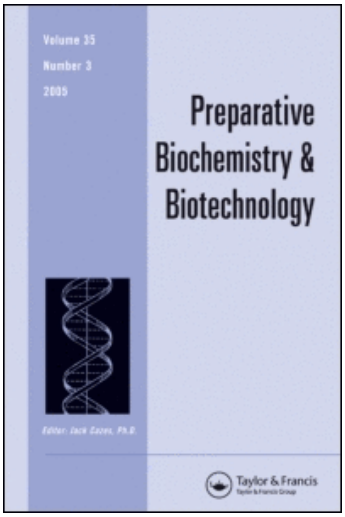

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Serban Fleschin[a]; Camelia Bala[a]; Andrei A. Bunaciu[b]; Alexandra Panait[c]; Hassan Y. Aboul-Enein[d]
[a] Department of Analytical Chemistry Faculty of Chemistry, University of Bucharest Sos, Bucharest, Romania [b] Analytical and Research Department, O. F. Systems - Mettler Toledo AG, Bucharest, Romania [c] Institutul Cantacuzino, Bucharest, Romania [d] Bioanalytical and Drug Development Laboratory Biological and Medical Research (MBC-03) King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia

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ENALAPRIL MICROBIAL BIOSENSOR

Serban Fleschin,[1] Camelia Bala,[1] Andrei A. Bunaciu,[2]
Alexandra Panait,[3] Hassan Y. Aboul-Enein[4,*]

[1] Department of Analytical Chemistry
Faculty of Chemistry
University of Bucharest
Sos. Panduri 90
76229 Bucharest-5, Romania

[2] O. F. Systems - Mettler Toledo AG
Analytical and Research Department
70644 - Str. Al. Serbănescu Nr. 25
P. O. Box 52-3
Bucharest 1, Romania

[3] Institutul Cantacuzino
Splaiul Independentei 102
Bucharest, Romania

[4] Bioanalytical and Drug Development Laboratory
Biological and Medical Research (MBC-03)
King Faisal Specialist Hospital and Research Centre
P. O. Box 3354
Riyadh 11211, Saudi Arabia

ABSTRACT

Enalapril maleate (EMa) belongs to a new class of antihypertensive agents known as angiotensin converting enzyme (ACE) inhibitors. This paper describes the development of a microbial biosensor for EMa using induced *Bacillus subitilis*

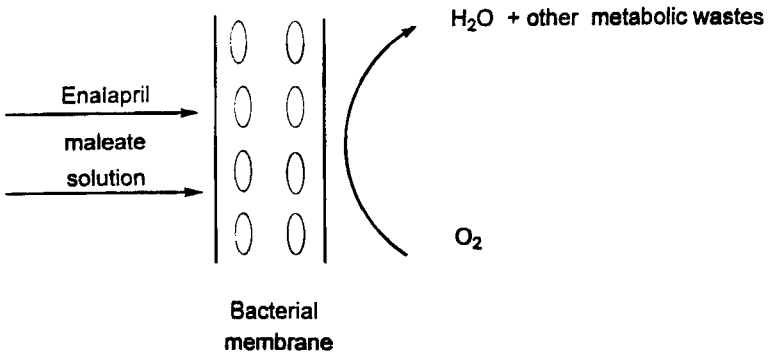

Figure 1. Schematic model for EMa transport through bacterial membrane.

cells. This biosensor measures the acceleration of respiration during specific metabolic pathways of this drug. It has been applied, with good results, for determination of the active ingredient in the pharmaceutical tablet formulations.

INTRODUCTION

The uses of biocatalysts, such as enzyme or whole cells, offers a remarkable arsenal of highly selective transformations for modern analytical chemistry. During the past decade, the methodology in the biosensor's field has generally been accepted as a complementary method to the already existing analytical tools.

Enalapril maleate (EMa), chemically known as (S)-1-[N-[1-(Ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline maleate, belongs to a new class of antihypertensive agents which causes inhibition of angiotensin converting enzyme (ACE).[1,2] Enalapril maleate (EMa) has been determined by spectrophotometric,[3] high performance liquid chromatographic (HPLC),[4,5] and gas chromatographic-mass spectrometric (GC-MS) methods.[6,7]

In analytical biochemical fields, many patents and scientific publications describe biosensors for the determination of about 150 substrates, including various pharmaceuticals such as antibiotics and other drugs.[8-14] All these substances could be determined by measuring O_2 during metabolic degradation.

