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Electro-oxidation of phenol on a new type of zeolite/graphite biocomposite electrode with horseradish peroxidase

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Abstract

A novel biocomposite electrode containing horseradish peroxidase (HRP) and zeolite co-immobilized on gelatin particles mixed with graphite was described.

Comparing the composite (zeolite/graphite) and the biocomposite (gelatin/HRP/zeolite/graphite) electrodes showed that the latter presented an enhancement on the electrodes diffusion properties and, apparently, the enzyme co-immobilized with zeolite on gelatin was electro-active and functioned as mediator in the electro-oxidation of phenol.

Various HRP/zeolite ratios were tested and the best result was obtained with the 1:100 m_{HRP}/m_{zeo} biocomposite electrode. These results confirm the existence of HRP–zeolite interactions, as previously observed in pseudo-homogeneous oxidation of phenol.

Keywords: Phenol; Electrochemical oxidation; Zeolites; Horseradish peroxidase; Gelatin entrapment; Cyclic voltammetry

1. Introduction

Phenolic compounds are among the major contaminants in medical, food and environmental matrices. For example, the maximal admissible concentration of phenolic compounds in drinking water should not exceed 0.5 μg/l [1]. However, from another point of view, phenol structures form a basis for many pharmaceutical preparations and/or used for human treatment.

Regardless of whether the phenolic compounds are treated as good or bad substances (a classification which is dependent on its concentration), the measurement of their concentration is of great importance [1–11].

Horseradish peroxidase (HRP) proved to be suitable for many practical applications for the determination of phenol; for example, it has been used as part of electrodes and amperometric biosensors [1–30]. HRP has been thoroughly studied and frequently used to exemplify the peroxidase reaction cycle (Change-George mechanism (1)–(3)) which is often involved in the elimination of phenolic compounds as it was used in previous works [31,32]:

$$\text{HRP} + \text{H}_2\text{O}_2 \rightarrow \text{compound I} + \text{H}_2\text{O} \quad (1)$$

$$\text{compound I} + \text{PhOH} \rightarrow \text{compound II} + \text{PhO}^{\bullet} \quad (2)$$

$$\text{compound II} + \text{PhOH} \rightarrow \text{HRP} + \text{PhO}^{\bullet} + \text{H}_2\text{O} \quad (3)$$

HRP affinity for hydrogen peroxide allowed the development of many electrodes or amperometric biosensors for the detection of hydrogen peroxide, either using direct [16–21] or mediated [14,22–30] electron transfers. Many of these peroxidase-modified electrodes are based on a direct electron transfer (DET) between the active site of the enzyme and an electrode, combining Eqs. (1) and (4):

$$\text{compound I} + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{HRP} + \text{H}_2\text{O} \quad (4)$$

However, electrode configurations based on various mediated electron transfer (MET) pathways, using diffusion mediators, have also been described. A variety of mediators can be used, such as ferrocenes, hexacyanoferrate(II), osmium complexed with pyridine or nickelocene [1].

The Change-George chain of reactions (1)–(3) has also been used for the development of biosensors for phenolic compounds

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detection [1–15], either by direct or mediated electron transfer; these biosensors usually involve the use of hydrogen peroxide to oxidise HRP.

Zeolite/graphite containing electrodes have been studied in various context, namely for the oxidation of phenol [33–35]. The enzymes and zeolites have also already produced some electrodes of great interest [36–45].

Often, the direct adsorption of proteins onto the electrodes surface can result in their denaturation and loss of activity; this effect has been reported for HRP onto zeolites in a previous work; however, the same study showed that the presence of zeolite in the vicinity of HRP can enhance the enzymatic activity of this enzyme and an heterogeneous catalyst prepared by the simultaneous entrapment of zeolite and enzyme in gelatin was shown to improve the enzyme's activity [32].

In the present work we have tried this type of heterogeneous catalyst for the construction of composite electrodes, combining both the zeolite and the enzyme. This new biocomposite zeolite/graphite electrode with HRP was studied for the direct electro-oxidation of phenol in the absence of added hydrogen peroxide.

2. Experimental

2.1. Materials

All reagents were of analytical grade.

Horseradish peroxidase (HRP-C [E.C.1.11.1.7], MW = 40 kDa) with a specific activity of 252 U/mg (according to pyrogallol method performed by the supplier) was obtained from Bioenzyme and stored at −18 °C. Gelatin, sodium phosphate and phenol were purchased from Merck. Distilled water was used in all the experiments and all solutions were stored at 4 °C.

