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Determination of organic peroxides in reversed micelles with a poly-*N*-methylpyrrole horseradish peroxidase amperometric biosensor

E. García-Moreno [a], M.A. Ruiz [a], C. Barbas [a,*], J.M. Pingarrón [b]

[a] *Facultad de CC, Experimentales y Técnicas, Universidad San Pablo C.E.U., Urbanizacion Montepríncipe, 28668 Madrid, Spain*
[b] *Department of Analytical Chemistry, Faculty of Chemistry, Complutense University of Madrid, 28040 Madrid, Spain*

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Abstract

The preparation of a peroxidase biosensor by immobilization of the enzyme during the electropolymerization of *N*-methylpyrrole and its use in the determination of organic peroxides in a predominantly nonaqueous medium, such as reversed micelles, is reported. Reversed micelles were formed with ethyl acetate as the continuous phase, 4% of 0.05 mol l^{-1} phosphate buffer solution of pH 7.4 as the dispersed phase, and 0.1 mol l^{-1} AOT as the emulsifying agent. Working variables affecting the polymer biosensor preparation, such as the polymerization potential, the constant accumulated charge to stop the polymerization process, the concentration of monomer and enzyme in the polymerization solution and the pH and concentration of the phosphate buffer solution, were optimized and discussed. Concerning the variables regarding the amperometric measurements in the reversed micellar medium, the potential value applied, the pH of the phosphate buffer solution, and the temperature were also optimized. Under the optimized conditions, the steady-state current for 2-butanone peroxide is reached in only 4 s. Linear calibration plots over the ranges 5–85 μmol l^{-1}, and 2–48 mmol l^{-1} were obtained for 2-butanone peroxide and *tert*-butylhydroperoxide, respectively. The limits of detection obtained were 0.086 μmol l^{-1}, and 0.03 mmol l^{-1}, respectively. The poly-*N*-methylpyrrole-HRP amperometric biosensor was used for the determination of the organic peroxide content in body lotion samples, by employing 2-butanone peroxide as a standard. Optimization of the peroxide extraction step from the sample was carried out, and recoveries approximating 100% were obtained.

Keywords: Poly-*N*-methylpyrrole; Peroxidase; Organic peroxides; Reversed micelles; Amperometry

1. Introduction

Determination of organic peroxides is of great interest in both the clinical field and food industry because these peroxides can be involved in ageing, mutagenic processes and in some types of diseases [1–3]. Reported methods for this purpose include fluorimetry [4], HPLC [5,6] and GC–MS [7]. Peroxidase-based electrochemical biosensors have also been extensively used for the determination of peroxides [8]. However, the scarce solubility of organic peroxides in water and the hydrophobicity of some samples (oils, for example) have led to the development of peroxidase electrodes able to work in predominantly nonaqueous media [9–11]. In this context, the usefulness of reversed micellar media for developing enzyme amperometric biosensors has recently been demonstrated [12–14]. These media, also called water-in-oil emulsions, are composed of an organic solvent acting as

* Corresponding author. Fax: +34-913510475.
E-mail address: cbarbas@ceu.es (C. Barbas).

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the continuous phase, a small amount of water (in our case, an aqueous solution of an appropriate supporting electrolyte) as the dispersed phase, and a surfactant as the emulsifying agent. These organized media can be considered as universal solubilization media for both hydrophilic and hydrophobic analytes, and allow the enzymatic determination of substances scarcely soluble in water. Furthermore, the content of the water needed for the hydration of the enzyme, and therefore, for enzyme operation, is easy to control and optimize.

On the other hand, enzyme immobilization during the electropolymerization of conducting polymers has attracted wide interest [15,16]. This methodology allows uniform and reproducible immobilization of enzymes over a small area, as well as control of the coating thickness, and consequently, of the amount of enzyme immobilized, by changing the polymerization conditions [17]. The use of electropolymerized films for the construction of amperometric biosensors has been reviewed [18,19].

