


Ferricyanide Confined in a Protonated Amine-Functionalized Silica Film on Gold: Application to Electrocatalytic Sensing of Nitrite Ions

Pedro L. Almeida Júnior^a, Carlos H. S. Mendes^a, Ingrid A. F. S. Lima^a, Mônica F. Belian^a, Severino C. B. Oliveira^a, Christopher M. A. Brett^b, and Valberes B. Nascimento^a 

^aDepartment of Chemistry, Federal Rural University of Pernambuco, Recife, Pernambuco, Brazil; ^bDepartment of Chemistry, Faculty of Science and Technology, University of Coimbra, Coimbra, Portugal

ABSTRACT

An amine-functionalized porous sol-gel silica film was shown to be an effective platform to immobilize small anionic redox mediators of high solubility on solid electrodes by electrostatic interaction. The highly soluble mediator hexacyanoferrate was used as a model. The film was grown and firmly anchored on a gold electrode surface via thiol groups of a self-assembled monolayer of (3-mercaptopropyl) trimethoxysilane. Film growth and thickness were controlled by electrochemical modulation of pH at the electrode/solution interface in a sol of a hydrolyzed solution of tetraethoxysilane and 3-[2-(2-aminoethylamino)ethylamino]propyltrimethoxysilane by the application of a negative potential to the electrode. Protonation of the amine groups made the amine-functionalized surface useful to immobilize hexacyanoferrate on gold. Thus, the immobilization is pH dependent, being highly effective in strongly acidic medium. Cyclic voltammetry and scanning electron microscopy were used to characterize the film and to optimize the experimental conditions. The stability of the film was demonstrated by applying the catalytic properties of the hexacyanoferrate containing surface for nitrite sensing using a flow injection analysis (FIA) system. Under the optimized conditions, the sensor exhibited high sensitivity, low detection limit, easy handling, and stability with a linear range from 1.0 to 40.0 $\mu\text{mol L}^{-1}$ and a detection limit of 0.53 $\mu\text{mol L}^{-1}$ based on a signal-to-noise ratio of 3. The sensor was successfully applied to nitrite determination in water samples using FIA with excellent recoveries.

ARTICLE HISTORY

Received 4 April 2017
Accepted 9 May 2017

KEYWORDS

Cyclic voltammetry; flow injection analysis; hexacyanoferrate; nitrite quantification; scanning electron microscopy; self-assembled monolayers; sol-gel

Introduction

Sol-gel materials find diverse application in electrochemistry, including the development of modified electrodes, electrochemical sensors, and biosensors (Lev et al. 1997). Nanostructures based on sol-gel-derived materials can be assembled and studied using electrochemical methods (Collinson 2007). The development of modified electrodes has taken advantage of progress in sol-gel technology such as electrochemical control of gelification by tuning of the pH at the electrode surface, where the generation of a localized alkaline region catalyzes the formation of a silica matrix directly on the electrified conducting surface (Shacham, Avnir, and Mandler 1999). The mild conditions involved in

CONTACT Valberes B. Nascimento  valberes@pq.cnpq.br  Department of Chemistry, Federal Rural University of Pernambuco, Recife, Pernambuco 52171-900, Brazil.

© 2017 Taylor & Francis

the sol-gel process and the wide-open structures of sol-gel-derived materials, usually containing many easily accessible active sites, make them particularly attractive to host recognition biomolecules, and other chemicals or more complex materials such as bacteria and cells in an active functional state that can be used in constructing (bio)sensors for analytical purposes (Walcarius et al. 2005). Among numerous other possibilities offered by the sol-gel chemistry, it has also been demonstrated that an amine-functionalized porous silica film can be used to reversibly preconcentrate cations in a pH-dependent way (Walcarius and Sibottier 2005).

Most of the research in developing electrochemical sensors has been devoted to confine important redox mediators on solid electrode surfaces. The electrochemical properties and the ability to catalyze heterogeneous oxidation and reduction processes of the redox couple hexacyanoferrate (III)/(II) ($[\text{Fe}(\text{CN})_6]^{3-/4-}$), usually referred to as hexacyanoferrate, has made it widely useful as a model system in both fundamental and applied electrochemistry (Moore, Banks, and Compton 2004; Xiang et al. 2008), being one of the most commonly used inorganic mediators in biosensors (Chaubey and Malhotra 2002). Nevertheless, such redox mediators immobilized on surfaces tend to leach and disperse into the bulk of the solution, which results instability and significant loss of the electrochemical signal. High-performance electrochemical sensors with confinement of low-molecular weight and soluble mediators remain a challenge and difficulties to confine hexacyanoferrate on electrode surfaces have been reported (Xiang et al. 2008).

Electrostatic interaction between cationic species and hexacyanoferrate anions has been largely used to confine this mediator as counter ion on polycation and conducting films deposited on electrode surfaces (Gros, Durliat, and Comtat 2000; Kumar et al. 2001; Gros and Comtat 2004; Raof, Ojani, and Nadimi 2004; Hübsch et al. 2005; Raof, Ojani, and Nadimi 2005; Vasantha and Chen 2005; Noguchi and Anzai 2006; Oukil, Makhloufi, and Saidani 2007; Takita, Yoshida, and Anzai 2007; Grieshaber et al. 2008; Lawal and Adeloju 2010; Zahn et al. 2010; Ding et al. 2013; Qin et al. 2013; Oukil et al. 2014; Wu et al. 2015). These examples involve confinement of hexacyanoferrate as the doping anion of electrochemically deposited conducting films of polypyrroles (Gros, Durliat, and Comtat 2000; Kumar et al. 2001; Gros and Comtat 2004; Raof, Ojani, and Nadimi 2004, 2005; Oukil, Makhloufi, and Saidani 2007; Lawal and Adeloju 2010; Ding et al. 2013; Oukil et al. 2014; Wu et al. 2015) and of polythiophenes (Vasantha and Chen 2005). Multilayer films of layers of oppositely charged polyelectrolytes, such as poly(L-glutamic acid)/poly(allylamine)hydrochloride (Hübsch et al. 2005; Takita, Yoshida, and Anzai 2007; Grieshaber et al. 2008; Zahn et al. 2010), and poly(L-glutamic acid)/poly(allylamine)hydrochloride alternated with the anionic polysaccharides carboxymethylcellulose or alginic acid (Noguchi and Anzai 2006) or poly(4-styrenesulfonate) (Li et al. 2010) have also been important approaches for the confinement of hexacyanoferrate. Composite films, such as poly(diallyldimethylammonium)-alginate have also been evaluated (Qin et al. 2013). Confinement of hexacyanoferrate ions on positively-charged sites arising from the protonated amino groups of organic polymer films has been discussed and demonstrated (Wang and Anzai 2007).

The driving force for the development of accurate, rapid, and simple methods for nitrite determination in water and food has been its potential toxicity to human health associated with the carcinogenicity of *N*-nitrosoamines formed by secondary amines with nitrite ions in the stomach, although a causative link between dietary exposure to nitrite/nitrate ions

and the risk of stomach cancer is still controversial (Bryan et al. 2012). Numerous routes for electrode modification by immobilization of electrocatalysts have been pursued in an attempt to overcome the poor sensitivity and lack of reproducibility in the electroanalysis of nitrite by direct oxidation or reduction at bare electrodes (Moorcroft, Davis, and Compton 2001; Kozub, Rees, and Compton 2010). Advantages and drawbacks of methods for nitrite determination have been reviewed (Moorcroft, Davis, and Compton 2001). The possible merits of using chemically modified electrodes rather than unmodified ones have been critically assessed (Kozub, Rees, and Compton 2010). Despite the important advances in nitrite sensing, a robust electrochemical method still remains a challenge and much research effort has been devoted to this issue (Moorcroft, Davis, and Compton 2001; Kozub, Rees, and Compton 2010; Dreyse et al. 2011; Qin et al. 2013; Jiang, Fan, and Du 2014; Rahim et al. 2014; Hui et al. 2016; Zuo et al. 2016).

