to interact with the co-reactant. In addition, it is observed that using a concentration of $Ru(bpy)_3^{2+}$ above 5.0 mmol L^{-1} can make the interaction with CQ difficult, thus decreasing signal intensity. Based on this information, a ruthenium complex concentration of 5 mmol L^{-1} was fixed for further studies.

The behavior of the CB analytical signal on the SPE surface for the $Ru(bpy)_3^{2+}$/CQ mixture was also evaluated for different concentrations of this material: 1.0; 3.0; 5.0; 7.0; and 10.0 mg mL^{-1} CB (Figure 3b). The insertion of Figure 3b shows that the ECL signal increases with the use of 1.0 to 5.0 mg mL^{-1} of CB and decreases for values above 5.0 mg mL^{-1}. However, the analytical signal tends to decrease when using a CB concentration above 5 mg mL^{-1}. This result can be associated with the fact that the excess of CB makes the electronic transfer process from the solution to the electrode surface difficult, thus decreasing the ECL signal strength. For this reason, the concentration of 5.0 mg mL^{-1} of CB was fixed for the following studies.

The effects of pH and supporting electrolyte were also investigated in order to assess at which pH value and under which type of buffer solution the $Ru(bpy)_3^{2+}$/CQ mixture

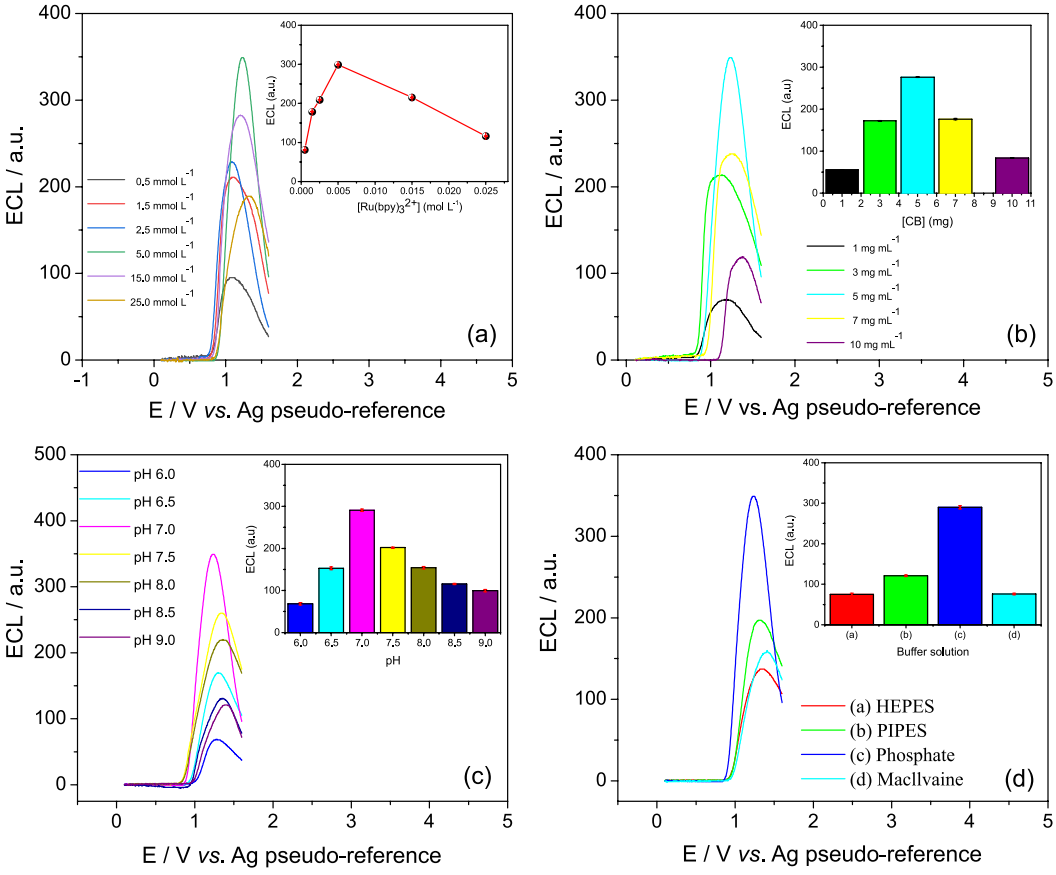


Figure 3. (a) ECL-potential curves for different concentrations of the $Ru(bpy)_3^{2+}$ complex. Inset: plot of the ECL signal *versus* [$Ru(bpy)_3^{2+}$]. Data obtained from Figure 3a. (b) ECL-potential curves for different concentrations of the carbon black (CB) with a [$Ru(bpy)_3^{2+}$]: 5 mmol L^{-1}. Inset: plot of the ECL intensity *versus* [CB]. Data obtained from Figure 3b. (c) ECL-potential curves for different pH values. Inset: plot of the ECL signal *versus* pH. Data obtained from Figure 3c. [$Ru(bpy)_3^{2+}$]: 5.0 mmol L^{-1}. (d) ECL-potential curves for different buffer solutions. Inset: plot of the ECL signal *versus* buffer solution. Data obtained from Figure 3d. Scan rate: 0.1 V s^{-1}. [CQ]: 300 µmol L^{-1}.

has the highest ECL signal intensity over the CB/SPE. Figure 3c shows the influence of the pH of the supporting electrolyte in the presence of 300 μmol L^{-1} of CQ at different pH values (6.0; 6.5; 7.0; 7.5; 8.0; 8.5; 9.0). According to the results shown in Figure 3c, it is observed that the ECL signal intensity increased at pH values from 6.0 to 7.0 and decreased from this pH value to higher pH values (inset of Figure 3c). According to the observed