Available online at www.sciencedirect.com
SCIENCE DIRECT
Analytica Chimica Acta 559 (2006) 152–158
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

On-line microfluidic sensor integrated with an enzyme-modified pre-cell for the monitoring of paracetamol in pharmaceutical samples

Germán A. Messina, Irma E. De Vito, Julio Raba *

Department of Chemistry, National University of San Luis, Chacabuco y Pedernera, 5700 San Luis, Argentina

Received 6 September 2005; received in revised form 21 November 2005; accepted 24 November 2005
Available online 30 January 2006

Abstract

An on-line microfluidic sensing device with an enzyme-modified pre-cell coupled to an amperometric detector for the monitoring of paracetamol in pharmaceutical formulations is described. Horseradish peroxidase (HRP) [EC 1.11.1.7], immobilized on a 3 μl pre-cell, in presence of hydrogen peroxide catalyses the oxidation of paracetamol to *N*-acetyl-*p*-benzoquinoneimine. The electrochemical reduction back to hydroquinone is detected on glassy carbon electrode surface at −0.10 V. The recovery of paracetamol from 10 samples ranged from 99.00 to 101.10%. This method could be used to determine paracetamol concentration in the range 0.35–100 μM ($r = 0.997$) with a limit of detection of 3.0×10^{-7} M and a relative standard deviation was less than 4.1% ($n = 8$). The method was successfully applied for the processing of as many as 20 samples per hour of paracetamol in pharmaceutical formulations.

Keywords: Paracetamol; Glassy carbon; Biosensor; Horseradish peroxidase; FIA

1. Introduction

Paracetamol (acetaminophen; PCT) is a popular analgesic and antipyretic medication that has few side effects and little toxicity when used in recommended doses. Consistent with the increased usage of acetaminophen, both accidental excessive ingestion can lead to toxic liver damage, and, less commonly, renal impairment. Thus, the development of a simple, precise and accurate procedure for the determination of this drug in pharmaceutical products is very useful [1–4].

Many analytical methodologies have been proposed for the determination of paracetamol including titrimetry [5], spectrophotometry including spectroflourometry, near infrared reflectance spectrometry, Raman spectrometry, Fourier transform infrared spectrophotometry [6–13], chromatography [14,15], chemiluminescence [16–18], enzymatic analysis [19,20] and electrochemical methods [21–23] have been proposed for the analysis of paracetamol in pharmaceuticals or biological fluids. The electrochemical methods are also popular for this application because the cost is low and time consuming is less.

Paracetamol can be readily oxidized at a carbon paste or glass carbon electrodes [24], but these amperometric procedures are non-selective, since the potential involved in this process ranges from 0.6 to 0.8 V and various substances are electroactive in this potential interval. However, the application of biosensors for this task can be operated at potentials much lower than those normally used, thus decreasing the interference.

Some papers describes enzyme-modified pre-column which can be coupled with amperometric or voltammetric techniques, showing inherent advantages as simplicity, low cost and rapidity [25,26]. In this work, a continuous flow sensing device with an enzyme-modified pre-cell coupled to an amperometric detector is proposed for determining PCT in pharmaceutical formulations, using a simple dissolution and filtration step before the measure. Horseradish peroxidase (HRP) in the presence of H_2O_2 catalyses the oxidation of PCT to *N*-acetyl-*p*-benzoquinoneimine (NAPQI) [19] whose electrochemical reduction back to PCT was obtained at peak potential of −0.10 V, proportionally to PCT concentration.

A large number of samples can be processed by means of the proposed method, which shows adequate sensitivity, low cost, versatility, simplicity and effectiveness.

* Corresponding author. Tel.: +54 2652 425385; fax: +54 2652 430224.
E-mail address: jraba@unsl.edu.ar (J. Raba).

0003-2670/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2005.11.072

In this paper, we show that the limit of detection for PCT can be lowered considerably, if the redox enzyme systems coupled to an amperometric detector are utilized rather than others electrochemical and enzymatic methods [19,20,22].

Our aim was develop a new method able to analyze pharmaceuticals formulations, avoiding or minimizing the number of steps needed to assess the concentration of the PCT. The results were compared with those obtained by HPLC-UV detection.