This work demonstrates for the first time that, just by tuning the pH, an amine-functionalized sol-gel silica film from 3-[2-(2-aminoethylamino)ethylamino]propyltrimethoxysilane, electrochemically grown on a modified gold electrode surface, can adsorb and efficiently confine the highly soluble anionic redox mediator hexacyanoferrate in an electroactive state. Positively charged sites, arising from the protonated amino groups, are responsible for the strong binding of hexacyanoferrate by electrostatic interaction (Wang and Anzai 2007). Thus, the confinement of hexacyanoferrate on amino groups is pH dependent, being effective in highly acidic medium. The electrochemical behavior of the assembly was studied by cyclic voltammetry, where cyclic voltammograms in blank supporting electrolyte solution showed that confined hexacyanoferrate maintains its redox activity. Catalytic reduction of nitrite ions in a flow system demonstrated the stability of the film.

Experimental

Chemicals and sample solutions

Chemicals were all of analytical reagent grade and were used as received. Tetraethoxysilane 98%, (3-mercaptopropyl)trimethoxysilane 95%, and (3-aminopropyl)trimethoxysilane were acquired from Sigma-Aldrich; 3-[2-(2-aminoethylamino)ethylamino]propyltrimethoxysilane was from Acros Organics and ethanol 99% was from F. Maia. Potassium hexacyanoferrate (III) and sodium nitrite were obtained from Merck and LabSynth, respectively. All solutions were prepared and subsequent dilutions made in ultra-pure water from a Millipore Milli-Q system (resistivity $\geq 18 \text{ M}\Omega \text{ cm}$). Buffers of acetate, pH 4.6, phosphate, pH 7.0, and ammonium, pH 9.1, (ionic strength $\sim 0.2 \text{ mol L}^{-1}$), were used as supporting electrolytes in the pH study. In all other experiments, unless specified, a $0.1 \text{ mol L}^{-1} \text{ HNO}_3$ solution was used as supporting electrolyte. Samples of ground water and of surface river water were freshly collected, and drinking water was acquired from local dealers. All samples were analyzed without pretreatment, except filtering to remove insoluble particles and diluting with the supporting electrolyte.

Apparatus

All voltammetric experiments were performed using an Autolab potentiostat/galvanostat (Model PGSTAT302 N, Metrohm-Autolab, Utrecht, Netherlands) with a three-electrode

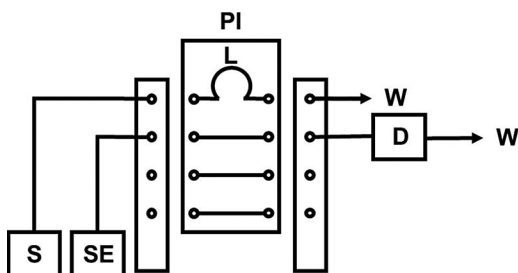


Figure 1. FIA system diagram. Note: S, sample or standard solutions; SE, supporting electrolyte; L, sampling loop of 100 μL (20 cm); PI, proportional injector; D, detector; W, waste; FIA, flow injection analysis.

system. The control of voltammetric parameters, acquisition and processing of data were performed by General Purpose Electrochemical System software version 4.9, Metrohm-Autolab, Utrecht, Netherlands.

A conventional 20 mL electrochemical cell was used in the voltammetric studies. Amperometric flow injection analysis (FIA) experiments were performed using a wall-jet flow cell configuration, as described in Lima et al. (1997), with an Ismatec peristaltic pump (Model IPC-8) and a manually-operated proportional injector, Figure 1. A gold disk ($\phi = 3$ mm) was used as the base-working electrode, with an Ag/AgCl (3 mol L^{-1} KCl) and a platinum wire as reference and auxiliary electrodes, respectively. All experiments were performed at room temperature ($25 \pm 1^\circ\text{C}$).

Preparation of the modified electrode

The procedure for modification of the gold electrode involved three successive steps: formation of a self-assembled monolayer of (3-mercaptopropyl)trimethoxysilane molecules on a gold surface; electrodeposition of an amine-functionalized silica thin-film on the monolayer; and electrostatic adsorption of hexacyanoferrate ions on the positively-charged silica film.

The gold electrode was first cleaned by immersing it for 20 min in a stirred 1:1 HF:H₂O mixture, then polished on a microcloth pad with an aqueous slurry of alumina (0.05 μm) for about 5 min to give a mirror finish, thoroughly rinsed with water and finally allowed to dry in air.

The formation of the self-assembled monolayer was performed by immersing the cleaned gold electrode in a quiescent 20 mmol L^{-1} (3-mercaptopropyl)trimethoxysilane in ethanol for 30 min at room temperature and rinsing with abundant water to remove unbound thiol molecules. After this, the electrode was immersed in a previously hydrolyzed sol (60 min under ultrasonic irradiation at 40°C) of the sol-gel precursors 3-[2-(2-aminoethylamino)ethylamino]propyltrimethoxysilane and tetraethoxysilane (15.8 $\mu\text{mol L}^{-1}$ and 300 mmol L^{-1} , respectively, in 50/50% (v/v) ethanol/0.1 mol L^{-1} HNO₃, pH 1.3). 3-[2-(2-aminoethylamino)ethylamino]propyltrimethoxysilane was replaced by (3-aminopropyl)trimethoxysilane for a comparative study. Formation and growth of the silica network on the surface of the self-assembled monolayer was performed potentiostatically by the application of a constant potential of -1.1 V vs. Ag/AgCl for 30 s to obtain the amine-functionalized silica thin-film electrode.

Hexacyanoferrate was confined in the silica network by immersing the amine-functionalized silica thin-film electrode in a 0.1 mol L^{-1} HNO_3 solution containing 10 mmol L^{-1} $\text{K}_3\text{Fe}(\text{CN})_6$ under stirring for 4 h to obtain the target modified electrode. Finally, the modified electrode was rinsed thoroughly with water to remove any unbound hexacyanoferrate and subjected to consecutive potential sweeps (-0.2 to 0.65 V vs. Ag/AgCl in 0.1 mol L^{-1} HNO_3 , scan rate 100 mV s^{-1}) until stabilization of the voltammetric signal (typically 3 scans).

An alternative procedure was also tested in which hexacyanoferrate was confined in the silica network by adding 10 mmol L^{-1} $\text{K}_3\text{Fe}(\text{CN})_6$ to the sol-gel precursor solution before the electrochemical formation and growth of the silica film.

Analytical procedures

Amperometric batch measurements were performed at an applied potential of 0.0 V vs. Ag/AgCl , at a stirring rate of 400 rev min^{-1} , adding microliter amounts of nitrite standard solutions or samples. The same potential was used to detect transient current signals in the FIA measurements, using a sampling volume of $100 \mu\text{L}$ injected into a carrier stream at a flow rate of 1.0 mL min^{-1} .