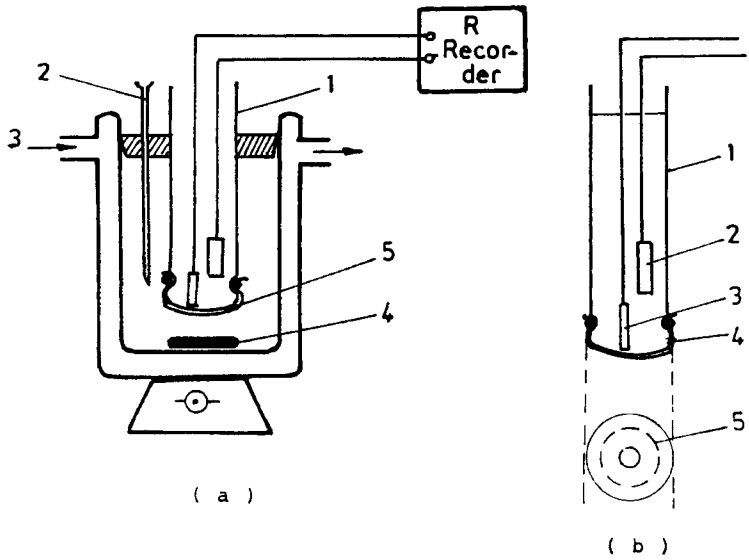

Figure 2. Experimental equipment and electrode construction. (a) Experimental equipment: 1) O2 electrode; 2) stainless steel syringe needle; 3) thermostatic bath; 4) magnetic stirrer; 5) bacterial membrane. (b) Electrode construction: 1) electrode body; 2) anode; 3) cathode; 4) oxygen membrane; 5) bacterial membrane.

This paper discusses the preparation, characteristics, and analytical applications of the EMa biosensor prepared from *Bacillus subtilis* cells, with high specificity in biochemical degradation of enalapril maleate (EMa) and an oxygen electrode (Figure 1).

EXPERIMENTAL

Reagents and Materials

Enalapril maleate substance (EMa) (Lot # L 154, 739-001DO70) and RENITEC® formulations were supplied by Merck Sharpe & Dohme (West Point, PA, USA). Standard solutions were made by dissolution in bidistilled water. All other chemicals were of analytical reagent grade. The microorganisms, culture cells of *Bacillus subtilis*, were obtained from Cantacuzino Institute of Bucharest and were grown in a nutrient liquid medium, containing: meat extract 3.5 g/L, peptone 5 g/L, NaCl 3 g/L,

phosphate buffer (pH 6.6) at 25°C for 18h. After this period, the meat extract and peptone was replaced with EMa 100 mg/L as a single source of carbon. At various time intervals, 10 mL samples were collected from the nutrient medium for determination of EMa by a spectrophotometric method at a fixed wavelength of 208 nm to prove the consumption of EMa.

Biosensor Preparation

The fresh bacterial cells were centrifuged from their medium and washed with distilled water and recentrifuged; these fresh cells were adsorbed on a cellulose acetate membrane. This membrane was placed over the Clark electrode membrane. The cell membrane was fastened with a dialysis membrane. It is of interest to mention that the lifetime of the prepared biosensor is about 5 days. After this period, the microbial membrane is denatured by autolysis; so, it is necessary to replace the bacterial cell in the membrane. The experimental equipment and the determination procedures are shown in Figure 2.

The system consists of the oxygen electrode (Cole Parmer Model 5946-Illinois, USA), vessel cell, a stainless steel syringe needle for washing the electrode and cells after determination, a magnetic stirrer and stirring bar. The surface of the oxygen electrode was covered with *Bacillus subtilis* cells embedded in the cellulose acetate membrane.

Recommended Procedure

For the biosensor calibration and analytical determination, 50 mL of sample solution, containing EMa in the range 1-25 mg/L, were injected through stainless steel needle. After 2-3 min. the current reached the stationary steady state corresponding to O_2 consumption of the bacterial cells. The base-line recovery ranged between 5-8 minutes. The experimental determinations were performed with fresh *Bacillus subtilis* cells at room temperature.

RESULTS AND DISCUSSION

Biosensor Response

Figure 3 presents a typical response for the biosensor. The samples was injected into the measuring cell at time zero.

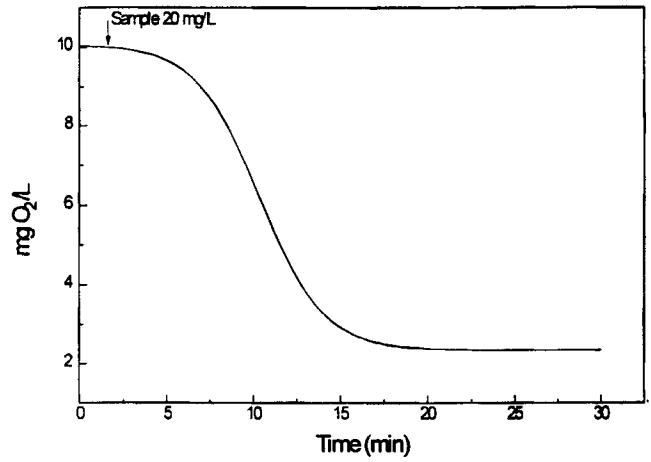

Figure 3. Response time of EMa biosensor.