results, higher signal intensity was observed only in the medium in which the concentration of hydroxyl ions and hydronium ions is the same. The decrease in the ECL signal for pH values below and above 7.0 can be caused by a limiting availability of the oxidation product of the ruthenium complex to maintain an efficient increase in the electrochemiluminescence signal, as the luminophore can act as a limiting reagent. In this sense, pH 7.0 was fixed for further studies.

Figure 3d shows the results for four different types of buffer solutions, prepared at a concentration of 0.1 mol L^{-1}, pH 7.0. This figure clearly shows that the ECL system presented the best response in phosphate buffer compared to the other buffers. This result can be associated with the smaller size of the ion, which may facilitate greater diffusion of chloroquine on the surface of the CB/SPE.

Analytical characterization and evaluation of the precision and accuracy of the proposed ECL method

After the optimization of the experimental parameters, the analytical characterization of the proposed ECL method was performed. For this purpose, the linear sweep voltammetry was used to obtain the ECL-potential curves by varying the concentration of CQ and fixing the concentration of the $Ru(bpy)_3^{2+}$ complex (5 mmol L^{-1}) (Figure 4a). Final CQ concentrations were: 0; 0.5; 25; 50; 75; 125; 250; 300; 400; and 500 μmol L^{-1} and from the ECL measurements, the ECL plot was constructed as a function of the CQ concentration, with regression equation Y_{ECL} (a.u.): 3.95 + 1.04 [CQ] (μmol L^{-1}) with a sensibility of 3.95 a.u. μmol^{-1} L and a correlation coefficient equal to 0.998 (Figure 4b). The limit of detection (LOD) and quantification (LOQ) were determined experimentally. The lowest detectable concentration was 0.5 μmol L^{-1} (signal-to-noise, S/N = 3), while the LOQ was 1.66 μmol L^{-1} (S/N = 10).

These results and the type of electrolyte and pH used were compared with other works in the literature (Table 1).[29-40] The proposed method provided good results compared to different methods for determining CQ, presenting a low LOD and wide linear response range. The ECL method is quite simple, it uses a low-cost carbon material to modify the electrode surface, it does not require specialized training for its operation, and it can be considered an environmentally correct method because it uses only 40 microliters of electrolyte solution to carry out the electrochemiluminescent measurements.