Results and discussion

Assembly of the modified electrode

A schematic representation of the electrode surface is illustrated in Figure 2. A three-step procedure was used to prepare the modified electrode. First, using a well-established procedure, the gold surface was covered with silanol groups by depositing a self-assembled monolayer of (3-mercaptopropyl)trimethoxysilane and, then, an amine-functionalized silica network was attached to the silanol groups of the self-assembled monolayer through

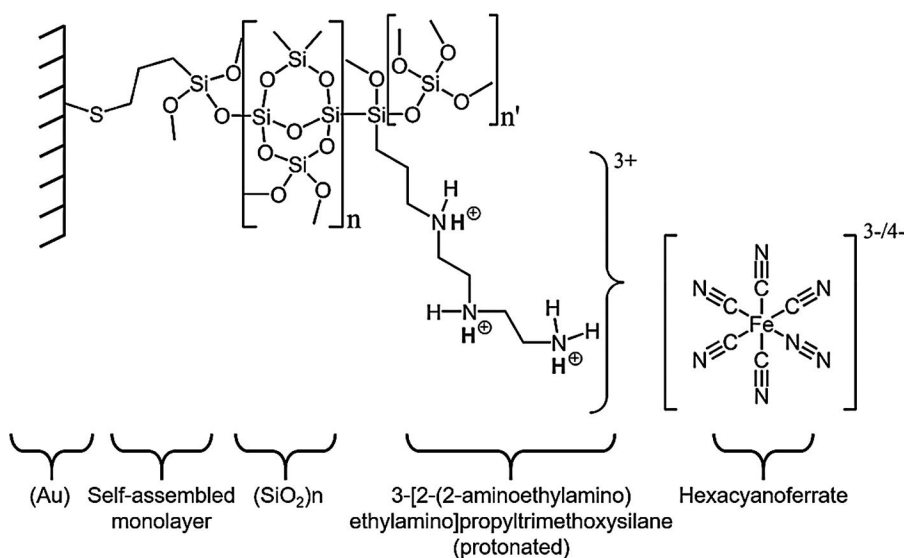


Figure 2. Schematic diagram of the proposed molecular architecture of the modified electrode.

electrochemically induced sol–gel process (Walcarius and Sibottier 2005). Finally, the electron mediator hexacyanoferrate was confined within the silica network by electrostatic interaction to protonated amine groups of the organosilica matrix.

Self-assembly of (3-mercaptopropyl)trimethoxysilane on gold

Chemisorption of (3-mercaptopropyl)trimethoxysilane on gold yields an appropriate base layer to build up highly adherent silica-based thin films on gold surfaces (Park and Weaver 2002; Shen et al. 2005). Under controlled conditions, a monolayer of (3-mercaptopropyl)trimethoxysilane can be formed on the gold surface with no significant participation of a polycondensation process, not imparting resistance to mass transport and/or charge transport (Park and Weaver 2002; Walcarius and Sibottier 2005).

It is well known that dense coverage obtained by a self-assembled monolayer film is formed in the first milliseconds to minutes, although structural imperfections can be self-corrected by a reorganization process, which can require extended times to the order of hours to maximize the density and minimize defects (Love et al. 2005). To optimize the time used for developing a well-structured self-assembled monolayer to anchor firmly the silica film, the electrode was dipped in a unstirred 20 mmol L⁻¹ (3-mercaptopropyl)trimethoxysilane ethanol solution for different periods and the effectiveness of the self-assembled monolayer was indirectly evaluated by the cyclic voltammetric hexacyanoferrate current response of the fully-modified gold electrode, in a supporting electrolyte solution. An immersion period of 30 min was sufficient to obtain a very stable signal. This immersion time was therefore used in subsequent experiments.

Electrodeposition of the amine-functionalized silica film

The silanol groups of (3-mercaptopropyl)trimethoxysilane make the self-assembled monolayer act as a “molecular glue” to anchor firmly the porous silica matrix generated on the gold surface via the sol–gel process (Park and Weaver 2002; Walcarius and Sibottier 2005). The electrochemical reduction of H⁺ (aq.) or of dissolved oxygen, at a sufficiently negative applied potential, produces an alkaline pH at the electrode-solution interface, promoting the formation, and growth of the silica network (Shacham, Avnir, and Mandler 1999). It has been demonstrated that the presence of amine groups in a sol–gel precursor also acts as a catalyst for polycondensation, contributing to the acceleration of the gelification step (Walcarius and Sibottier 2005), where a ratio of 1:9 (pH 3) was the best compromise to prepare a stable and effective sol of (3-aminopropyl)trimethoxysilane and tetraethoxysilane to optimize the electrochemical deposition of the silica film functionalized with amine groups on silanol-containing gold surfaces. Since the amine groups are expected to be highly protonated at acidic pH, the above strategy served as the starting point in the present work, where the objective was to form a positively-charged thin silica matrix suitable for immobilizing anions by electrostatic interaction. Furthermore, instead of using a mixture of (3-aminopropyl)trimethoxysilane and tetraethoxysilane as sol–gel precursors, which was not successful, a mixture of the amine-rich 3-[2-(2-aminoethylamino)ethylamino]propyltrimethoxysilane and tetraethoxysilane was used with the aim of increasing the availability of amine groups in the film. A highly amine-functionalized material should favor the immobilization of hexacyanoferrate ions.

Optimization of the ratio of the sol–gel precursors 3-[2-(2-aminoethylamino)ethylamino]propyltrimethoxysilane and tetraethoxysilane was done by using mixtures of the precursors in selected ratios in ethanol solution, pH 3, under sonication, to identify visually the maximum ratio that would not lead to excessively rapid condensation. The maximum possible ratio for 3-[2-(2-aminoethylamino)ethylamino]propyltrimethoxysilane:tetraethoxysilane to obtain a sol stable for up to 3 h was 1:18, unlike the 1:9 ratio for (3-aminopropyl)trimethoxysilane/tetraethoxysilane under the same conditions (hydrochloric acid, ethanolic solution, pH 3) described in Walcarius and Sibottier (2005). Such a difference may be ascribed to the higher content of amine groups in 3-[2-(2-aminoethylamino)ethylamino]propyltrimethoxysilane compared to (3-aminopropyl)trimethoxysilane.

Further experiments demonstrated a significant improvement in the stability of the sol (up to 2 days) by changing the 1:18 ratio of 3-[2-(2-aminoethylamino)ethylamino]propyltrimethoxysilane:tetraethoxysilane (hydrochloric acid, ethanolic solution, pH 3) to a 1:19 ratio in nitric acid, ethanolic solution, pH 1.3, and the as-prepared sol was also appropriate for the silica film preparation. This condition was chosen for further experiments.

Electrostatic confinement of hexacyanoferrate

The negatively charged electron mediator hexacyanoferrate can be efficiently confined within a porous silica network by electrostatic interaction to positively charged protonated amine groups of an organosilica matrix. The confinement can be achieved either by including it in the sol-precursor of the sol–gel process or by growing the silica film from the sol-precursor and afterwards immersing it in an acid solution of hexacyanoferrate.