In this work, we have combined the advantages of reversed micelles as the working media with those of the preparation of biochemical sensors, by forming conducting polymer layers via electropolymerization, to develop a peroxidase electrochemical biosensor for the determination of organic peroxides. Although polypyrrole is the most widely used conducting polymer in the preparation of enzyme electrodes [20,21], the use of this polymer has some practical drawbacks: the need for monomer distillation, solution deaeration (to avoid the oxidation of pyrrole during the electropolymerization) and careful handling of the polypyrrole film (to avoid its oxidation during transfer from the polymerization solution to the measurement cell). Consequently, we have chosen to use *N*-methylpyrrole, whose polymer film is more difficult to oxidize, as an appropriate monomer for constructing the polymer peroxidase electrodes.

2. Experimental

2.1. Apparatus

Both electropolymerization and amperometric measurements were performed on an Autolab PGSTAT 20 potentiostat, equipped with the ECD low current module, and using the GPES 4.0 electrochemical analysis software. A magnetic stirrer (P-Selecta), an ultrasonic bath (P-Selecta), a vortex MS1 minishaker (IKA), an XL2020 microtip probe-equipped ultrasonic liquid processor (Misonix), a Centronic centrifuge (P-Selecta), and a thermostatic bath (P-Selecta) were also used.

2.2. Electrodes and electrochemical cell

A Metrohm 6.1204.120 Pt disk electrode (2 mm diameter) was used as the electrode substrate to prepare the polymer peroxidase biosensor. A BAS RE-5B (Mod. 2052) Ag/AgCl reference electrode, a Pt wire auxiliary electrode, and a double-walled electrochemical cell (Pobel) were also used.

2.3. Reagents and solutions

Stock solutions of 2-butanone peroxide (0.10 mol l^{-1}, Fluka), *tert*-butyl hydroperoxide (0.50 mol l^{-1}, Sigma), ferrocene (0.10 mol l^{-1}, Sigma), and dioctyl sulphosuccinate (AOT, 0.10 mol l^{-1}, Sigma) were prepared in ethyl acetate (Prolabo). Horseradish peroxidase (HRP) EC 1.11.1.7, type II, activity 240 U mg^{-1} was obtained from Sigma (cat. no. P8250). HRP stock solutions were freshly prepared by dissolving 0.0083 g of enzyme in 100 μl of 0.2 mol l^{-1} phosphate buffer (pH 7.0).

An aqueous 0.4 mol l^{-1} *N*-methylpyrrole (Aldrich) solution in a 0.2 mol l^{-1} phosphate buffer solution of pH 8.5, containing 80 U ml^{-1} HRP, was used in the enzyme immobilization process by electropolymerization. Other chemicals were of analytical reagent grade, and water was obtained from a Millipore Milli-Q purification system.

2.4. Sample

The sample analyzed was a commercial body lotion (Johnson & Johnson) purchased in a local supermarket.

2.5. Procedures

2.5.1. Preparation of the HRP bioelectrode

Prior to polymerization, the Pt electrode was polished for 1–2 min with diamond powder (1 μm particle size, BAS MF-2054), rinsed with deionized water in

the ultrasonic bath, polished with alumina (Metrohm, 6.2802.000), and rinsed again. Electropolymerization was accomplished in the above mentioned stirred solution by applying a constant potential of +0.85 V. The amount of polymer electrodeposited was controlled by monitoring the accumulated charge during the film growth and stopping the deposition after a charge of 15 mC cm^{-2} had been passed. After polymerization, the enzyme electrode was rinsed with deionized water and let dry before using.

2.5.2. Amperometric measurements

The enzyme electrode was immersed in the electrochemical cell containing 10.0 ml of the corresponding reversed micelle solution (prepared with ethyl acetate, 0.1 mol l^{-1} AOT, 5.0×10^{-4} mol l^{-1} ferrocene and 4% of 0.05 mol l^{-1} phosphate buffer of pH 7.4), and amperometric measurements under constant stirring conditions were performed at constant temperature by applying a potential of +0.10 V, and allowing the steady-state current to be reached.

2.5.3. Determination of organic peroxides in a body lotion sample

About 1 g of sample was accurately weighed into a centrifuge tube. Next, 2 ml of a 1:1 ethyl acetate:isopropanol mixture were added. The mixture was homogenized by shaking with the vortex mini-shaker at 2200 rpm for 1 min. Then, the mixture was sonicated using the microtip at the highest power and in pulse mode (pulsar duty cycle 25% s^{-1}). In order to avoid evaporation of the organic solvent, the centrifuge tube was immersed in a glass with ice during sonication. After centrifugation at 4000 rpm for 15 min, a 40 μl aliquot of the upper resulting phase was transferred to the electrochemical cell containing 10 ml of the above mentioned reversed micelle. Determination of the peroxide content was performed by applying the standard additions method, which involved five successive 40 μl additions of a 1.25×10^{-3} mol l^{-1} 2-butanone peroxide stock solution in ethyl acetate (5.0–25.0 μmol l^{-1} 2-butanone peroxide additions).