For the construction of the pellets, graphite with a high degree of purity was used for mixing with zeolites or silica in powder form; electrodes obtained by mixing graphite with the gelatin particles described below were also constructed.

The zeolite used in this work was the LZY-52 (NaY) with Si/Al = 2.5 (which will, hereafter, be referenced as $NaY_{2.5}$) from Union Carbide. For the purpose of performing control experiments a porous silica (EP10X from Crosfield), hereafter referenced as $(SiO_2)_p$, was used. The zeolite and the silica samples were calcined, under air at 793 K, prior to use, to ensure a clean surface. After calcination they were stored in a closed vessel under a constant and high humidity atmosphere.

2.2. Instrumentation and equipment

Electrochemical measurements were performed on a DEA101 Digital Electrochemical Analyser coupled to an IMT102 Electrochemical Interface from Radiometer/Copenhagen.

The pellets were produced using a Graseby/Specac press.

Thermogravimetric measurements were performed on an SDT 2960 Simultaneous DSC–TGA from TA Instruments. These measurements were used to determine the amount of water that was adsorbed in the zeolite under storage conditions (so as to establish a suitable dry calculation basis for the weight of the zeolites that were used in the experiments) and to confirm the total water and protein content in the gelatin particles.

A B. Braun Biotech International Christ® Alpha 2-4 equipped with a Pfeiffer Balzers Duo 008 B vacuum pump was used to lyophilise the gelatin samples.

2.3. Methods

2.3.1. Gelatin–enzyme entrapment

The standard procedure for the enzyme entrapment, as described in previous work [32] (adapted from [46]), was as follows: 0.5 g of gelatin was dissolved in 4.5 ml of sodium phosphate buffer solution and mixed with 0.5 ml of HRP-C solution (15.14 mg/ml). The final solution was divided in two parts to ensure that both had the same enzyme/gelatin ratio. In one part the adequate zeolite or silica quantity was added to reach a final proportion of 1:100, 2:100, 5:100 of m_{HRP}/m_{zeo} and 1:100 of $m_{HRP}/m_{(SiO_2)_p}$ ratio. The other part was used as reference (hereafter, these samples will be referenced as Refs. 1–4, respectively).

With soft stirring, the solution was solidified at 4 °C and the resulting solid was washed with the buffer solution.

All the samples were powdered by lyophilisation. These powders were sieved through a mesh with an aperture of 0.141 mm, to standardise particle dimensions.

Thermogravimetric experiments were made to confirm the amount of protein in each preparation. Table 1 shows the relative composition of each gelatin sample.

Also for control purposes, particles containing only gelatin and zeolite, in the same proportions as the ones containing the entrapped enzyme, were also prepared. With these particles, electrodes maintaining the general structure and properties of the biocomposite ones, but with no enzyme present, were constructed. Table 2 shows the composition for all gelatin/zeolite samples.

2.3.2. Electrodes construction

The composite electrodes were prepared by a simple mechanical mixture of the powder of the zeolite, silica, gelatin/zeolite or gelatin/HRP/zeolite (or silica) particles with graphite in a 2:1 (w/w) proportion. The pellets had a total mass of around 5 mg of the previously homogenised mixture and were subjected to a pressure of 0.5 tonnes for 5 min.

Table 1
Composition (wt%) of all gelatin samples [32]

	Samples							
	Ref. 1	1:100	Ref. 2	2:100	Ref. 3	5:100	Ref. 4	1:100
H_2O	16.5	20.0	13.3	16.1	17.6	19.1	16.6	10.2
Gelatin	82.3	31.5	85.4	47.3	81.2	61.4	82.2	35.5
HRP-C	1.2	0.5	1.3	0.7	1.2	0.9	1.2	0.5
$NaY_{2.5}$	–	48.0	–	35.9	–	18.6	–	–
$(SiO_2)_p$	–	–	–	–	–	–	–	53.8

Refs. 1–4 correspond to the sample reference, i.e., without the zeolite or the porous silica.

Table 2
Composition (wt%) of all the gelatin/zeolite samples

	gel/zeo$_{(1:100)}$	gel/zeo$_{(2:100)}$	gel/zeo$_{(5:100)}$
H_2O	21.0	17.1	17.9
Gelatin	34.0	45.6	59.9
$NaY_{2.5}$	45.0	37.3	22.2

Written in parentheses (subscript) the corresponding sample with enzyme is indicated.

The pellet, which was 3 mm in diameter, was placed in a special support where it contacted with the electric circuit by means of a platinum disc that was pressed against the pellet, as described in previous works [33–35,47].