Figure 3 shows the results of a study of the influence of the variation of several parameters on the formation and growth of the silica film on the thiol monolayer to obtain modified electrode by adsorption of hexacyanoferrate during film growth (Figure 3a) or after film formation (Figure 3c). Figure 3a presents cyclic voltammograms obtained after the potential-assisted deposition of the film incorporating hexacyanoferrate for different periods (10, 20, 30, 40, 50, and 60 s—from left to right). Figure 3b shows the cyclic voltammogram for a film following 30 s deposition, after thoroughly rinsing the electrode to remove any unbound hexacyanoferrate and exchanging the medium to a supporting electrolyte solution ($0.1 \text{ mol L}^{-1} \text{ HNO}_3$).

Figure 3c presents cyclic voltammograms in the supporting electrolyte solution for a film after 30 s deposition, grown in the absence of hexacyanoferrate, before (flat line) and after (peak-shaped line) immersion for 4 h in a stirred solution of 20 mmol L^{-1} hexacyanoferrate in $0.1 \text{ mol L}^{-1} \text{ HNO}_3$. It is clearly demonstrated that, for both procedures, the modified electrode exhibits the ideal electrochemical response of hexacyanoferrate (Ojani, Raoof, and Zarei 2006). However, incorporation of hexacyanoferrate into the previously formed silica film leads to an eight times higher response. This significant gain in sensitivity reveals a film that is much more densely populated in hexacyanoferrate. Figure 3a also shows the existence of interplay between silica film thickness and the resistance to charge transfer of the modified electrode, since the current increases up to a certain thickness and then decreases as the film gets thicker. Moreover, the negative potential applied to the electrode during formation and growth of the silica film disfavors the simultaneous electrostatic interaction of hexacyanoferrate anions, thus giving

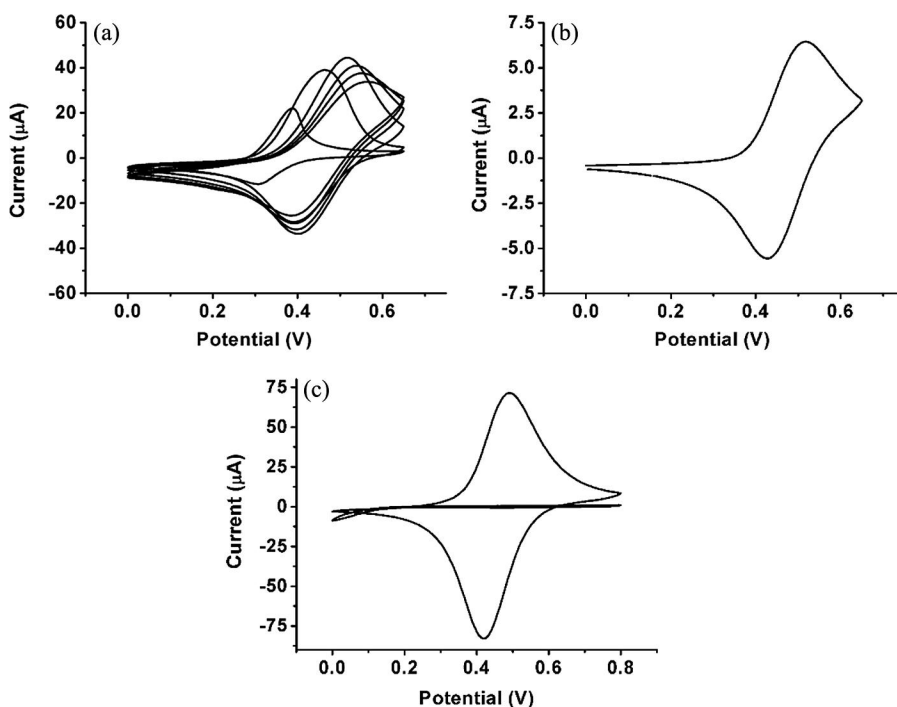


Figure 3. Cyclic voltammograms of the self-assembled monolayered gold electrode: (a) after immersion in a stirred sol–gel precursor containing hexacyanoferrate, applying a potential of -1.1 V vs. Ag/AgCl for 10, 20, 30, 40, 50 or 60 s—from left to right; (b) equal to “a” with for 30 s deposition, and medium exchange to an hexacyanoferrate-free solution of $0.1 \text{ mol L}^{-1} \text{ HNO}_3$; (c) in a solution of $0.1 \text{ mol L}^{-1} \text{ HNO}_3$ (flat line) and after 30 s deposition in a stirred sol–gel precursor solution followed by immersion for 4 h in a stirred solution of 20 mmol L^{-1} hexacyanoferrate in $0.1 \text{ mol L}^{-1} \text{ HNO}_3$ (peak-shaped line). The scan rate was 100 mV s^{-1} .

rise to a film less densely populated in hexacyanoferrate. The second procedure, illustrated in Figure 3c, was thus chosen for preparation of the modified electrode at all following experiments.

Since the confinement process is reversible and pH dependent, being favored at very strongly acidic pH, when the amine groups are protonated and positively charged, the loading of hexacyanoferrate increases with decreasing pH. Thus, the length of time of immersion of the amine-functionalized silica thin-film electrode in the hexacyanoferrate solution to obtain a maximum amount of hexacyanoferrate in the film was investigated. The maximum uptake of hexacyanoferrate was attained by keeping the electrode immersed for 4 h in a stirred 20 mmol L^{-1} hexacyanoferrate solution in strongly acid medium ($0.1 \text{ mol L}^{-1} \text{ HNO}_3$).

Electrochemical behavior of the modified electrode

The electrochemical behavior of the modified electrode in electrolyte solutions of different pH values was assessed as shown in Figure 4a. The typical cyclic voltammogram waves of hexacyanoferrate (III/II) are seen in HNO_3 at pH 1.3, while, as expected, there is a

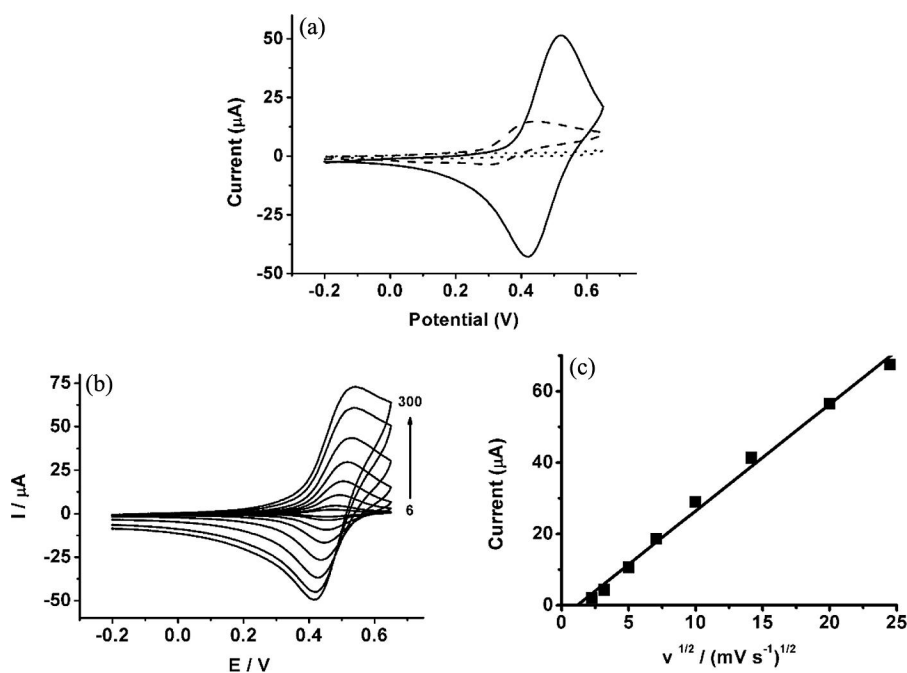


Figure 4. (a) Cyclic voltammograms of the modified electrode at different pH values 1.3 (---), 4.6 (— — —), and 7.0 (· · · · ·) at a scan rate of 100 mV s^{-1} . (b) Cyclic voltammograms of the modified electrode at increasing scan rates (5, 10, 25, 50, 100, 200, 400, and 600 mV s^{-1}) in $0.1 \text{ mol L}^{-1} \text{ HNO}_3$. (c) Plot of peak current vs. The square root of scan rate.