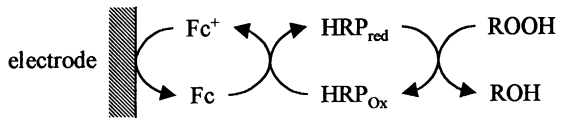
Recovery studies were done by adding about 500 μg g^{-1} of 2-butanone peroxide to approximately 10 g of sample (accurately weighed). After homogenization, the same procedure described above was followed.

3. Results and discussion

As indicated in the introduction, we benefited from the advantages of reversed micelles as suitable working media for developing amperometric enzyme electrodes, to construct an HRP electrochemical biosensor for the determination of organic peroxides. The composition of the reversed micellar system employed was the same as that optimized previously [22], i.e. ethyl acetate as the organic solvent, 4% of 0.05 mol l^{-1} phosphate buffer solution of pH 7.4 as the aqueous dispersed phase, and 0.1 mol l^{-1} AOT as the emulsifying agent. The enzymatic reaction used involved catalytic reduction of peroxides in the presence of ferrocene as a mediator, and consequently, the resulting amperometric signal corresponded to the electrochemical reduction of ferricinium (Scheme 1). A ferrocene concentration of 5.0×10^{-4} mol l^{-1} in the reversed micelle was used in all cases.

3.1. Optimization of working variables affecting the polymer biosensor preparation

The *N*-methylpyrrole was selected as an appropriate monomer to construct the polymer peroxidase electrodes for the practical reasons mentioned in the introduction. The optimization of the variables affecting the polymer bioelectrode construction was accomplished using concentrations of 2-butanone peroxide as standard organic peroxide, between 5.0 and 25.0 μmol l^{-1} in the reversed micellar working medium. In all cases, the optimum response was considered as that which provided the highest sensitivity, i.e. the highest slope of the calibration plot obtained for the previously mentioned 2-butanone peroxide concentration range. The working variables checked were the polymerization potential, the constant accumulated charge to stop the polymerization process, the concentration of monomer and enzyme in the polymerization solution, and the pH and concentration of the phosphate buffer solution.



Scheme 1. Reaction sequence for the poly-*N*-methylpyrrole-HRP amperometric biosensor

The effect of the electropolymerization potential was evaluated by carrying out the oxidative process in a 0.2 mol l^{-1} phosphate buffer solution of pH 7.0, containing the monomer at a concentration of 0.4 mol l^{-1} and 100 U ml^{-1} of the enzyme HRP. The potentiostatic polymerization was performed for the period of time necessary to reach an accumulated charge of 10 mC cm^{-2}, the potential ranging between 0.70 and 0.95 V. Once the polymer bioelectrode was prepared, amperometric measurements of 2-butanone peroxide in the reversed micelles were carried out at +0.10 V. The slope of the 2-butanone peroxide calibration graph increased slightly up to 0.85 V and then levelled off. This was the expected behavior, because the final accumulated charge was the same, and consequently, the amount of polymer formed and, therefore, of the enzyme immobilized, should also be the same. Nevertheless, as expected, the time needed to reach such a charge decreased as the polymerization potential increased. A potential of 0.85 V was then selected for subsequent work.

Fig. 1 shows the results obtained for the optimization of the accumulated charge during the electropolymerization process. As observed, the sensitivity of the 2-butanone peroxide calibration plot increased as the polymerization process was carried out, from 2 to 15 mC cm^{-2}, as a consequence of the higher amount of HRP immobilized. However, from 15 mC cm^{-2} the sensitivity decreased slightly, which was attributed to the fact that a very thick polymer coating slows down the electron transfer through the film. Of course, the polymerization time increased as the accumulated charge increased, with periods of 10 min for 30 mC cm^{-2}. Consequently, a charge of 15 mC cm^{-2} was selected to obtain an appropriate amount of the electrodeposited polymer for analytical purposes.