The precision of the method was evaluated considering the relative standard deviation (RSD) of ECL measurements of the CB/SPE for the $Ru(bpy)_3^{2+}$/CQ mixture. Measurements were performed on the same working day (Figure 5a) and also on different days (Figure 5b) under the optimized experimental conditions (0.1 mol L^{-1} of phosphate buffer solution, pH 7.0). As can be seen in Figure 5a, the measurements obtained presented a RSD of 0.92%, indicating good precision for measurements performed on the same day. The RSD was calculated for the measurements shown in Figure 5b, and it was observed that the proposed method has excellent reproducibility with a RSD of 2.12%.

Additionally, the selectivity of the method was investigated using six possible interferents: ascorbic acid, citric acid, glucose, potassium chloride, sodium chloride and oflaxicin. Measurements were made under optimized conditions in the presence of CQ/interferent in the 1:1

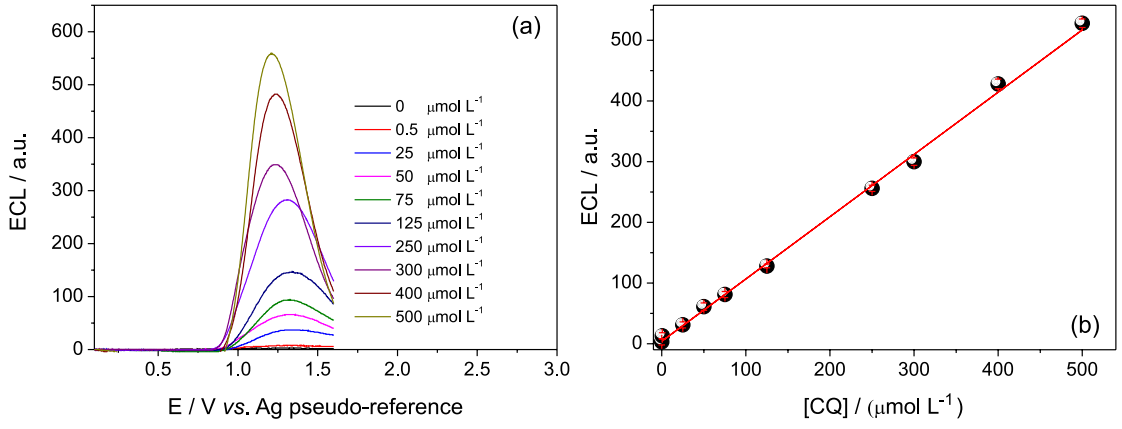


Figure 4. (a) ECL intensity for different CP concentrations (0-500 μmol L^{-1}). (b) Analytical curve obtained from the data in Figure 4a. [$Ru(bpy)_3^{2+}$]: 5.0 mmol L^{-1}. Scan rate: 0.1 V s^{-1}.

Table 1. Comparison of analytical parameters of different methods for determining chloroquine

Method	Electrolyte/pH	LOD	Linear range	Reference
DPV	phosphate/pH 5.5	0.01 μg mL^{-1}	0.068-6.88 μg mL^{-1}	29
Spectrometry UV-Vis	water	0.073 μg mL^{-1}	10.88-30.56 μg mL^{-1}	30
HPLC	triethylamine:methanol (25:75), phosphate buffer/pH 3.0	6 μg mL^{-1}	30-360 μg mL^{-1}	31
RP-HPLC	methanol:water (70:30)/pH 2.8	0.1 μg mL^{-1}	1-100 μg mL^{-1}	32
Spectrophotometric	monossodium phosphate/pH 6.8	–	7.2-19.2 μg mL^{-1}	33
Spectrophotometric	acetate/pH 4.5	0.128 μg mL^{-1}	1.25-8.75 μg mL^{-1}	34
LC	–	0.17 μg mL^{-1}	0.08-5.70 μg mL^{-1}	35
CV	phosphate/pH 6.0	0.04 μmol L^{-1}	0.5-82.4 μmol L^{-1}	36
DPV	PBS/pH 7.0	4 μmol L^{-1}	5-75 μmol L^{-1}	37
SWV	Britton-Robson/pH 6.0	0.002 μmol L^{-1}	0.01-0.25 μmol L^{-1}	38
DPV	acetate/pH 4.0	3×10^{-8} mol L^{-1}	1×10^{-7}-1×10^{-5} mol L^{-1}	39
Potentiometric	acetate/pH 5.0	7.1×10^{-6} mol L^{-1}	–	40
ECL (CB/SPE)	phosphate/pH 7.0	0.5 μmol L^{-1} [a] 0.26 mg L^{-1} [a] 0.00026 mg mL^{-1} [a] 0.26 μg mL^{-1} [a]	0.5-500 μmol L^{-1} 0.26-260 mg L^{-1} 0.00026-0.26 mg mL^{-1} 0.26-260 μg mL^{-1}	this work