2. Experimental

2.1. Reagents and solutions

All reagents used, were of analytical reagent grade. The enzyme horseradish peroxidase, HRP, [EC 1.11.1.7] Grade II, were purchased from Sigma Chemical Co., St. Louis, Glutaraldehyde (25% aqueous solution) and hydrogen peroxide were purchased from Merck, Darmstadt. Acetonitrile (ACN) was from Sintorgan, HPLC grade. 3-Aminopropyl-modified controlled-pore glass, 1400 Å mean pore diameter and 24 $m^2\,m\,g^{-1}$ surface area, was from Electro-Nucleonics (Fairfield, NJ) and contained 48.2 $\mu mol\,g^{-1}$ of amino groups. PCT was purchased from Sigma Chemical Co., St. Louis, and all other reagents employed were of analytical grade and used without further purifications. Aqueous solutions were prepared using purified water from a Milli-Q-system.

2.2. Horseradish peroxidase immobilization

The enzyme-modified 3 μl pre-cell was prepared by immobilizing HRP on 3-aminopropyl-modified controlled-pore glass (APCPG). The APCPG, smoothly spread on one side of a double-coated tape affixed to the disk surface, and was allowed to react with an aqueous solution of 5% (w/w) glutaraldehyde at pH 10.00 (0.20 M carbonate) for 2 h at room temperature. After washing with purified water and 0.10 M phosphate buffer of pH 7.00, the enzyme (2.0 mg of enzyme preparation in 0.10 ml of 0.10 M phosphate buffer, pH 7.00) was coupled to the residual aldehyde groups in phosphate buffer (0.10 M, pH 7.00) overnight at 5 °C. The immobilized enzyme preparation was finally washed with phosphate buffer (pH 7.00) and stored in the same buffer at 5 °C between uses. The immobilized HRP preparations were perfectly stable for at least 1 month of daily use.

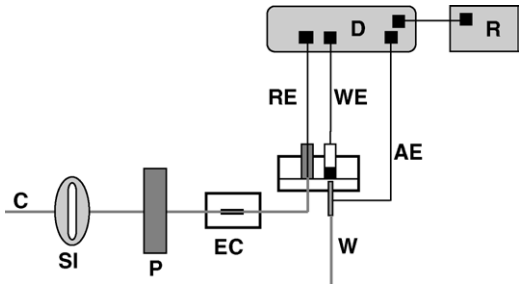

Fig. 2. Block diagram of the continuous-flow system and detection arrangement. P: pump; C: carrier buffer line; SI: sample injection; W: waste line; EC: enzyme-modified pre-cell; WE: GCE; RE: reference electrode; AE: auxiliary electrode; D: potentiostat/detection unit; R: recorder.

2.3. Flow-through cell/detector unit

The body of the pre-cell and amperometric detector was made of Teflon (Fig. 1). Fig. 2 illustrates schematically the components of the single-line continuous-flow setup containing the enzyme-modified pre-cell and the amperometric detector system. Glassy carbon electrode (GCE) is on the top of the amperometric detec-

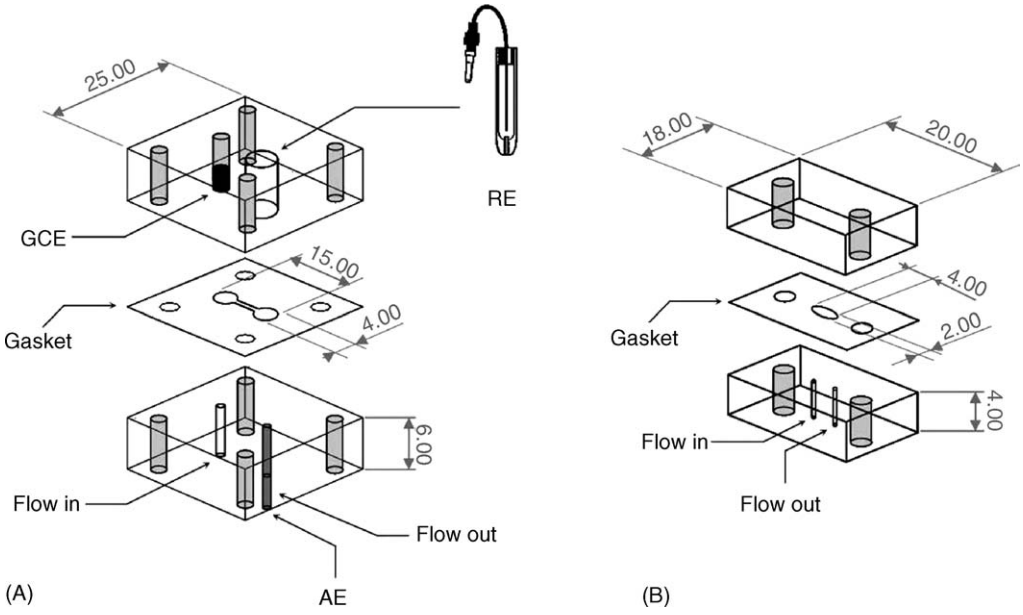

Fig. 1. Schematic representation of components in the pre-cell and amperometric detector. (A) Amperometric detector cell. GCE: glassy carbon electrode. RE: reference electrode. AE: auxiliary electrode. (B) Pre-cell. All measurements are given in millimeters.

tor cell. The potential applied to the working electrode was −0.10 V versus Ag/AgCl, 3.0 M NaCl reference electrode and a Pt wire was used as counter electrode. At this potential, a catalytic current was well established.