2.3.3. Electrochemical behaviour

All the electrochemical measurements were performed as described in previous works [33–35,47] where the conditions were optimised.

Cyclic voltammetry studies were carried out in a three-electrode cell, at a platinum disc or at a composite working electrode (prepared as described in the previous section), probed by a Luggin capillary connected to a silver/silver chloride reference electrode (Ag/AgCl). A platinum wire auxiliary electrode was employed.

All electrochemical measurements were performed at room temperature and with a scanning rate of 10 mV/s (unless otherwise indicated) in a 100 mM sodium phosphate buffer solution, so as to ensure a pH of 7.0. Prior to each experiment a base line was recorded using a clean buffer solution.

Unless otherwise specified, the measurements were carried out using a solution containing 5 mM of phenol in the sodium phosphate buffer solution.

2.3.4. HRP-C enzyme concentration

The HRP-C concentration in the enzyme solutions that were used to prepare the composite catalysts was determined spectrophotometrically at 404 nm using a molar absorption coefficient value of 102,000 M^{-1} cm^{-1} [48–51].

3. Results and discussion

Electro-oxidation of phenol with zeolite/graphite composite electrodes was well studied in previous works [33–35]. In those studies, $NaY_{2.5}$ was the zeolite that showed the best performance, because it presented the oxidation wave with the highest current intensity and the lowest potential.

With the same type of electrode, containing only the $NaY_{2.5}$ zeolite/graphite composite, the simple addition of HRP to the phenol solution was attempted. In Fig. 1 we can observed that when HRP is added to the solution containing phenol (25 mM), a small wave can be observed with the $NaY_{2.5}$/graphite electrode, at a potential just bellow the wave corresponding to the oxidation of phenol on the zeolite containing electrode. This wave increases when the amount of HRP in solution is increased from 0.5 to 1 mg/l.

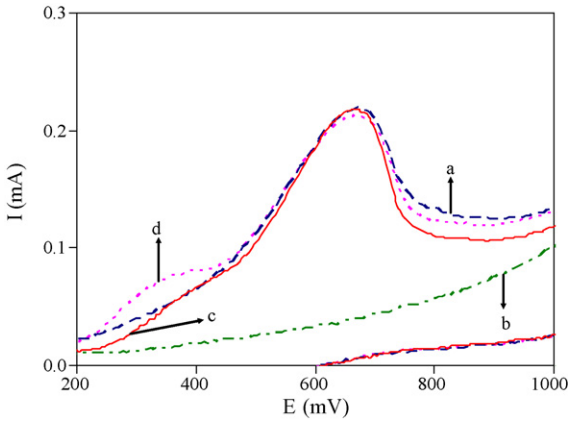

Fig. 1. Cyclic voltammograms with $NaY_{2.5}$/graphite working electrode in the presence of only 25 mM of phenol (a), only 1 mg/l of HRP (b), with 25 mM of phenol and 0.5 mg/l of HRP (c) and with 25 mM of phenol and 1 mg/l of HRP (d), all in phosphate buffer, with a scan rate of 10 mV/s.

This new wave is clearly due to the interaction of HRP with phenol, since when no phenol is present in solution no significant oxidation of HRP can be detected, probably because the enzyme concentration is very small (1 mg/l = 0.025 mM). Thus it is conceivable that the enzyme will act as a mediator in the oxidation of phenol by the electrode, since the oxidation wave potential is somewhat lower than without HRP. This assumption is reinforced by the fact that the ratio between the second (direct oxidation of phenol on the zeolite electrode) and the first waves is only 4.5 times when the molar ratio between phenol and HRP is 1000 (for the 1 mg/l HRP solution), clearly pointing to the existence of a catalytic process.

In a previous work we have shown that the $NaY_{2.5}$ zeolite can enhance the horseradish peroxidase activity, despite the fact that when HRP adsorbs itself directly on the surface of the zeolite it looses all activity [32]. It is, thus, conceivable that the simple addition of HRP on the solution in contact with the zeolite containing electrode will imply some adsorption of the enzyme onto the zeolite surface, thus reducing its apparent activity.

Following these experiments, the same type of electrode was constructed but with the particles containing both entrapped enzyme and zeolite (in which HRP was active).