[a]Experimental limit of detection. LOD: limit of detection; DPV: differential pulse voltammetry; HPLC: high performance liquid chromatography; RP-HPLC: reversed-phase high performance liquid chromatography; LC: liquid chromatography; CV: cyclic voltammetry; SWV: square-wave voltammetry; ECL (CB/SPE): carbon black/screen printed electrode.

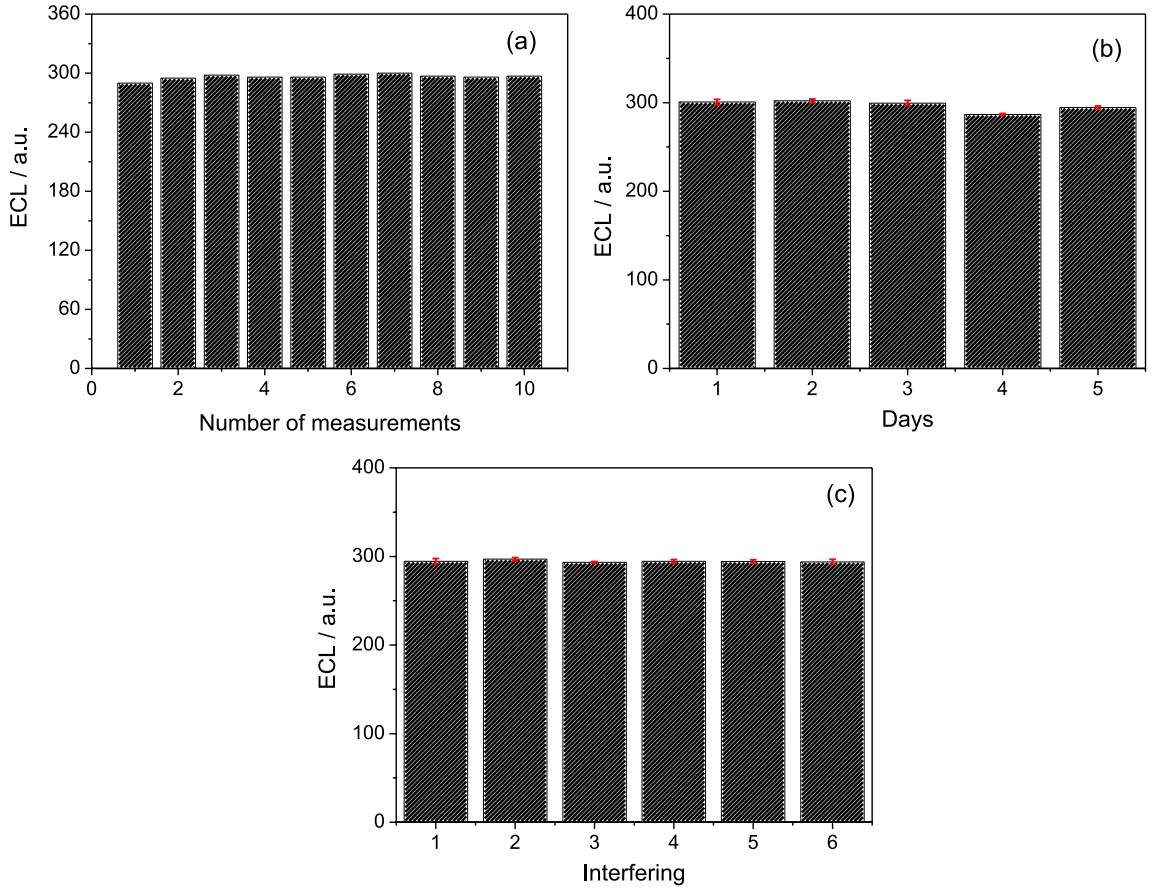


Figure 5. (a) ECL intensity measurements performed on the same working day and (b) on different days. Influence of possible interferences on the ECL signal strength for: (1) CQ + ascorbic acid; (2) CQ + citric acid; (3) CQ + glucose; (4) CQ + KCl; (5) CQ + NaCl; and (6) CQ + oflaxacin. The experiments were carried out in 0.1 mol L^{-1} of phosphate buffer, pH 7.0.

ratio. In Figure 5c none of the species showed significant interference in the ECL signal intensity, which demonstrates the excellent selectivity of the proposed method.

Finally, the proposed method was applied to drug samples in which six different concentrations of CQ (1, 10, 50, 100, 150, and 200 mg L^{-1}) and in artificial urine samples (four different concentrations of CQ), to assess the accuracy of the proposed method (Tables 2 and 3). For the different concentrations of CQ in tablet and artificial urine samples, concentration percentages from 100 to 103% and 99 to 103% were obtained, respectively, demonstrating that the proposed method has good accuracy.

Table 2. Study of chloroquine addition and recovery in chloroquine tablet

[CQ] added / (mg L^{-1})	[CQ] expected / (mg L^{-1})	[CQ] found / (mg L^{-1})	Recovery[a] / %
0	0	152 ± 1	–
1	153	153 ± 1	100
10	162	164 ± 2	101
50	202	208 ± 2	103
100	252	254 ± 1	101
150	302	302 ± 2	100
200	352	359 ± 4	102