Change in enzyme-modified pre-cell volume was accomplished by addition of a gasket of several thickness that changed the relative position of the upper pre-cell body with respect to the lower pre-cell body with the unit assembled. The volume of the pre-cell can change from 3 to 25 μl.

Amperometric detection was performed using a BAS LC4C amperometric detector and BAS 100 B (electrochemical analyzer Bioanalytical System, West Lafayette, IN) was used to voltammetric determinations. The potential applied to the GCE was −0.10 V versus Ag/AgCl, 3.0 M NaCl reference electrode BAS RE-6, and a Pt wire counter electrode. At this potential, a catalytic current was well established.

A pump (Gilson Minipuls 3 peristaltic pump, Gilson Electronics, Inc. Middleton, WI) was used for pumping, sample introduction. The pump tubing was Tygon (Fisher AccuRated, 0.3 mm i.d., Fisher Scientific Co., Pittsburgh, PA) and the remaining tubing used was Teflon, 1.00 mm i.d. from Cole-Parmer (Chicago, IL).

All pH measurements were made with an Orion Expandable Ion Analyzer (Orion Research Inc., Cambridge, MA) Model EA 940 equipped with a glass combination electrode (Orion Research Inc., Cambridge, MA).

The HPLC experiments were performed using the procedure of reference [27] with the following modifications. HPLC experiments were performed with a Beckman model 332 liquid chromatograph equipped with a variable wavelength detector model 164 operated at λ = 254 nm. The retention times as well as peak area measurements were obtained with a Varian 4290 integrator. The operating temperature was ambient and a Phenosphere 5 mμ ODS-2 C18 column (250 mm × 4.6 mm) was used in all experiments with a flow rate of 1.0 ml min^{-1}. The mobile phase consisted of 20 mM phosphate buffer, pH 7.0—ACN (in a range of 90/10 (v/v) to 70/30 (v/v) in according to the sample). Quantitative data were calculated from the linear regression of external standard of PCT, relating peak area and concentration.

2.4. Analysis of pharmaceutical samples

Ten tablets or the contents of 10 tablets were weighed form each dosage forms and powdered. Equivalent amount to one tablet was weighed and transferred to a 100 ml volumetric flask. The flask was sonicated for 10 min and filled with 0.1 M phosphate–citrate buffer, pH 5.00. Appropriated solutions were prepared by taking suitable aliquots of the clear supernatant and diluting them with 0.1 M phosphate–citrate buffer, pH 5.0. In the oral drops preparations, the entire content was directly put into a 100 ml volumetric flask and the procedure described above was followed.

Sample preparation for HPLC assay was similar, 10 tablets or the contents of 10 tablets were powdered and the amount corresponding to one tablet of PCT was dissolved in 100 ml volumetric flask with 20 mM phosphate buffer, pH 7.0—ACN (70:30, v/v) and subjected to sonication for 15 min. The volume was filtered though a 0.45 μm PTFE Whatman to the vials for injection into the HPLC system.

2.5. Preparation of synthetic samples

Synthetic samples were prepared into a 100 ml calibrated flasks by spiking a placebo (mixture of tablet excipients such as lactose, starch, citric acid, magnesium stearate, sucrose, glucose, saccharin, sodium lauryl sulphate, bicarbonate sodium salt, cyclamate sodium salt, sorbitol,) with accurately amount of PCT (Sigma Chemical Co.) at a concentration similar to formulation concentration (0.2–10 g). Then, the procedure described above was followed.

2.6. Dosage of PCT in different pharmaceutical formulations

(1) Paracetamol Raffo 500 mg tablets. (2) Paracetamol Raffo oral drops. (3) Paracetamol Lazar® 500 mg tablets. (4) Paracetamol Lazar® oral drop. (5) Termofrem Paracetamol Ped. Two hundred milligrams tablets (Roemers). (6) Termofrem Paracetamol oral drops (Roemers). (7) Mejoral Paracetamol 500 mg tablets (Elisium). (8) Paracetamol Northia® oral drops. (9) Tafirol® Paracetamol 500 mg tablets. (10) Tafirol® Paracetamol 1 g tablets.