In Fig. 2 we intend to compare, for two different phenol concentrations (0.5 and 5 mM), the effects due to porosity and HRP presence on the electrode performance. All the electrodes had a 2:1 weight ratio of gelatin particles (gelatin/zeolite, gelatin/HRP, gelatin/HRP/zeolite or gelatin/HRP/$(SiO_2)_p$) to graphite (since all electrodes have the same proportion of gelatin particles to graphite, hereafter, graphite will not be mentioned). As it can be seen, with 0.5 mM in phenol (Fig. 2a) the electrode based on Ref. 1 (which contains only gelatin/HRP), shows an oxidation wave that has a lower current intensity than the electrode constructed with gelatin/HRP/$(SiO_2)_p$ ($m_{HRP}/m_{(SiO_2)_p}$ = 1:100) and this one lower than gelatin/HRP/zeolite (m_{HRP}/m_{zeo} = 1:100). Since the gelatin particles that have only HRP are essentially non-porous, it seems that the existence of some porosity on the electrode, either given by the zeolite or by the porous silica, is very important to ensure access of the species to the electrode; nevertheless,

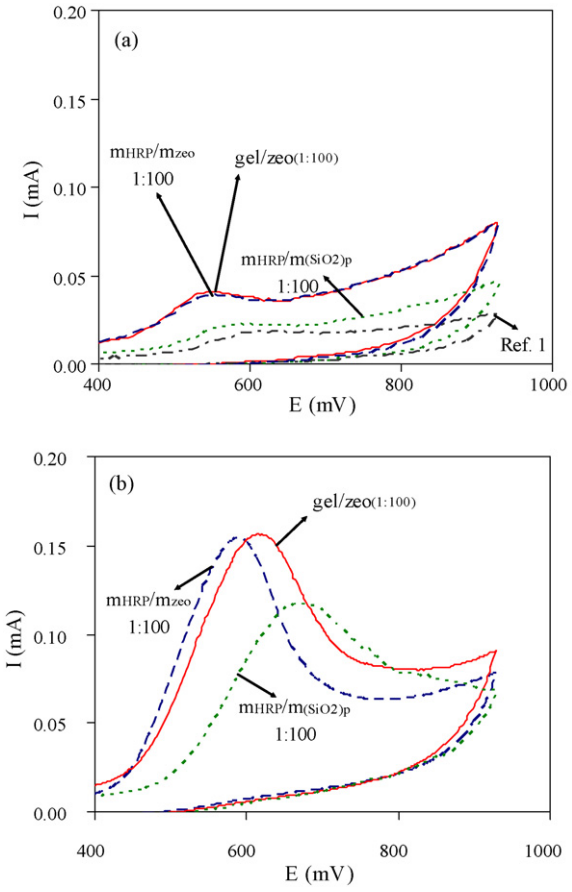

Fig. 2. Cyclic voltammograms with different gelatin-based composite working electrode; zeolite in gelatin (gel/zeo$_{(1:100)}$), zeolite + HRP in gelatin (m_{HRP}/m_{zeo} = 1:100), silica + HRP in gelatin ($m_{HRP}/m_{(SiO_2)_p}$ = 1:100) and HRP in gelatin (Ref. 1) in the presence of 0.5 mM (a) and 5 mM (b) of phenol in phosphate buffer, with a scan rate of 10 mV/s.

the zeolite containing electrode is able to achieve higher currents and lower potentials for the oxidation of phenol than the one containing silica, in accordance with the observations in previous works [33–35].

The sample containing both HRP and zeolite (m_{HRP}/m_{zeo} = 1:100) shows more or less the same current intensity as the corresponding electrode without HRP (gel/zeo$_{(1:100)}$). At 5 mM (Fig. 2b) the differences are more clear; although the intensities are practically the same for the samples containing zeolite, with and without HRP, the sample containing the enzyme presents a slightly lower oxidation potential, indicating that the electro-oxidation of phenol is easier. Again, the sample with silica and HRP (gelatin/HRP/$(SiO_2)_p$, $m_{HRP}/m_{(SiO_2)_p}$ = 1:100) shows lower current intensity and a higher potential on this type of electrode.

These results indicate that the combination of HRP with zeolite has a distinct influence on the redox activity. To have a clearer picture of the effect of adding the enzyme to the zeolite system the scan rate was varied. Fig. 3a and b compares the behaviour of electrodes with (gelatin/HRP/zeolite continuous lines) and without HRP (gelatin/zeolite dashed lines) for the two different ratios m_{HRP}/m_{zeo} (1:100 and 5:100, respectively) and for three different scan rates (10, 50 and 100 mV/s).

As it can be seen in Fig. 3a and b, the current intensity is higher in all experiments where the electrodes include the enzyme.

Once again, the best result was achieved with the 1:100 sample, as it was previously observed for the oxidation of phenol with hydrogen peroxide in solution [32].