substantial lowering in the signal on increasing the pH, with the oxidation and reduction peak potentials also being shifted in the positive direction, in agreement with the literature for low pH values (Park and Weaver 2002). No response is observed at neutral pH due to a complete release of hexacyanoferrate from the deprotonated amine sites. In strongly acidic solutions, the modified electrode exhibits a very stable response, evidencing the importance of the protonation of the amine-functionalized silica film for hexacyanoferrate confinement. The best performance of the electrode in strongly acidic media was in $0.1 \text{ mol L}^{-1} \text{ HNO}_3$ solution; this was chosen as the supporting electrolyte.

Cyclic voltammograms recorded at different scan rates in $0.1 \text{ mol L}^{-1} \text{ HNO}_3$ were analyzed to assess the nature of the hexacyanoferrate redox process, Figure 4b. There is a linear dependence of peak current on square root of scan rate ($r = 0.9985$), Figure 4c, so the confined hexacyanoferrate exhibits diffusion-controlled electrochemical behavior. This can be explained by the hexacyanoferrate ions being loosely packed, with some freedom of movement within the porous silica matrix, although they do not leach out of the silica matrix when it is brought in contact with an acidic pure electrolyte solution due to the strong electrostatic interaction. In addition, the immobilization efficiency is not dependent on the applied potential, as usually happens for the immobilization of ionic mediators as counter ions of conducting polymers (Gros and Comtat 2004; Raouf, Ojani, and Nadimi 2004, 2005; Oukil, Makhloufi, and Saidani 2007; Oukil et al. 2014). For all these reasons, such a strategy can also be promising for the immobilization of other small highly soluble anionic redox mediators.

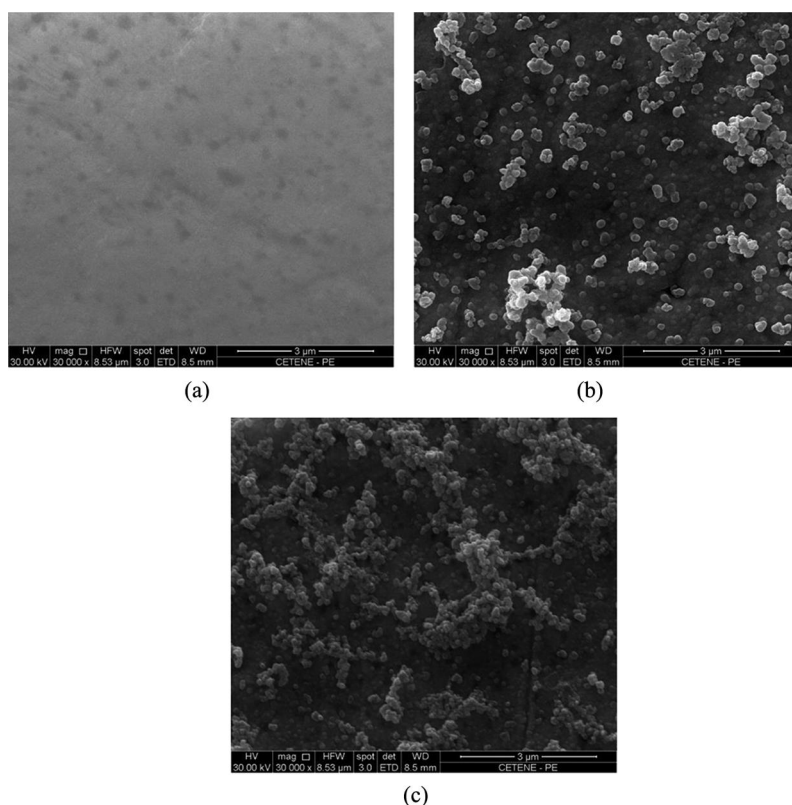


Figure 5. SEM images of the gold electrode surface (a) before, (b) after deposition of the self-assembled monolayer followed by electrodeposition of the silica film and (c) after confinement of hexacyanoferrate in the silica matrix. *Note:* SEM, scanning electron microscopy.

Surface morphology characterization of the modified electrode

The surface morphology of the bare and modified electrodes was examined by scanning electron microscopy. A clear difference is observed between the morphologies of the bare and modified gold electrode, which can be attributed to the silica network formation shown in Figure 5. The silica film (Figure 5b) is formed by aggregates more or less uniformly distributed over the gold surface, suggesting the occurrence of some molecular organization processes during silica film formation. After uptake of hexacyanoferrate in the silica film (Figure 5c), the uniformly distributed aggregate structure remains very similar, with some small differences in the morphology at the submicrometer scale.

Catalytic reduction of nitrite on the modified electrode

The catalytic activity of the modified electrode, toward electrochemical reduction of nitrite ions, was studied by cyclic voltammetry in a solution containing 20 mmol L^{-1} nitrite as shown in Figure 6a. A substantial increase in the cathodic peak height and a corresponding decrease in anodic peak height are clearly observed for the modified electrode besides a complete absence of signal for the unmodified electrode. Such behavior corresponds to a typical electrocatalytic reduction mechanism, in which the reversible electrochemical

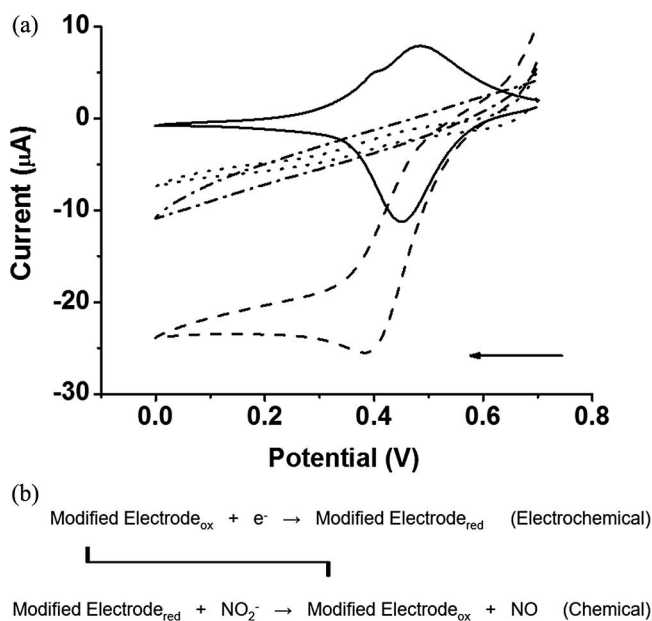


Figure 6. (a) Cyclic voltammograms (scan rate 10 mV s^{-1}) of the modified electrode in the absence (—) and presence (---) of 20 mmol L^{-1} NaNO_2 in 0.1 mol L^{-1} HNO_3 solution; (····) and (-·-·) are the corresponding responses at a bare gold electrode. (b) Reaction mechanism of the irreversible chemical reduction reaction of nitrite ions.

reaction of hexacyanoferrate immobilized at the electrode surface is coupled to the irreversible chemical reduction reaction of nitrite ions. The reaction mechanism is shown in Figure 6b.