3. Results and discussion

3.1. Study of the enzymatic process

Reactions catalyzed by enzymes have long been used for analytical purposes in the determination of different analytes such as substrates, inhibitors, and also the enzymes. The catalysis mechanism of HRP was explained in reference [28].

HRP in presence of hydrogen peroxide catalyses the oxidation of paracetamol to *N*-acetyl-*p*-benzoquinoneimine which at a potential of −0.10 V was electrochemically reduced to PCT, providing a peak current related to its concentration. A schematic representation of this process is shown in Fig. 3.

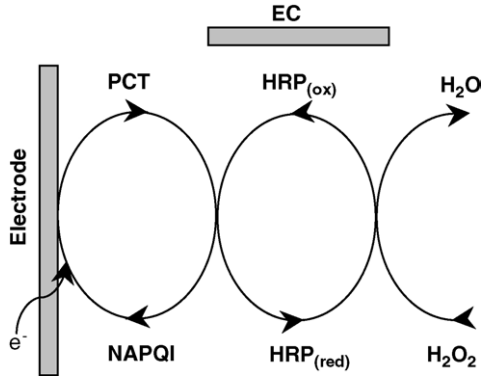

Fig. 3. Schematic representations of the reduction wave of the enzymatic process between paracetamol (PCT), *N*-acetyl-*p*-benzoquinoneimine (NAPQI), hydrogen peroxide (H_2O_2), and peroxidase (HRP). EC: enzyme-modified pre-cell.

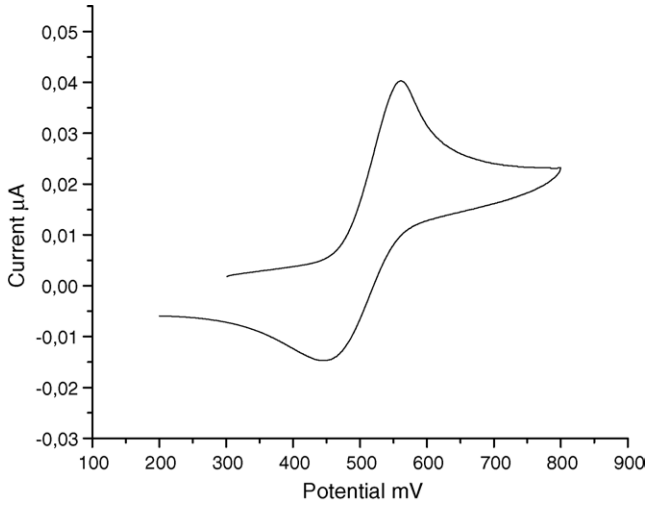

Fig. 4. Cyclic voltammogram in aqueous solution containing 0.1 M phosphate–citrate buffer (pH 5.0) of PCT 5×10^{-5} M. Scan rate: 100 m V s^{-1}, T: 25 ± 1 °C.

3.2. Cyclic voltammetry of PCT on GCE

A cyclic voltammetric study was performed by scanning the potential from +1.0 to 0.0 V versus Ag/AgCl at a scan rate of 100 mV s^{-1}. Cyclic voltammetry (first cycle) of 5.0×10^{-5} mol l^{-1} of PCT in aqueous solution containing 0.1 M phosphate–citrate buffer (pH 5.0), shows one anodic (A_1) and corresponding cathodic peak (C_1), which corresponds to the transformation of PCT to NAPQI and vice versa within a quasi-reversible two-electron process (Fig. 4). A peak current ratio (I_{C1}/I_{A1}) of nearly unity, particularly during the recycling of potential, can be considered as criteria for the stability of NAPQI produced at the surface of electrode under the experimental conditions.

3.3. Effect of continuous-flow operation

The implementation of continuous-flow programming permits: (a) utilization of relatively low enzyme loading conditions; (b) instantaneous operation under high initial rate conditions; (c) easy detection of products; (d) facilitating the arrival of substrate at the active sites and the release of products from the same sites. The main advantages of this system are its simplicity, and the ease with which it can be applied to the determination of PCT at low levels.

The effects of varying the flow rate was studied in the range 75 µl to 1 ml min^{-1} and show a maximum value of activity at flow rate 100 µl min^{-1}. Fig. 5 shows the effect of different flow rate under continuous flow conditions. These responses indicate that the utilization of the biocatalytic action of the immobilized enzyme preparations is better under small flow rate. The current developed at the detector was directly proportional to the concentration of PCT in the sample.

3.4. Effect of enzyme-modified pre-cell volume and sample size

Depending on the volume of the enzyme-modified pre-cell in contact with the amperometric detector, the overall process becomes controlled by diffusion of the PCT towards the active sites of the enzyme (large volumes) or by the chemical kinetics of the enzyme-catalyzed reactions (small volumes).