Regarding the monomer concentration in the polymerization solution, no influence on the sensitivity for concentrations between 0.2 and 0.4 mol l^{-1} was observed. Concentrations lower than 0.2 mol l^{-1} prolonged the polymerization time considerably, and concentrations higher than 0.4 mol l^{-1} caused solubility problems. Taking into account that the time needed to reach the selected charge was lower as the *N*-methylpyrrol concentration was higher, a 0.4 mol l^{-1} monomer solution was used for further work.

An interesting study is that concerned with the enzyme concentration and the pH in the electropolymerization solution. Different authors have discussed the mechanisms for enzyme immobilization by in situ electrochemical polymerization, which are based on physical entrapment within the polymer matrices

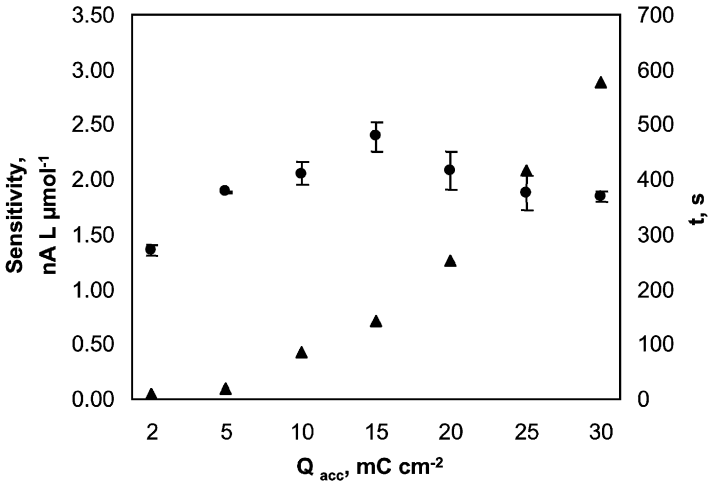


Fig. 1. Influence of the accumulated charge during the electropolymerization process on the (●) sensitivity (see text) of a poly-*N*-methylpyrrole-HRP amperometric biosensor, and (▲) the polymerization time. $E_{app} = +0.85$ V. Polymerization solution: 0.2 mol l^{-1} phosphate buffer of pH 7.0, 0.4 mol l^{-1} *N*-methylpyrrole, and 100 U ml^{-1} HRP; test solution: 5.0–25.0 μmol l^{-1} 2-butanone peroxide in reversed micelles formed with ethyl acetate, a 4% of 0.05 mol l^{-1} phosphate buffer solution of pH 7.4, and 0.1 mol l^{-1} AOT; 5×10^{-4} mol l^{-1} ferrocene; $E_{app} = +0.10$ V.

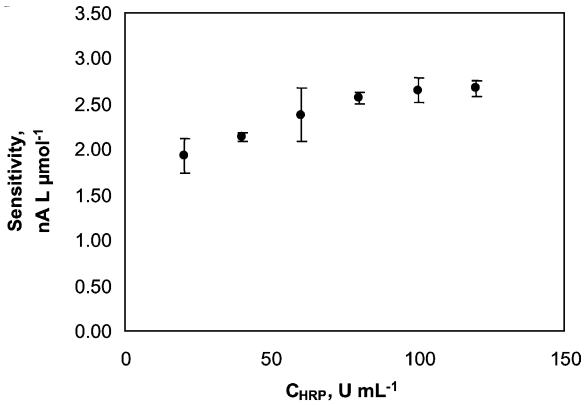


Fig. 2. Effect of the enzyme concentration in solution during the electropolymerization process on the sensitivity of a poly-*N*-methylpyrrole-HRP amperometric biosensor in reversed micelles. $Q_{acc} = 15\,mC\,cm^{-2}$. Other conditions concerning both the polymerization solution and the reversed micellar working medium as in Fig. 1.