Values obtained after considering the dilution factor. [a]RSD ≤ 1%. CQ: chloroquine diphosphate.

Table 3. Study of chloroquine addition and recovery in chloroquine artificial urine

[CQ] added / (mg L^{-1})	[CQ] expected / (mg L^{-1})	[CQ] found / (mg L^{-1})	Recovery[a] / %
30	30	33 ± 3	103
50	50	51 ± 1	102
80	80	79 ± 4	99
130	130	130 ± 2	100

Values obtained after considering the dilution factor. [a]RSD ≤ 5%. CQ: chloroquine diphosphate.

Conclusions

The present work describes a simple method based on electrogenerated chemiluminescence to determine chloroquine in a drug sample used to treat many diseases and an artificial biological fluid sample. The carbon black modified screen-printed electrode showed good precision and accuracy for determining chloroquine. Raman spectroscopy has shown that the carbon black sample presented an elevated degree of graphitization which can contribute to the exposition of edge planes of CB particles leading to a better electrochemical performance of CB/SPE electrode compared to bare SPE. The method showed a wide linear working range, good precision and accuracy in drug sample used in the treatment of malaria and artificial urine sample.

Acknowledgments

The authors are grateful to FAPEMA (INFRA-02021/21; INFRA-02050/21; UNIVERSAL-01057/19; UNIVERSAL-06535/22; POS-GRAD-02432/21), CNPq (308204/2018-2; 309828/2020-1; 305806/2020-3), and Instituto Nacional de Ciência e Tecnologia em Bioanalítica (465389/2014-7) for financial support. Authors would like to thank to the Laboratories of Impedance Spectroscopy and Multiuser Centre for Research in Materials and Biosystems (CeMatBio) of the Federal University of Maranhão (UFMA), for the support with the SEM measurements.