Moreover, Fig. 3a shows that increasing the scan rate (50 and 100 mV/s) clearly reveals the catalytic properties of the HRP in the electrode. At 50 mV/s, the current intensity is much higher with the enzyme containing electrode than in the corresponding electrode without the enzyme; additionally, for the 100 mV/s experiment, not only the intensity was much higher, but also the phenol oxidation wave of the electrode without the enzyme was displaced to much higher potentials.

Changing the scan rates allows us to have an idea of the rate of the reactions involved and the fact that the current is higher at higher scan rates when HRP is present clearly indicates that the HRP present in the electrode is effectively participating in the reaction, decreasing the phenol oxidation potential and working as a mediator in its electro-oxidation.

From the results presented above we can also see that the mass ratio m_{HRP}/m_{zeo} is significant. In the case of the sample 5:100, where less zeolite was put into the particles (Table 1), we can see that the current is always lower, probably due to the fact

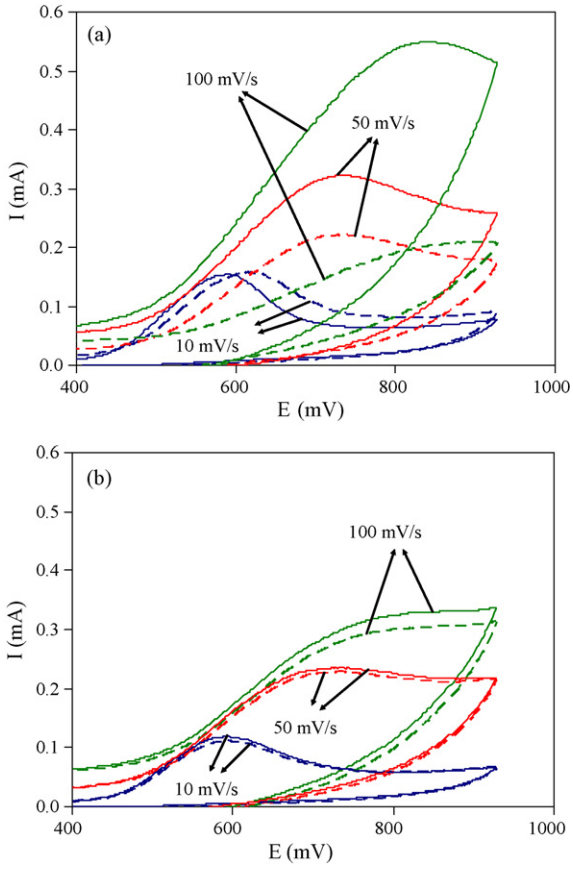

Fig. 3. Comparison of voltammograms between the biocomposite electrode with HRP (gelatin/HRP/zeolite/graphite continuous lines) and the corresponding composite electrode without enzyme (gelatin/zeolite/graphite dashed lines) for two different ratios m_{HRP}/m_{zeo} 1:100 (a) and 5:100 (b) using three different scan rates and 5 mM of phenol solution.

that the electrode itself is less porous, and the presence of HRP has a less pronounced effect.

The variation of the scan rate also allows us to see that electron transfer seems to be controlled by diffusion. In fact, in a cyclic voltammetric process that is diffusion controlled the observed current can be related to the scan rate by Eq. (5) (written in a more concise form for 25 °C, where the constant value is 2.99×10^5 C mol^{-1} V$^{-1/2}$) [52]:

$$I = (2.99 \times 10^5) n(\alpha n_a)^{1/2} A C_b D_e^{1/2} v^{1/2} \quad (5)$$

where I is the peak height, n the number of electrons, α the transfer coefficient, n_a the number of electrons involved in the rate-determining charge-transfer step, C_b the bulk concentration of phenol in solution, D_e the diffusion coefficient and v is the scan rate.

It can be seen in Fig. 4 that all samples containing HRP presented a linear I versus $v^{1/2}$ relationship which is indicative of a diffusion controlled process; the non-zero intercepts may be due to uncompensated resistance in the electrode, or to other non-faradic components in the current that seem not to be dependent on the scan rate. In contrast, it was seen in previous work that the $NaY_{2.5}$/graphite electrodes present a current that is essentially due to the adsorbed phenol and has only a limited contribution from species that diffuse to the electrode during the actual cyclic voltammogram [33,35].

Thus, the biocomposite electrode with gelatin seems to improve the rate of diffusion in relation to the original $NaY_{2.5}$/graphite.