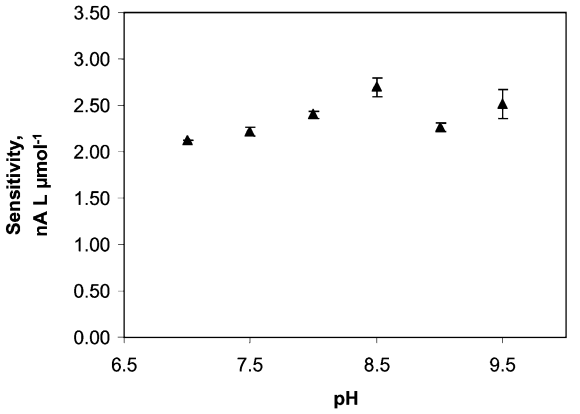


Fig. 3. Influence of the electropolymerization solution pH on the sensitivity of a poly-*N*-methylpyrrole-HRP amperometric biosensor in reversed micelles; $Q_{acc} = 15\,mC\,cm^{-2}$, $80\,U\,ml^{-1}$ HRP. Other conditions as in Fig. 1.

during electropolymerization [23,24], and the use of negatively charged enzymes as a dopant for conductive polymers [25,26]. If the enzyme immobilization mechanism was governed only by physical entrapment during the polymer growth, it could be expected that the amount of immobilized enzyme would be proportional to its concentration in solution and consequently, the biosensor sensitivity would increase as this concentration increased. Furthermore, the amount of entrapped enzyme should be independent of pH in the usual working range [27].

Fig. 2 shows the trend observed when the HRP concentration in solution was varied between 20 and $120\,U\,ml^{-1}$. As seen, the sensitivity increased with the enzyme content, up to $80\,U\,ml^{-1}$, and then levelled off. This behavior was attributed to an incorporation of the enzyme molecules into the polymer by electrostatic attraction to compensate for the positive charge appearing in the electropolymerization process. At low enzyme concentrations, not all the positive charges can be compensated for with enzyme molecules (probably the remaining sites would be occupied by phosphate anions). The amount of HRP entrapped increased as the enzyme concentration in solution also increased. However, when most of the positive sites could be compensated for with the charged enzyme molecules, an increase in the HRP concentration did not imply a higher amount of immobilized enzyme.

This assumption was supported by the effect of pH observed on the biosensor sensitivity. Fig. 3 shows the results obtained in the 7.0–9.5 pH range, using a constant HRP concentration of $80\,U\,ml^{-1}$. The pH range was selected taking into account the data found in the literature on the HRP isoelectric point [28,29]. An increase in sensitivity was observed with a pH up to 8.5, which indicated that an increase in the net enzyme charge gave rise to a higher amount of immobilized enzyme. These results allow us to conclude that the enzyme immobilization mechanism is governed not only by physical entrapment during the polymer growth, but also by electrostatic attraction. A pH value of 8.5 was selected for further work.

Finally, as phosphate anions can compete with the enzyme molecules for the positive sites in the polymer, the effect of the phosphate supporting electrolyte concentration in the polymerization solution was also checked at two pH values, 7.0 and 8.0, in the $0.1–0.3\,mol\,l^{-1}$ concentration range. Again, the sensitivity was higher at pH 8.5, but no significant variation was observed when changing the phosphate concentration. This may mean that the enzyme concentration in solution was high enough to ensure a preferential incorporation of HRP in the polymer film.

Under the optimized working conditions, the polymer-HRP biosensor exhibited a rapid response to the changes in the 2-butanone peroxide concentration in the reversed micelles, reaching the steady-state current in only 4 s.

Table 1
Analytical characteristics for the determination of 2-butanone peroxide and *tert*-butylhydroperoxide in reversed micelles at a poly-*N*-methylpyrrole-HRP amperometric biosensor

Organic peroxide	Linear range (μmol l^{-1})	Slope (nA l μmol^{-1})	Intercept (nA)	r	Detection limit (μmol l^{-1})	Quantification limit (μmol l^{-1})	R.S.D. (%)
2-Butanone peroxide	5–85	1.49 ± 0.05	0.9 ± 3	0.9998	0.086	0.3	5.1
Tert-butylhydroperoxide	$(2–48) \times 10^3$	$(5.2 \pm 0.1) \times 10^{-4}$	0.3 ± 0.3	0.9990	30	90	6.2

3.2. Optimization of working variables affecting the organic peroxide amperometric measurements

Concerning the variables regarding the amperometric measurements in the reversed micellar medium, the potential value applied, as well as the pH of the aqueous dispersed phosphate buffer solution and the temperature, were optimized. Further, the slope of the calibration plot for 2-butanone peroxide was taken as the evaluation criterion to select the corresponding variable.