Author Contributions

Jeovana C. Pacheco was responsible for conceptualization, data curation; formal analysis, investigation, methodology, validation, visualization, writing-original draft, review and editing; Jhonathas A. R. Brito for conceptualization, formal analysis, investigation, methodology, validation, visualization, writing-original draft, review and editing; Clenilton Costa dos Santos for conceptualization, formal analysis, funding acquisition, resources, writing - review and editing; Cícero W. B. Bezerra for conceptualization, formal analysis, resources, writing - review and editing; Flávio Santos Damos for conceptualization, funding acquisition, project administration, resources, writing - original draft, writing - review and editing; Rita C. S. Luz for conceptualization, data curation, formal analysis, funding acquisition, project administration, resources, supervision, visualization, writing-original draft, review and editing.

References

1. Tönnesmann, E.; Kandolf, R.; Lewalter, T.; *Immunopharmacol. Immunotoxicol.* **2013**, *35*, 434. [Crossref]
2. Juurlink, D. N.; *Can. Med. Assoc. J.* **2020**, *192*, E450. [Crossref]
3. Restrepo-Valencia, C. A.; Domínguez, E. H.; Orozco-De La Hoz, C.; *AMC, Acta Med. Colomb*. **2022**, *47*, 49. [Crossref]
4. Chatre, C.; Roubille, F.; Vernhet, H.; Jorgensen, C.; Pers, Y. P.; *Drug Saf.* **2018**, *41*, 919. [Crossref]
5. Goldman, A.; Bomze, D.; Dankner, R.; Hod, H.; Meirson, T.; Boursi, B.; Maor, E.; *Br. J. Clin. Pharmacol.* **2020**, *87*, 1432. [Crossref]
6. Kelly, J. C.; Wasserman, G. S.; Bernard, W. D.; Schultz, C.; Knapp, J. F.; *Ann. Emerg. Med*. **1990**, *19*, 47. [Crossref]
7. Gustafsson, L. L.; Walker, O.; Álvan, G.; Beermann, B.; Estevez, F.; Gleisner, L.; Lindstrom, B.; Sjoqvist, F.; *Br. J. Clin. Pharmacol.* **1983**, *15*, 471. [Crossref]