This demonstrates clearly the potential of the modified electrode as a sensor for the amperometric determination of nitrite. By keeping the potential applied to the modified electrode below 0.4 V vs. Ag/AgCl , where the hexacyanoferrate ions are in the reduced state in a highly acidic medium, nitrite ions are chemically reduced to NO (Ojani, Raoof, and Zarei 2006; Qin et al. 2013) by the hexacyanoferrate; the reduced form is regenerated and leads to a cathodic current.

Amperometric response of the modified electrode to nitrite ions

Important parameters affecting the electroanalytical performance of the sensor in amperometric mode were identified and tuned to optimize the analytical response, namely the operational potential and pH. As discussed above (Figure 4a), the pH has a strong influence on sensor stability and must be fixed at highly acidic values, while the operational potential must be chosen in the reduction range of the hexacyanoferrate voltammogram (Figure 6a). Nitrite reduction is also favored by highly acidic medium (Qin et al. 2013). Thus, a 0.1 mol L^{-1} HNO_3 solution and 0.0 V applied potential were chosen as supporting electrolyte and operational potential, respectively. The potential of 0.0 V has the additional advantage of minimizing possible interferences from other electroactive species. Figure 7a displays the amperometric response of the sensor toward successive additions of nitrite ion. The sensor responds very rapidly, which can be attributed to the highly porous film. The resulting

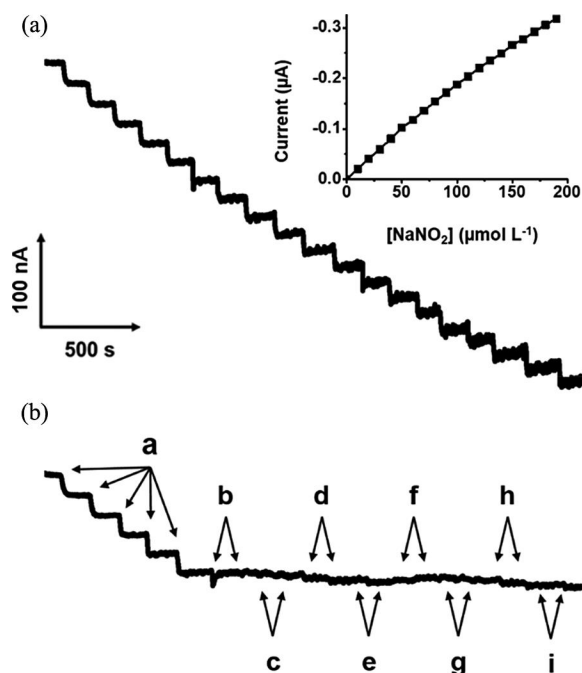


Figure 7. Amperometric response of the modified electrode in stirred $0.1 \text{ mol L}^{-1} \text{ HNO}_3$ solution at 0.0 V vs. Ag/AgCl . (a) Successive additions of $10 \text{ } \mu\text{mol L}^{-1} \text{ NaNO}_2$; the inset shows the corresponding analytical curve ($r = 0.9993$, for $0\text{--}100 \text{ } \mu\text{mol L}^{-1}$). (b) Additions of “a” $10 \text{ } \mu\text{mol L}^{-1}$ of NaNO_2 , “b–i” $150 \text{ } \mu\text{mol L}^{-1}$ of Cu(II) , Fe(III) , Mn(II) , Ni(II) , Cd(II) , Al(II) , Mg(II) , and Zn(II) , respectively.

calibration plot (also shown) indicates a highly linear response for nitrite sensing with a linear range up to $100 \text{ } \mu\text{mol L}^{-1}$ ($r = 0.9993$).

Interferences in the response to nitrite for ionic species normally found in waters were tested. No interference was observed from Cu(II) , Fe(III) , Mn(II) , Ni(II) , Cd(II) , Al(II) , Mg(II) , and Zn(II) up to 10 times the concentration of nitrite ion, (Figure 7b).

Application of the modified electrode to nitrite sensing in real samples by FIA

Flow injection analysis was used to evaluate application of the modified electrode to nitrite sensing in real samples. To ensure a better electrochemical performance of the sensor, the modified electrode was cycled 10 times between $+0.6$ and 0.0 V before use. This conditioning provides greater stability to the sensor by freeing the excess of unbound hexacyanoferrate ions. Figure 8 displays FIA signals for injections of standard solutions and samples. The resulting calibration plot (also shown) indicates a highly linear response for nitrite sensing with a linear range from 1.0 to $40.0 \text{ } \mu\text{mol L}^{-1}$ ($r = 0.9995$) and a detection limit of $0.53 \text{ } \mu\text{mol L}^{-1}$ based on a signal-to-noise ratio of 3.

Since hexacyanoferrate is confined on the electrode by electrostatic interaction, low flow rates are preferable to avoid hexacyanoferrate leaching. Flow rates of around 1.0 mL min^{-1} were satisfactory, even though a small baseline drift can be seen (Figure 8) caused by leaching of hexacyanoferrate from the electrode surface. Fortunately, this does not hinder use in flow analysis, as demonstrated by the performance of the sensor for measuring nitrite in

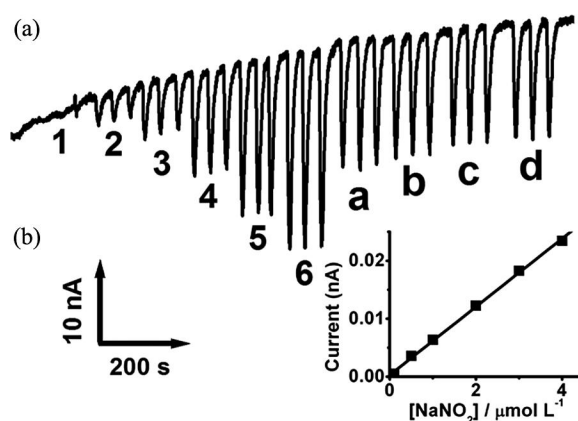


Figure 8. FIA signals for injections of standard solutions (1) 1.0, (2) 5.0, (3) 10.0, (4) 20.0, (5) 30.0, and (6) 40.0 $\mu\text{mol L}^{-1}$ of nitrite ions and samples of (a) rain, (b) river, (c) ground, and (d) drinking water, spiked with 25 $\mu\text{mol L}^{-1}$ of nitrite ions in a 0.1 mol L^{-1} HNO_3 carrier solution at a flow rate of 1.0 mL min^{-1} . The corresponding analytical curve ($r = 0.9995$) is shown in the inset. *Note:* FIA, flow injection analysis.

Table 1. Recovery of nitrite in natural water samples obtained with the modified electrode ($n = 3$).