The increase rate of diffusion can also be seen in Fig. 5. In this experiment the electrode was saturated by plunging the electrode in a 100 mM phenol solution for about 10 min, so that the pores of the electrode are filled with this solution; the electrode was then placed in the cell containing only a clear buffer solution and fast cyclic voltammograms were recorded as a function of the time of contact with this fresh solution. These voltammograms allow us to measure the amount of phenol that is still inside the electrode and, thus, we can follow the diffusion of the phenol out of the electrode into the clear buffer solution. Fig. 5 compares

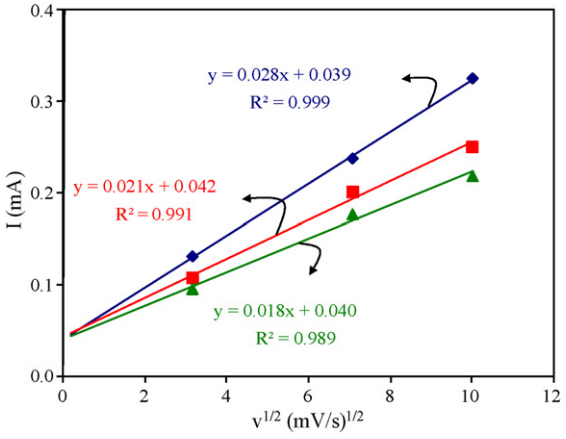

Fig. 4. Current intensity of the oxidation wave as a function of the square root of the scan rate for gelatin/HRP/zeolite/graphite 1:100 (♦), 2:100 (■) and 5:100 (▲) biocomposite electrodes. Empirical equations are given.

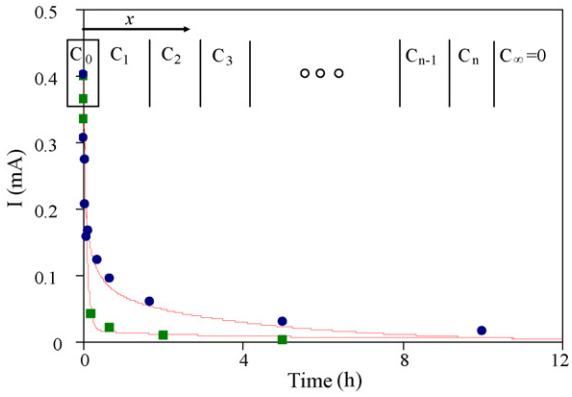

Fig. 5. Mass transfer of phenol in $NaY_{2.5}$/graphite composite electrode (●) and gelatin/HRP/$NaY_{2.5}$/graphite 1:100 biocomposite electrode (■) (the line represents the diffusional model that is described by Eqs. (6)–(8)).

the results obtained in these experiments for a zeolite/graphite [35] and a gelatin/HRP/zeolite/graphite electrode.

A simple diffusion model, in which the electrode particle is described as a slab and the solution immediately in contact with it is divided into n sections (according to Eqs. (6)–(8)), was used to compute the effective diffusivity of phenol, as previously done for the $NaY_{2.5}$/graphite electrode [35]. Fitting this model to the experimental data by taking A/V and D_e as parameters gave an estimated value for the effective diffusivity of phenol of $D_e = 21.6$ mm^2/h, which can be compared with the one previously obtained for $NaY_{2.5}$/graphite electrode ($D_e = 1.6$ mm^2/h) [35]:

$$V\frac{\partial C_0}{\partial t} = -D_e A \frac{\partial C}{\partial x} \approx -D_e A \frac{C_0 - C_1}{\Delta x/2} \quad (6)$$

$$\frac{\partial C_1}{\partial t} = \left(\frac{C_0 - C_1}{\Delta x/2} - \frac{C_1 - C_2}{\Delta x}\right)\frac{D_e}{\Delta x} \quad (7)$$

$$\frac{\partial C_n}{\partial t} = \left(\frac{C_{n-1} - C_n}{\Delta x} - \frac{C_n - C_\infty}{\Delta x}\right)\frac{D_e}{\Delta x} \quad (8)$$

The biocomposite electrode has an effective phenol diffusivity which is one order of magnitude higher than the zeolite/graphite one. It should be noted, however, that the mechanical stability of the biocomposite electrode decreases in comparison with the composite electrode.

4. Conclusions

From the results presented, it can be seen that HRP can be directly involved in the electro-oxidation of phenol with a composite electrode containing $NaY_{2.5}$/graphite even in a homogeneous solution bulk.