In the case of the applied potential, taking into account both the sensitivity and the background current, a value of +0.10 V was selected to carry out the amperometric monitoring of the enzyme reaction. At this potential, no deaeration of the measurement solution was needed.

On the other hand, no significant variation in the 2-butanone calibration slope was found when the pH of the dispersed aqueous phase was varied between 7.0 and 7.6. However, a considerable decrease in sensitivity was observed for higher pH values. Consequently, a pH of 7.4 was chosen for analytical purposes.

The effect of temperature on the biosensor response was checked in the 20–40°C range. Sensitivity increased with the temperature up to 30°C, and decreased for temperatures higher than 35°C, due to the possible deactivation of the enzyme. As the highest sensitivity was obtained between 30–35°C, we decided to work at controlled temperatures within this range.

3.3. Calibration plots and analytical characteristics

Under the optimized working conditions, calibration plots for 2-butanone peroxide and *tert*-butylhydroperoxide were constructed in the reversed micellar system. The analytical characteristics for both calibration graphs are summarized in Table 1. As observed, the sensitivity is much higher for 2-butanone peroxide than for *tert*-butylhydroperoxide, as expected, taking into account that peroxidase, in general, rapidly reacts with alkyl peroxides. Secondary, tertiary and endoperoxides have proved to be unable to serve as peroxidase substrates [8]. The limits of quantification and detection were estimated according to the $10 \times$ S.D. on and $3\,s_b/m$ criteria, respectively, where m is the slope of the corresponding calibration graph and s_b is the standard deviation of the blank current, which was measured each second for a period of time 10-fold the width of the registered analytical signal. It should be noted that the detection limits achieved were good when they were compared with those reported in the literature for other peroxidase biosensors working in aqueous solutions [8]. Relative standard deviations shown in Table 1 were calculated from 10 different solutions of each substrate at a concentration level of 5.0×10^{-6} mol l^{-1} for 2-butanone peroxide, and 2.0×10^{-3} mol l^{-1} for *tert*-butylhydroperoxide.

The reproducibility of the responses obtained from different polymer-HRP bioelectrodes constructed in the same manner was evaluated by comparing the slope values of the corresponding 2-butanone peroxide calibration plots obtained with five different electrodes. An R.S.D. value of 5.7% was found, indicating that the preparation procedure of the polymer biosensors is reliable and allows reproducible electroanalytical responses to be obtained with different electrodes.

Calculation of the apparent Michelis–Menten constants ($K_{m,app}$) and the maximum rate of the reaction (V_m) were carried out from the corresponding Lineweaver–Burk plots for each substrate. $K_{m,app}$ values were 206 μmol l^{-1} and 2640 mmol l^{-1} for 2-butanone peroxide and *tert*-butylhydroperoxide,

respectively, whereas the V_m values obtained were 238 and 1429 nA, respectively.

3.4. Determination of organic peroxides in cosmetic samples

The HRP biosensor developed was used for the determination of the organic peroxide content in a cosmetic sample, a body lotion. Formation of peroxides in this type of samples occurs as a consequence of the oxidation of the long-chain lipids in which they contain, which in turn gives rise to the irreversible deterioration of the product. Consequently, the detection and monitoring of the peroxide formation can be used as a quality indicator for these products.

As occurred with other methods which do not involve separation steps, the determination of the peroxide content was done by using one of them (2-butanone peroxide) as a standard. Therefore, the concentrations obtained are actually an indication of the real content, because, as was shown above, not all the organic peroxides gave the same response at the bioelectrode.

Due to the hydrophobic nature of the analytes, peroxides were extracted using an organic solvent, and hence, different solvents were tested in order to optimize this step. Using ethyl acetate, *n*-hexane, chloroform nor isopropanol alone, no satisfactory results could be obtained. Then, taking into account that ethyl acetate is the solvent employed to prepare the reversed micelles, mixtures of this solvent with the others were tested. Only by following the procedure described in the Section 2 (using focused ultrasonication) and using an ethyl acetate:isopropanol mixture (containing more than 40% isopropanol) as extracting solvent, could a total disegregation of the sample with two well-separated phases be obtained. No significant differences in the amount of extracted peroxides were found for isopropanol percentages in the mixture higher than 50%. Therefore, a 1:1 ethyl acetate:isopropanol mixture was used as the extracting agent. Furthermore, the sonication time was also optimized in order to avoid a possible degradation of the extracted peroxides. Using the working conditions of the microtip ultrasonic processor described in the Section 2, the amount of peroxides extracted was maximum for a sonication time of 3 min, which was selected for further work. Assuming that under the above mentioned conditions the amount of peroxides extracted was 100% (which was further confirmed), the rest of the solvent mixtures checked gave extraction performances of around 40%.