Sample	NO_2^- added ($\mu\text{mol L}^{-1}$)	NO_2^- found ($\mu\text{mol L}^{-1}$)	Recovery (%)
River water	25.0	25.1 (± 0.02)	100.3
Rainwater	25.0	25.3 (± 0.04)	101.2
Groundwater	25.0	23.9 (± 0.01)	95.7
Drinking water	25.0	24.2 (± 0.05)	96.6

pure electrolyte and in natural-water samples, where modified electrode responded efficiently to increments in the nitrite concentration. Recoveries of 96–101% were found for 25 $\mu\text{mol L}^{-1}$ nitrite in the real samples studied, Table 1.

Conclusion

A new amine-functionalized sol–gel silica film was developed and demonstrated to be a useful platform to immobilize the highly soluble hexacyanoferrate anion, without loss of redox activity or slower kinetics. The immobilization of hexacyanoferrate is completely controlled by the adjustment of pH. A self-assembled monolayer of (3-mercaptopropyl) trimethoxysilane served to chemically bond a sol–gel silica matrix to the surface of the gold electrode. Electrochemically induced alkaline pH at the electrode–solution interface, by application of a sufficiently negative potential, promoted the sol–gel process, and yielded a very thin film of amine-functionalized silica bonded to the silanol groups of the self-assembled monolayer. It was demonstrated that hexacyanoferrate ions can be efficiently confined within the rich amine-functionalized silica matrix. Cyclic voltammetry demonstrated that the uptake and incorporation of hexacyanoferrate in the film is reversible and is governed by electrostatic interaction. The diffusion of hexacyanoferrate in and out of the film is strongly pH dependent because hexacyanoferrate ions are unable to diffuse out of the film in strongly acidic electrolyte solution, thus retaining their electrochemical activity. Based on this, a sensitive amperometric sensor for the catalytic reduction

of nitrite ions was developed with a linear range from 1.0 to 40.0 $\mu\text{mol L}^{-1}$ ($r = 0.9995$) and a detection limit of 0.53 $\mu\text{mol L}^{-1}$ based on a signal-to-noise ratio of 3. This concept of amine-functionalized silica-modified networks on electrodes as a confinement framework for hexacyanoferrate, can be extended, through judicious tailoring of the silica matrix with different silylation agents, to efficient confinement of other highly soluble ionic redox mediators.

Funding

The authors acknowledge financial support from Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (APQ-1459-01.06/10, AMD-0071-1.06/13, APQ-0535-1.06/14, APQ-456725/14-8), Fundação para a Ciência e a Tecnologia (FCT), Portugal, UID/EMS/00285/2013 (co-financed by the European Community Fund FEDER), and CETENE and CENAPESQ-UFRPE for instrumental support.

ORCID

Valberes B. Nascimento  <http://orcid.org/0000-0002-8274-3291>

References

- Bryan, N. S., D. D. Alexander, J. R. Coughlin, A. L. Milkowski, P. Boffetta. 2012. Ingested nitrate and nitrite and stomach cancer risk: An updated review. *Food and Chemical Toxicology* 50:3646–65. doi:10.1016/j.fct.2012.07.062
- Chaubey, A., and B. D. Malhotra. 2002. Mediated biosensors. *Biosensors and Bioelectronics* 17:441–56. doi:10.1016/S0956-5663(01)00313-X
- Collinson, M. M. 2007. Electrochemistry: An important tool to study and create new sol-gel-derived materials. *Accounts of Chemical Research* 40:777–84. doi:10.1021/ar600029p
- Ding, S.-N., J.-F. Chen, J. Xia, Y.-H. Wang, and S. Cosnier. 2013. Voltammetric detection of heparin based on anion exchange at electropolymeric film of pyrrole-alkylammonium cationic surfactant and MWCNTs composite. *Electrochemistry Communications* 34:339–43. doi:10.1016/j.elecom.2013.07.036
- Dreyse, P., M. Isaacs, K. Calfumán, C. Cáceres, A. Aliaga, M. J. Aguirre, and D. Villagra. 2011. Electrochemical reduction of nitrite at poly-[Ru(5-NO₂-phen)₂Cl]tetrapyrrolylporphyrin glassy carbon modified electrode. *Electrochimica Acta* 56:5230–37. doi:10.1016/j.electacta.2011.03.028
- Grieshaber, D., J. Vörös, T. Zambelli, V. Ball, P. Schaaf, J.-C. Voegel, and F. Boulmedais. 2008. Swelling and contraction of ferrocyanide-containing polyelectrolyte multilayers upon application of an electric potential. *Langmuir* 24:13668–76. doi:10.1021/la801875u
- Gros, P., and M. Comtat. 2004. A Bioelectrochemical polypyrrole-containing Fe(CN)₆³⁻ interface for the design of a NAD-dependent reagentless biosensor. *Biosensors and Bioelectronics* 20:204–10. doi:10.1016/j.bios.2004.02.023
- Gros, P., H. Durliat, and M. Comtat. 2000. Use of polypyrrole film containing Fe(CN)₆³⁻ as pseudo-reference electrode: Application for amperometric biosensors. *Electrochimica Acta* 46:643–50. doi:10.1016/S0013-4686(00)00644-7
- Hübsch, E., G. Fleith, J. Fatissou, P. Labbé, J. C. Voegel, P. Schaaf, and V. Ball. 2005. Multivalent ion/polyelectrolyte exchange processes in exponentially growing multilayers. *Langmuir* 21:3664–69. doi:10.1021/la047258d
- Hui, N., F. Chai, P. Lin, Z. Song, X. Sun, Y. Li, S. Niu, and X. Luo. 2016. Electrodeposited conducting polyaniline nanowire arrays aligned on carbon nanotubes network for high performance supercapacitors and sensors. *Electrochimica Acta* 199:234–41. doi:10.1016/j.electacta.2016.03.115