The pre-cell volume was changed by increasing the thickness gasket between the top and the lower pre-cell body from 3 to 25 µl, with a flow rate of 100 µl min^{-1}. The smallest pre-cell volume of 3 µl was adopted for further studies (Fig. 6). The rate of response, as expected, decreased linearly with an increase in cell volume due to the dilution effect and the fact that the measured current is directly proportional to bulk concentration.

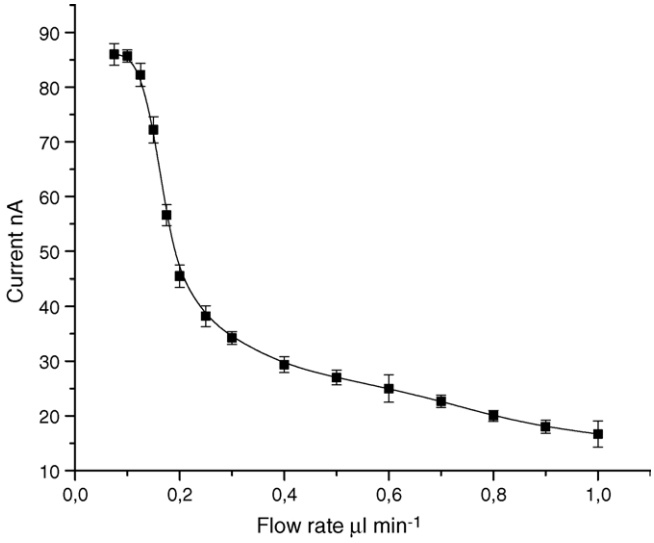

Fig. 5. Effect of flow rate. Pre-cell volume was 3 µl, 0.1 M phosphate–citrate buffer, pH 5.0 solution containing 1.0×10^{-3} mol l^{-1} H_2O_2, and 5.0×10^{-3} mol l^{-1} PCT.

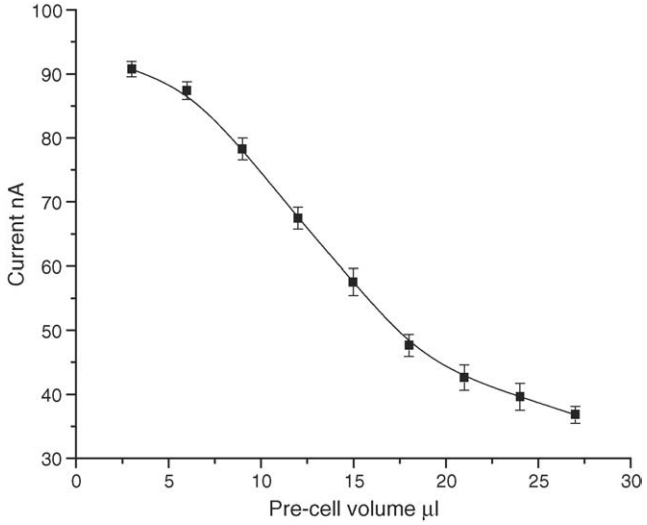

Fig. 6. Pre-cell volume. Flow rate 100 µl min^{-1}. 0.1 M phosphate–citrate buffer, pH 5.0 solution containing 1.0×10^{-3} mol l^{-1} H_2O_2, and 5.0×10^{-3} mol l^{-1} PCT.

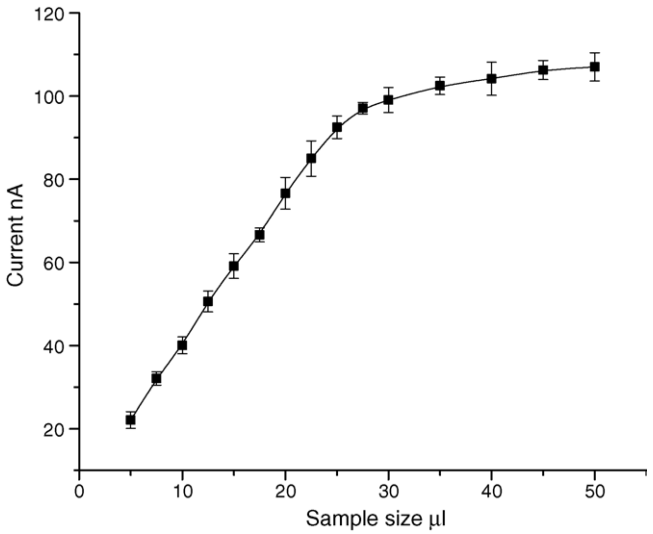

Fig. 7. Effect of sample size. Each value of initial rate based on triplicate of six determinations. Flow rate 100 µl min^{-1}. Pre-cell volume was 3 µl, 0.1 M phosphate–citrate buffer, pH 5.0 solution containing 1.0×10^{-3} mol l^{-1} H_2O_2, and 5.0×10^{-3} mol l^{-1} PCT.