8. Martins, Y. A.; Gonçalves, T. M.; Lopez, R. F. V.; *DARU, J. Pharm. Sci*. **2021**, *29*, 223. [Crossref]
9. Maciel, T. R.; Pacheco, C. O.; Ramos, P. F.; Ribeiro, A. N. C. F.; dos Santos, R. B.; Haas, S. E.; *J. Appl. Pharm. Sci.* **2023**, *13*, 106. [Crossref]
10. Cheomung, A.; Na-Bangchang, K.; *J. Pharm. Biomed. Anal.* **2011**, *55*, 1031. [Crossref]
11. Erarpat, S.; Bodur, S.; Öner, M.; Günkara, O. T.; Bakirdere, S.; *J. Chromatogr. A* **2021**, *1651*, 462273. [Crossref]
12. Annuryanti, F.; Darmawati, A.; Miatmoko, A.; Kustiawan.; *Res. J. Pharm. Technol.* **2020**, *13*, 1293. [Crossref]
13. Sharma, S.; Simsek, H.; *Chemosphere* **2019**, *221*, 630. [Crossref]
14. Lu, M.; Deng, Y.; Luo, Y.; Lv, J.; Li, T.; Xu, J.; Chen, S. W.; Wang, J.; *Anal. Chem.* **2019**, *91*, 888. [Crossref]
15. Li, L.; Chen, Y.; Zhu, J. J.; *Anal. Chem.* **2017**, *89*, 358. [Crossref]
16. Huang, W.; Hu, G. B.; Yao, L. Y.; Yang, Y.; Liang, W. B.; Yuan, R.; Xiao, D. R.; *Anal. Chem.* **2020**, *92*, 3380. [Crossref]
17. Ma, C.; Cao, Y.; Gou, X.; Zhu, J. J.; *Anal. Chem.* **2020**, *92*, 431. [Crossref]
18. Wei, H.; Wang, E.; *Luminescence* **2011**, *26*, 77. [Crossref]
19. Squissato, A. L.; Almeida, E. S.; Silva, S. G.; Richter, E. M.; Batista, A. D.; Munoz, R. A. A.; *TrAC, Trends Anal. Chem.* **2018**, *108*, 210. [Crossref]
20. e Pavão, D. P.; Botelho, C. N.; Fernandes, R. N.; dos Santos, C. C.; Damos, F. S.; Luz, R. C. S.; *Electroanalysis* **2020**, *32*, 1498. [Crossref]
21. Talarico, D.; Arduini, F.; Costantino, A.; Carlo, M. D.; Compagnone, D.; Moscone, D.; Palleschi, G.; *Electrochem. Commun.* **2015**, *60*, 78. [Crossref]
22. Ali, M. R.; Bacchu, M. S.; Al-Mamun, M. R.; Ahhomed, M. S.; Aly Saad Aly, M.; Khan, M. Z. H.; *RSC Adv.* **2021**, *11*, 15565. [Crossref]
23. Caetano, L. P.; Lima, A. P.; Tormin, T. F.; Richter, E. M.; Espindola, F. S.; Botelho, F. V.; Munoz, R. A. A.; *Electroanalysis* **2018**, *30*, 1870. [Crossref]
24. Bard, A. J.; Faulkner, L. R.; *Electrochemical Methods*, 2nd ed.; John Wiley & Sons: New York, USA, 2001.
25. Laube, N.; Mohr, B.; Hesse, A.; *J. Cryst. Growth* **2001**, *233*, 367. [Crossref]
26. Baldissarelli, V. Z.; Benetoli, L. O. B.; Cassini, F. A.; de Souza, I. G.; Debacher, N. A.; *J. Braz. Chem. Soc.* **2014**, *25*, 126. [Crossref]
27. Pawlyta, M.; Rouzaud, J. N.; Duber, S.; *Carbon* **2015**, *84*, 479. [Crossref]
28. Bokobza, L.; Bruneel, J.; Couzi, M.; *J. Carbon Res.* **2015**, *1*, 77. [Crossref]
29. Mashhadizadeh, M. H.; Akbarian, M.; *Talanta* **2009**, *78*, 1440. [Crossref]
30. Desta, K.; Amare, M.; *Chem. Int.* **2017**, *3*, 288. [Crossref]
31. Coelho, A. S.; Chagas, C. E. P.; de Pádua, R. M.; Pianetti, G. A.; Fernandes, C.; *J. Pharm. Biomed. Anal.* **2017**, *145*, 248. [Crossref]
32. Shamshad, H.; Sayqal, A.; Zeb, J.; Mirza, A. Z.; *J. Chromatogr. Sci.* **2021**, *59*, 923. [Crossref]
33. e Souza, M. A. C.; Reis, N. F. A.; Batista, L. S.; César, I. C.; Fernandes, C.; Pianetti, G. A.; *Curr. Pharm. Anal*. **2020**, *16*, 5. [Crossref]
34. Nagaraja, P.; Shrestha, A. K.; Shivakumar, A.; Gowda, A. K.; *J. Food Drug Anal.* **2010**, *18*, 239. [Crossref]
35. Rivas-Granizo, P.; Santos, S. R. C. J.; Ferraz, H. G.; *Chromatographia* **2009**, *69*, 137. [Crossref]
36. Srivastava, M.; Tiwari, P.; Mall, V. K.; Srivastava, S. K.; Prakash, R.; *Microchim. Acta* **2019**, *186*, 415. [Crossref]
37. Silva, M. K. L.; Sousa, G. S.; Simoes, R. P.; Cesarino, I.; *J. Solid State Electrochem.* **2022**, *26*, 581. [Crossref]
38. Oliveira, G. G.; Azzi, D. C.; Silva, T. A.; de Oliveira, P. R.; Fatibello-Filho, O.; Janegitz, B. C.; *J. Carbon Res.* **2020**, *6*, 75. [Crossref]
39. Radi, A.; *Talanta* **2005**, *65*, 271. [Crossref]
40. Saad, B.; Kanapathy, K.; Ahmad, M. N.; Hussin, A. H.; Ismail, Z.; *Talanta* **1991**, *38*, 1399. [Crossref]

Submitted: December 12, 2022
Published online: April 14, 2023

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