The use of gelatin/HRP/zeolite/graphite biocomposite electrodes, in comparison with the simple zeolite/graphite ones, not only reduced diffusion limitations but also showed that the HRP was electro-active and participated in the electro-oxidation of phenol, decreasing the oxidation potential and increasing the current, namely for higher scan rates.

From the experimental results we can see that this electrode does not require the presence of hydrogen peroxide to

oxidise phenol, since the oxidation of the enzyme can be made directly within the composite electrode, which constitutes a clear advantage, not only because hydrogen peroxide is an expensive reactant, but also because it can react with other compounds present in the solution. The mechanism, by which the enzyme is oxidised, however, still requires clarification.

These results indicate that HRP–zeolite interactions do exist and potentiate the use of systems that rely on these two components, as it is the case of these biocomposite electrodes.

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References

[1] T. Ruzgas, E. Csöregi, J. Emnéus, L. Gorton, G. Marko-Varga, Anal. Chim. Acta 330 (1996) 123–138.
[2] B. Serra, B. Benito, L. Agüí, A.J. Reviejo, J.M. Pingarrón, Electroanalysis 13 (2001) 693–700.
[3] T. Ruzgas, L. Gorton, J. Emnéus, G. Marko-Varga, J. Electroanal. Chem. 391 (1995) 41–49.
[4] A. Lindgren, J. Emnéus, T. Ruzgas, L. Gorton, G. Marko-Varga, Anal. Chim. Acta 347 (1997) 51–62.
[5] L. Gorton, A. Lindgren, T. Larsson, F.D. Munteanu, T. Ruzgas, I. Gazaryan, Anal. Chim. Acta 400 (1999) 91–108.
[6] T. Ruzgas, J. Emnéus, L. Gorton, G. Marko-Varga, Anal. Chim. Acta. 311 (1995) 245–253.
[7] S.S. Rosatto, P.T. Sotomayor, L.T. Kubota, Y. Gushikem, Electrochim. Acta 47 (2002) 4451–4458.
[8] S.S. Rosatto, L.T. Kubota, G.O. Neto, Anal. Chim. Acta 390 (1999) 65–72.
[9] S.A. Kane, E.I. Iwuoha, M.R. Smyth, Analyst 123 (1998) 2001–2006.
[10] S.-I. Imabayashi, Y.-T. Kong, M. Watanabe, Electroanalysis 13 (2001) 408–412.
[11] S. Yang, Y. Li, X. Jiang, Z. Chen, X. Lin, Sens. Actuators B 114 (2006) 774–780.
[12] F.-D. Munteanu, A. Lindgren, J. Emnéus, L. Gorton, T. Ruzgas, E. Csöregi, A. Ciucu, R.B. Van Huystee, I.G. Gazaryan, L.M. Lagrimini, Anal. Chem. 70 (1998) 2596–2600.
[13] A. Morrin, A. Guzman, A.J. Killard, J.M. Pigarron, M.R. Smyth, Biosens. Bioelectron. 18 (2003) 715–720.
[14] C.-X. Lei, S.-Q. Hu, G.-L. Shen, R.-Q. Yu, Talanta 59 (2003) 981–988.
[15] M.C. Ramos, M.C. Torijas, A.N. Díaz, Sens. Actuators B 73 (2001) 71–75.
[16] E. Csöregi, G. Jönsson-Pettersson, L. Gorton, J. Biotechnol. 30 (1993) 315–337.
[17] A. Morales, F. Céspedes, J. Muñoz, E. Martínez-Fábregas, S. Alegret, Anal. Chim. Acta 332 (1996) 131–138.
[18] C. Fan, H. Wang, D. Zhu, G. Wagner, G. Li, Anal. Sci. 17 (2001) 273–276.
[19] S.-Q. Liu, H.-X. Ju, Anal. Biochem. 307 (2002) 110–116.
[20] Y.-T. Kong, M. Boopathi, Y.-B. Shim, Biosens. Bioelectron. 19 (2003) 227–232.
[21] J. Tang, B. Wang, Z. Wu, X. Han, S. Dong, E. Wang, Biosens. Bioelectron. 18 (2003) 867–872.