The sample size was studied in the range 5–50 µl and shows a maximum rate of response at 25 µl. For convenience a sample size of 25 µl was used to evaluate other parameters. The rate of response increased linearly with sample size up to 5 µl in a pre-cell with a volume of 3 µl. Sensitivity is almost tripled in the range between 5 and 25 µl (Fig. 7).

3.5. Optimal conditions for the enzymatic products determination

The rate of enzymatic response under flow conditions was studied in the pH range 3–8 and show a maximum value of activity at pH 5.0 (Fig. 8). The pH value used was 5.0 in 0.1 M phosphate–citrate buffer.

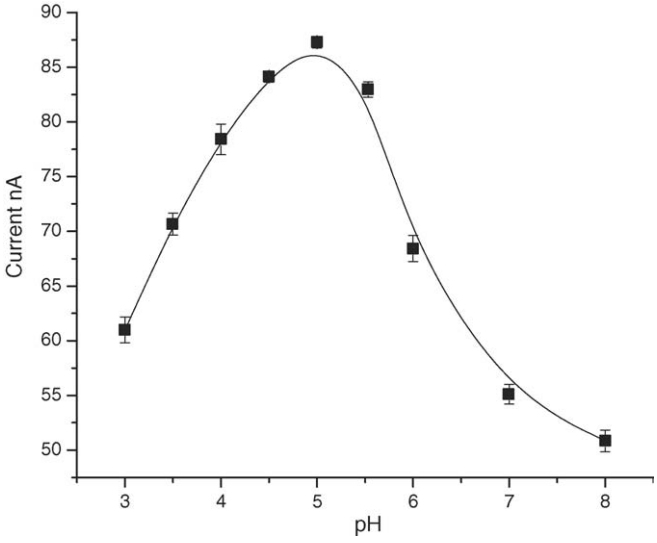

Fig. 8. Effect of pH on the rate of enzymatic response. Flow rate 100 µl min^{-1}. Pre-cell volume was 3 µl, 0.1 M phosphate–citrate buffer, pH 5.0 solution containing 1.0×10^{-3} mol l^{-1} H_2O_2, and 5.0×10^{-3} mol l^{-1} PCT.

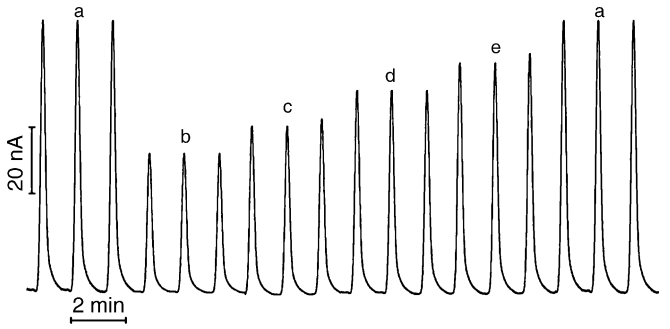

Fig. 9. Response of the biosensor for PCT determination. (a) PCT 5.0×10^{-5} mol l^{-1}, and H_2O_2 1.0×10^{-3} mol l^{-1}. From (b) to (e) is shown the response for several PCT concentrations: (b) 2.0×10^{-5}; (c) 2.75×10^{-5}; (d) 3.5×10^{-5}; (e) 4.25×10^{-5} mol l^{-1}.

The effect of varying H_2O_2 concentration from 7.0×10^{-4} to 5.0×10^{-3} mol l^{-1}, for 5×10^{-5} mol l^{-1} PCT solution on the biosensor response was evaluated. The optimum H_2O_2 concentration found was 1.0×10^{-3} mol l^{-1}. This concentration was then used.

Enzyme exhibits the best activity over the temperature range of 20–25 °C; higher temperature would be harmful for its activity [29].

A linear relation (Eq. (1)) was observed between the rate of response and the PCT concentration in the range of 1.0×10^{-4} mol l^{-1} to 3.5×10^{-7} mol l^{-1}.

$$\text{Rate of response (nA/min)} = 13.96 + 1.49[C_{\text{PCT}}] \quad (1)$$

The correlation coefficient for this type of plot was typically 0.997. Detection limit (DL) taken as the concentration that gives a signal three times the standard deviation of the blank, the DLs for amperometric detection 0.3 µM. Reproducibility assays were made using repetitive standards ($n = 5$) of 50 µM; the percentage standard error was less than 4.5%.