Table 2
Determination of the organic peroxide content in body lotion samples at a poly-*N*-methylpyrrole-HRP amperometric biosensor in reversed micelles; 2.0 ml of 1:1 ethyl acetate:isopropanol as extracting agent; sonication time, 3 min

Sample	Body lotion weight (g)	Organic peroxide concentration ($\mu g\,g^{-1}$)
1	1.1239	305 ± 76
2	1.0590	309 ± 9
3	0.9969	305 ± 20
4	1.1533	327 ± 64
5	0.9720	336 ± 17
6	1.0645	316 ± 84
7	0.9801	324 ± 18
8	1.0119	297 ± 51
9	1.0207	280 ± 23
10	0.9480	293 ± 59
11	1.0007	290 ± 50
12	1.0142	326 ± 25
Mean ($\mu g\,g^{-1}$)		309 ± 11
R.S.D. (%)		5.6

The determination of the organic peroxide content was carried out by applying the standard additions method, which involved the addition of 2-butanone peroxide concentrations between 5.0 and 25.0 $\mu mol\,l^{-1}$. Twelve determinations of the body lotion sample were effected, and each extract was analyzed in triplicate. The results obtained are summarized in Table 2, the confidence intervals calculated for a significance level of 0.05. As can be seen, a mean concentration of 309 ± 11 μg of 2-butanone peroxide per g of sample was found, with an R.S.D. value of 5.6%, which indicates a good precision of the method.

In order to evaluate the accuracy of the proposed method and taking into account the impossibility of applying a reference method, we decided to carry out recovery studies by adding an exact amount of 2-butanone peroxide, around 500 $\mu g\,g^{-1}$, to the sample. Following the simple experimental procedure commented on above, six different samples were analyzed, each of them in duplicate. The results are summarized in Table 3. As can be observed, recoveries were near 100% in all cases, with a mean value of $100 \pm 3\%$.

Table 3
Recovery studies for the determination of the organic peroxide content in body lotion samples at a poly-*N*-methylpyrrole-HRP amperometric biosensor in reversed micelles. Other conditions as in Table 2

Weight of sample (g)	Peroxide content (μg)	Peroxide added (μg)	Total (μg)	Found (μg)	Recovery (%)
1.0540	325.7	435.6	761.3	762.9	100.2
1.0590	327.2	437.7	764.9	776.2	101.5
0.9405	290.6	388.7	679.3	703.9	103.6
1.0135	313.2	418.9	732.1	727.3	99.3
0.9453	292.1	390.7	682.8	692.5	101.4
0.9689	299.4	400.4	699.8	667.8	95.4
Mean (%)					100 ± 3

Finally, the sample linearity was also checked in order to ensure a homogeneous distribution of the analyte in the sample. To do this, different amounts of body lotion, ranging between 25 and 150% of the sample weight used in former studies (1 g), were subjected to the same sample treatment procedure described above, and the amperometric responses were measured in triplicate. A linear relationship ($r = 0.9991$) was obtained, thus confirming that the response obtained was proportional to the sample amount in such a range.

Therefore, although the results obtained should be considered only as an indication of the organic peroxide content in the samples, and not as the real value, the easy and rapid methodology developed is suitable for monitoring the evolution of the concentration of these substances in the cosmetic samples, and, consequently, for use in their quality control.

4. Conclusions

All the above results demonstrate that peroxidase amperometric electrodes prepared by immobilizing the enzyme in an electrodeposited poly-*N*-methylpyrrole film constitute suitable biosensors that give a rapid and reliable indication of the organic peroxide content in different samples. The compatibility of the polymer-HRP bioelectrode with predominantly nonaqueous media, such as the reversed micelles, permits the development of easy, rapid and sensitive analytical methodologies for that purpose, as has been shown in the case of cosmetic samples.

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