[22] M.G. Garguilo, N. Huynh, A. Proctor, A.C. Michael, Anal. Chem. 65 (1993) 523–528.
[23] J. Qian, Y. Liu, H. Liu, T. Yu, J. Deng, J. Electroanal. Chem. 397 (1995) 157–162.
[24] J. Qian, Y. Liu, H. Liu, T. Yu, J. Deng, Anal. Biochem. 236 (1996) 208–214.
[25] J. Li, S.N. Tan, H. Ge, Anal. Chim. Acta 335 (1996) 137–145.
[26] C. Lei, Z. Zhang, H. Liu, J. Kong, J. Deng, Anal. Chim. Acta 332 (1996) 73–81.
[27] P.C. Pandey, S. Upadhyay, B. Upadhyay, Anal. Biochem. 252 (1997) 136–142.
[28] V. Rajendran, E. Csöregi, Y. Okamoto, L. Gorton, Anal. Chim. Acta 373 (1998) 241–251.
[29] Y. Xiao, H.-X. Ju, H.-Y. Chen, Anal. Chim. Acta 391 (1999) 73–82.
[30] S. Gaspar, I. Popescu, I.G. Gazaryan, A.G. Bautista, I.Y. Sakharov, B. Mattiasson, E. Csöregi, Electrochim. Acta 46 (2000) 255–264.
[31] R.H. Carvalho, F. Lemos, M.A.N.D.A. Lemos, V. Vojinović, L.P. Fonseca, J.M.S. Cabral, Bioproc. Biosyst. Eng. 29 (2006) 99–108.
[32] R.H. Carvalho, F. Lemos, J.M.S. Cabral, F. Ramôa Ribeiro, J. Mol. Catal. B: Enzym. 44 (2007) 39–47.
[33] R.H. Carvalho, F. Lemos, M.A.N.D.A. Lemos, J.M.S. Cabral, F. Ramôa Ribeiro, J. Mol. Catal. A: Chem. 248 (2006) 48–52.
[34] R.H. Carvalho, M.A.N.D.A. Lemos, F. Lemos, J.M.S. Cabral, F. Ramôa Ribeiro, J. Mol. Catal. A: Chem. 253 (2006) 170–175.
[35] R.H. Carvalho, M.A.N.D.A. Lemos, F. Lemos, J.M.S. Cabral, F. Ramôa Ribeiro, Catal. Today, in press.
[36] H. Kotte, B. Gründig, K.-D. Vorlop, B. Strehlitz, U. Stottmeister, Anal. Chem. 67 (1995) 65–70.
[37] G. Marko-Varga, E. Burestedt, C.J. Svensson, J. Emnéus, L. Gorton, T. Ruzgas, M. Lutz, K.K. Unger, Electroanalysis 8 (1996) 1121–1126.
[38] T. Yu, Y. Zhang, C. You, J. Zhuang, B. Wang, B. Liu, Y. Kang, Y. Tang, Chem. Eur. J. 12 (2006) 1137–1143.
[39] Z. Dai, S. Liu, H. Ju, Electrochim. Acta 49 (2004) 2139–2144.
[40] B. Liu, F. Yan, J. Kong, J. Deng, Anal. Chim. Acta 386 (1999) 31–39.
[41] B. Liu, Z. Liu, D. Chen, J. Kong, J. Deng, Fresenius J. Anal. Chem. 367 (2000) 539–544.
[42] B. Liu, R. Hu, J. Deng, Anal. Chem. 69 (1997) 2343–2348.
[43] C.F. Chen, C.M. Wang, J. Electroanal. Chem. 466 (1999) 82–99.
[44] J. Wang, A. Walcarius, J. Electroanal. Chem. 404 (1996) 237–242.
[45] M.L. Hamlaoui, K. Reybier, M. Marrakchi, N. Jaffrezic-Renault, C. Martelet, R. Kherrat, A. Walcarius, Anal. Chim. Acta 466 (2002) 39–45.
[46] A. Nighojkar, S. Srivastava, A. Kumar, J. Ferm. Bioeng. 80 (1995) 346–349.
[47] M.A.N.D.A. Lemos, P. Sousa, F. Lemos, A.J.L. Pombeiro, F. Ramôa Ribeiro, Stud. Surf. Sci. Catal. 122 (1999) 443–446.
[48] J.A. Nicell, J. Chem. Technol. Biotechnol. 60 (1994) 203–215.
[49] P.K. Patel, M.S. Mondal, S. Modi, D.V. Behere, Biochim. Biophys. Acta 1339 (1997) 79–87.
[50] D.I. Metelitza, A.V. Litvinchuk, M.I. Savenkova, J. Mol. Catal. 67 (1991) 401–411.
[51] K.J. Baynton, J.K. Bewtra, N. Biswas, K.E. Taylor, Biochim. Biophys. Acta 1206 (1994) 272–278.
[52] A.J. Bard, L.R. Faulkner, Electrochemical Methods. Fundamentals and Applications, John Wiley & Sons Inc., New York, 1980, p. 222.