The stability of the bioreactor was tested for nearly 3 h of continuous use in the FIA system. The long-term stability of the enzymatic system to pharmaceutical formulations was study. In this experiment, after every five samples, a standard of 50 µM PCT is injected to test the electrode response. In the FIA system using an enzymatic reactor, there is practically no decay in the catalytic current after six samples (Fig. 9).

Table 1
Specificity results of the proposed method[a]

Sample number	Pure sample 10 (µM)	Synthetic tablet sample ($n = 5$) X (µM)
1	10.02	10.26
2	9.971	10.18
3	10.07	9.92
4	9.987	9.90
5	9.994	10.13
$X \pm$ S.E.	10.01 ± 0.017	10.078 ± 0.071
S.D.	0.038	0.16

[a] X (µM), mean ± S.E., standard error; S.D., standard deviation.

Table 2
Accuracy and precision dates for PCT obtained by proposed method (level of significance $\alpha = 0.05$)

Added (g)	Found (g)	Recovery (%)	Precision (g)	Accuracy[b] (% relative error)
0.1	0.099	99.00	X[a] = 0.099 ± 0.012 S.D.[c] = 0.004 VC[d] = 4.04%	−1
0.5	0.498	99.60	X = 0.498 ± 0.01 S.D. = 0.009 VC = 1.80%	−0.4
1.00	1.011	101.10	X = 1.011 ± 0.06 S.D. = 0.016 VC = 1.58%	1.1
10.00	10.013	100.13	X = 10.013 ± 0.023 S.D. = 0.058 VC = 1.38%	0.57

[a] X = mean.
[b] Accuracy = [(found − added)/added] × 100.
[c] Standard deviation.
[d] Variation coefficient.

Table 3
Results obtained in the measurement of PCT in pharmaceuticals formulations analyzed by two techniques, based on triplicate of six determinations

Sample	FIA biosensor X ± S.D.	HPLC ± S.D.
Paracetamol Raffo 0.5 g/tablets	0.501 ± 0.008	0.497 ± 0.005
Paracetamol Raffo oral drops 0.1 g ml^{-1}	0.99 ± 0.002	0.1013 ± 0.0085
Paracetamol Lazar® 0.5 g/tablets	0.499 ± 0.001	0.496 ± 0.0032
Paracetamol Lazar® oral drop. 0.1 g ml^{-1}	0.101 ± 0.0036	0.102 ± 0.0045
Termofrem Paracetamol Ped. (Roemers) 0.2 g/tablets	0.199 ± 0.0019	0.198 ± 0.003
Termofrem Paracetamol oral drops (Roemers) 0.1 gml^{-1}	0.1003 ± 0.0027	0.992 ± 0.0042
Mejoral Paracetamol (Elisium) 0.5 g/tablets	0.501 ± 0.0012	0.503 ± 0.002
Paracetamol Northia® oral drops 0.1 g ml^{-1}	0.102 ± 0.004	0.101 ± 0.003
Tafirol® Paracetamol 0.5 g/tablets	0.505 ± 0.0036	0.497 ± 0.006
Tafirol® Paracetamol 1 g/tablets	1.01 ± 0.026	1.017 ± 0.051

3.6. Determination of PCT in pharmaceuticals formulations

Specificity is the ability of the method to measure the analyte response in the presence of all the potential interference. For the specificity test, FIA of standard solution of tablet excipients were recorder at selected conditions. The response of the analyte with excipients, were compared with the response of pure PCT. It was found that assay results were not changed. Therefore, the excipients did not interfere with the quantization of PCT as such in synthetic as commercial tablet samples. In Table 1 the results are showed.

Recovery studies were performed by adding a synthetic mixture prepared according to the manufacturer's batch formula to known amount of PCT. The recovery was 101.0%. The results are showed in Table 2.

The precision for PCT was <4.1% within the range 0.1–10.0 g (Table 2). Precision studies were performed by adding a synthetic mixture prepared according to the manufacturer's batch formula to known amount of PCT. The accuracy for PCT was <1.2% (Table 2).

The developed FIA-biosensor method for the PCT determination was applied to 10 commercial preparations. There is no need for any extraction procedure before FIA analysis. No change of the peak height in the presence of the excipients was observed. The HPLC method was employed as a comparison to evaluate the validity of the developed method. Table 3 gives the results obtained using the two methods for six separate determinations starting from different groups of pharmaceutical preparations of PCT. The results were compared and there was no significant difference between the methods.