- Jiang, J., W. Fan, and X. Du. 2014. Nitrite electrochemical biosensing based on coupled graphene and gold nanoparticles. *Biosensors and Bioelectronics* 51:343–48. doi:10.1016/j.bios.2013.08.007
- Kozub, B. R., N. V. Rees, and R. G. Compton. 2010. Electrochemical determination of nitrite at a bare glassy carbon electrode: Why chemically modify electrodes? *Sensors and Actuators B: Chemical* 143:539–46. doi:10.1016/j.snb.2009.09.065
- Kumar, A., Rajesh, A. Chaubey, S. K. Grover, and B. D. Malhotra. 2001. Immobilization of cholesterol oxidase and potassium ferricyanide on dodecylbenzene sulfonate ion-doped polypyrrole film. *Journal of Applied Polymer Science* 82:3486–91. doi:10.1002/app.2210
- Lawal, A. T., and S. B. Adeloju. 2010. Comparison of polypyrrole-based xanthine oxidase amperometric and potentiometric biosensors for hypoxanthine. *Journal of Molecular Catalysis B: Enzymatic* 66:270–75. doi:10.1016/j.molcatb.2010.06.002
- Lev, O., Z. Wu, S. Bharathi, V. Glezer, A. Modestov, J. Gun, L. Rabinovich, and S. Sampath. 1997. Sol-gel materials in electrochemistry. *Chemistry of Materials* 9:2354–75. doi:10.1021/cm970367b
- Li, F., C. Tang, S. Liu, and G. Ma. 2010. Development of an electrochemical ascorbic acid sensor based on the incorporation of a ferricyanide mediator with a polyelectrolyte-calcium carbonate microsphere. *Electrochimica Acta* 55:838–43. doi:10.1016/j.electacta.2009.09.049
- Lima, A. W. O., V. B. Nascimento, J. J. Pedrotti, and L. Angnes. 1997. Coconut-based plant tissue reactor for biosensing of catechol in flow injection analysis. *Analytica Chimica Acta* 354:325–31. doi:10.1016/S0003-2670(97)00428-5
- Love, J. C., L. A. Estroff, J. K. Kriebel, R. G. Nuzzo, and G. M. Whitesides. 2005. Self-assembled monolayers of thiolates on metals as a form of nanotechnology. *Chemical Reviews* 105:1103–69. doi:10.1021/cr0300789
- Moorcroft, M. J., J. Davis, and R. G. Compton. 2001. Detection and determination of nitrate and nitrite: A review. *Talanta* 54:785–803. doi:10.1016/S0039-9140(01)00323-X
- Moore, R. R., C. E. Banks, and R. G. Compton. 2004. Basal plane pyrolytic graphite modified electrodes: Comparison of carbon nanotubes and graphite powder as electrocatalysts. *Analytical Chemistry* 76:2677–82. doi:10.1021/ac040017q
- Noguchi, T., and J.-I. Anzai. 2006. Redox properties of the ferricyanide ion on electrodes coated with layer-by-layer thin films composed of polysaccharide and poly(allylamine). *Langmuir* 22:2870–75. doi:10.1021/la053226u
- Ojani, R., J.-B. Raoof, and E. Zarei. 2006. Electrocatalytic reduction of nitrite using ferricyanide: Application for its simple and selective determination. *Electrochimica Acta* 52:753–59. doi:10.1016/j.electacta.2006.06.005
- Oukil, D., L. Benhaddad, R. Aitout, L. Makhloufi, F. Pillier, and B. Saidani. 2014. Electrochemical synthesis of polypyrrole films doped by ferrocyanide ions onto iron substrate: Application in the electroanalytical determination of uric acid. *Sensors and Actuators B: Chemical* 204:203–10. doi:10.1016/j.snb.2014.07.086
- Oukil, D., L. Makhloufi, and B. Saidani. 2007. Preparation of polypyrrole films containing ferrocyanide ions deposited onto thermally pre-treated and untreated iron substrate: Application in the electroanalytical determination of ascorbic acid. *Sensors and Actuators B: Chemical* 123:1083–89. doi:10.1016/j.snb.2006.11.014
- Park, S., and M. J. Weaver. 2002. A versatile surface modification scheme for attaching metal nanoparticles onto gold: Characterization by electrochemical infrared spectroscopy. *The Journal of Physical Chemistry B* 106:8667–70. doi:10.1021/jp025957y
- Qin, C., W. Wang, C. Chen, L. Bu, T. Wang, X. Su, Q. Xie, and S. Yao. 2013. Amperometric sensing of nitrite based on electroactive ferricyanide-poly(diallyldimethylammonium)-alginate composite film. *Sensors and Actuators B: Chemical* 181:375–81. doi:10.1016/j.snb.2013.01.052
- Rahim, A., L. S. S. Santos, S. B. A. Barros, L. T. Kubota, R. Landers, and Y. Gushikem. 2014. Electrochemical detection of nitrite in meat and water samples using a mesoporous carbon ceramic SiO₂/C electrode modified with in situ generated manganese(II) phthalocyanine. *Electroanalysis* 26:541–47. doi:10.1002/elan.201300468
- Raoof, J.-B., R. Ojani, and S. R. Nadimi. 2004. Preparation of polypyrrole/ferrocyanide films modified carbon paste electrode and its application on the electrocatalytic determination of ascorbic acid. *Electrochimica Acta* 49:271–80. doi:10.1016/j.electacta.2003.08.009

- Raouf, J.-B., R. Ojani, and S. R. Nadimi. 2005. Voltammetric determination of ascorbic acid and dopamine in the same sample at the surface of a carbon paste electrode modified with polypyrrole/ferrocyanide films. *Electrochimica Acta* 50:4694–98. doi:10.1016/j.electacta.2005.03.002
- Shacham, R., D. Avnir, and D. Mandler. 1999. Electrodeposition of methylated sol-gel films on conducting surfaces. *Advanced Materials* 11:384–88. doi:10.1002/(sici)1521-4095(199903)11:5<384::aid-adma384>3.3.co;2-d
- Shen, Y., T. Wu, Y. Zhang, and J. Li. 2005. Comparison of two-typed (3-mercaptopropyl)trimethoxysilane-based networks on Au substrates. *Talanta* 65:481–88. doi:10.1016/j.talanta.2004.06.030
- Takita, R., K. Yoshida, and J.-I. Anzai. 2007. Redox properties of ferricyanide ion on layer-by-layer deposited poly(glutamic acid) film-coated electrodes and its use for electrocatalytic sensing of ascorbic acid. *Sensors and Actuators B: Chemical* 121:54–60. doi:10.1016/j.snb.2006.09.003
- Vasantha, V. S., and S.-M. Chen. 2005. Electrochemical preparation and electrocatalytic properties of PEDOT/ferricyanide film-modified electrodes. *Electrochimica Acta* 51:347–55. doi:10.1016/j.electacta.2005.04.029
- Walcarius, A., D. Mandler, J. A. Cox, M. M. Collinson, and O. Lev. 2005. Exciting new directions in the intersection of functionalized sol-gel materials with electrochemistry. *Journal of Materials Chemistry* 15:3663–89. doi:10.1039/b504839g
- Walcarius, A., and E. Sibottier. 2005. Electrochemically-induced deposition of amine-functionalized silica films on gold electrodes and application to Cu(II) detection in (hydro)alcoholic medium. *Electroanalysis* 17:1716–26. doi:10.1002/elan.200503300
- Wang, B., and J.-I. Anzai. 2007. Redox reactions of ferricyanide ions in layer-by-layer deposited polysaccharide films: A significant effect of the type of polycation in the films. *Langmuir* 23:7378–84. doi:10.1021/la0701199mm
- Wu, X.-Y., W.-J. Niu, S. Cosnier, S.-Y. Deng, X.-J. Zhang, and D. Shan. 2015. Ferricyanide confined into the integrative system of pyrrolic surfactant and SWCNTs: The enhanced electrochemical sensing of paracetamol. *Electrochimica Acta* 186:16–23. doi:10.1016/j.electacta.2015.10.156
- Xiang, L., Z. Zhang, P. Yu, J. Zhang, L. Su, T. Ohsaka, and L. Mao. 2008. In situ cationic ring-opening polymerization and quaternization reactions to confine ferricyanide onto carbon nanotubes: A general approach to development of integrative nanostructured electrochemical biosensors. *Analytical Chemistry* 80:6587–93. doi:10.1021/ac800733t
- Zahn, R., F. Boulmedais, J. Vörös, P. Schaaf, and T. Zambelli. 2010. Ion and solvent exchange processes in PGA/PAH polyelectrolyte multilayers containing ferrocyanide. *The Journal of Physical Chemistry B* 114:3759–68. doi:10.1021/jp9106074
- Zuo, J., Z. Zhang, J. Jiao, H. Pang, D. Zhang, and H. Ma. 2016. Sensitive and selective nitrite sensor based on phosphovanadomolybdates $H_6[PMo_9V_3O_{40}]$, poly(3,4-ethylenedioxythiophene) and Au nanoparticles. *Sensors and Actuators B: Chemical* 236:418–24. doi:10.1016/j.snb.2016.05.159