4. Conclusions

The usefulness of an on-line microfluidic sensing device with a enzyme-modified pre-cell with amperometric detection used for the determination of very low concentrations of PCT is demonstrated. The merits of FIA-enzyme-modified pre-cell-amperometric detector are useful if we think in inexpensive enzyme and miniaturized systems.

In practice the biosensor developed in this work is able to operate as a fast, selective and sensitive detection unit when is incorporated into a FIA system, also minimizes the wastage of expensive reagents, shows physical and chemical stability, low background current, wide working potential range, accuracy and does not require highly skilled technicians or expensive and dedicated equipment. This method is very simple and straightforward, its the good applicability in pharmaceutical industry as a routine method.

Acknowledgements

The authors wish to thank the financial support from the Universidad Nacional de San Luis and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). One of the authors Germán A. Messina acknowledges support in the form of a fellowship from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

References

[1] J. Ambre, M. Alexander, J. Assoc. Am. 238 (1977) 500.
[2] M. Black, Gastroenterology 78 (1980) 382.
[3] L.F. Prescott, Brit. J. Pharmacol. Clin. 7 (1979) 443.
[4] M.A. Campanero, B. Calahorra, E. Garcia-Quatglas, A. Lopez-Ocariz, J. Honorato, J. Pharm. Biomed. Anal. 20 (1999) 327.
[5] M.K. Srivastava, S. Ahmed, D. Singh, I.C. Shukala, Analyst 110 (1985) 735.
[6] Z. Boushsain, S. Garrigues, A. Morales-Rubio, M. de la Guardia, Anal. Chim. Acta 330 (1996) 59.
[7] G. Amann, G. Gubitz, R.W. Frei, W. Santi, Anal. Chim. Acta 116 (1980) 119.
[8] K. Molt, M. Egelkraut, GIT Fachz. Lab. 32 (1988) 1311.
[9] T.H. King, C.K. Mann, T.J. Vickers, J. Pharm. Sci. 74 (1985) 443.
[10] Z. Boushsain, S. Garrigues, M. de la Guardia, Analyst 121 (1996) 635.
[11] M.A. Evans, R.D. Harbinson, J. Pharm. Sci. 66 (1977) 1628.
[12] A. Ivaska, T.H. Ryan, Collect. Chem. Commun. 46 (1981) 2865.
[13] M.L. Ramos, J.F. Tyson, D.J. Curran, Anal. Chim. Acta 364 (1998) 107.
[14] M.J. Stewart, P.I. Adriaenssens, D.R. Jarvie, L.F. Prescott, Ann. Clin. Biochem. 16 (1979) 89.
[15] A.N. Papas, M.Y. Alpert, S.M. Marchese, J.W. Fitzgerald, Anal. Chem. 57 (1985) 1408.
[16] I.I. Koukli, A.C. Calokerinos, T.P. Hadjiioannou, Analyst 114 (1989) 711.
[17] A.G. Alapont, L.L. Zamora, J.M. Calatayud, J. Pharm. Biomed. Anal. 21 (1999) 311.
[18] K. Vandyke, M. Sacks, N. Qazi, J. Biolum. Chemilum. 13 (1998) 339.
[19] I.C. Vieira, K.O. Lupetti, O. Fatibello-Filho, Quim. Nova 26 (2003) 39.
[20] O. Fatibello-Filho, K.O. Lupetti, I.C. Vieira, Talanta 55 (2001) 685.
[21] J. Wang, T. Golden, P. Tuzhi, Anal. Chem. 59 (1987) 740.
[22] P. Masawat, S. Liawruangrath, Y. Vaneesorn, B. Liawruangrath, Talanta 58 (2002) 1221.
[23] W. Peng, T. Li, H. Li, E. Wang, Anal. Acta Chim. 298 (1994) 415.
[24] B. Eggins, Biosensors an Introduction, Wiley Teubner, New York, 1996, p. 167.
[25] K. Hayashi, Y. Iwasaki, R. Kurita, K. Sunagawa, O. Niwa, A. Tate, J. Electroanal. Chem. 579 (2005) 215.
[26] T. You, O. Niwa, M. Tomita, S. Hirono, Anal. Chem. 75 (2003) 2080.
[27] R.A. Horvitz, P.I. Jatlowt, Clin. Chem. 23 (1977) 1596.
[28] G.A. Messina, A. Torriero, I.E. De Vito, J. Raba, Talanta 64 (2004) 1009.
[29] G.D. Liu, J.T. Yan, G.L. Shen, R.Q. Yu, Sens. Actuators B 80 (2001) 95.