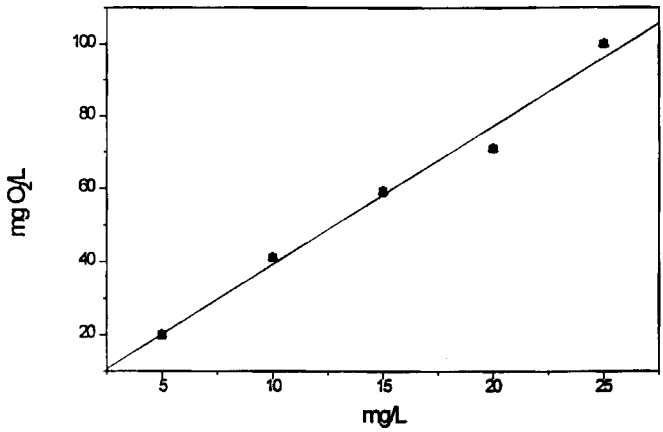

Figure 4. Calibration graph for EMa biosensor.

When a solution of 20 mg/L EMa was injected into the measuring cell, the current began to decrease, owing to oxygen consumption, and reached a steady state after 17-20 min. The biosensor response to different EMa solutions is linear in the range of 1-25 mg/L as shown in Figure 4.

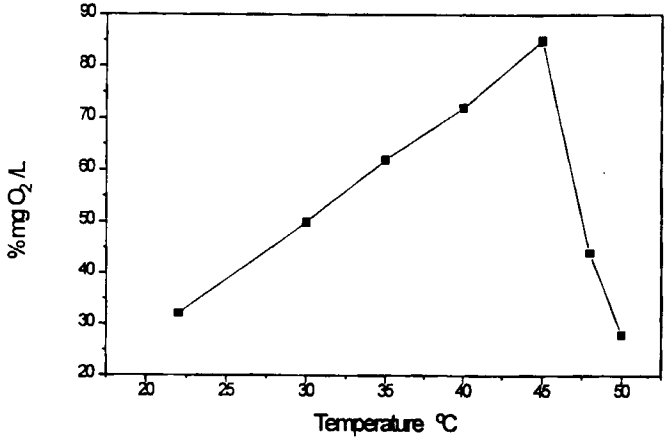

Figure 5. Effect of temperature on EMa biosensor response.

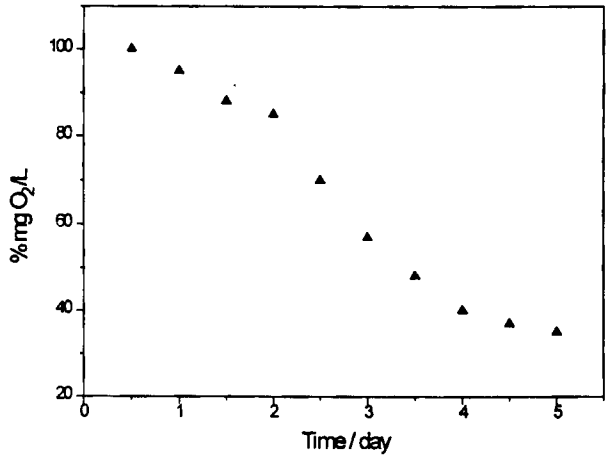

Figure 6. Stability of EMa biosensor.

Effect of Temperature

The influence of temperature was investigated after the biosensor was kept in a solution of the microbiological medium at each temperature for 2h. As shown in Figure 5, the response of the biosensor increased until 45°C but decreased for temperatures greater than 45°C (Figure 5).

Table 1

Results of the Quantitative Determination of EMa with the Biosensor Method

Pharmaceutical Preparations	Sample	Found (% of Nominal)* Biosensor	Spectrophotometric
EMa raw-material	1	109.5	107.5
	2	100.5	107.3
	3	106.2	105.1
RENITEC 5 (5 mg/tablet)	1	101.2	101.8
	2	108.1	107.0
	3	104.5	108.3

*All values are averages of four determinations. Similar results were obtained for other preparations (RENITEC 10 and RENITEC 20) too.

Stability of Biosensor

The stability of the biosensor was monitored for 5 days, after which the microbial membrane should be replaced. The experimental measurements were carried at 27°C in EMa solution containing 15 mg/L. During this period, the biosensor was stored in microbiological solution at room temperature. During the experimental period, the biosensor response decreased to 40% of its initial response (Figure 6).

pH Optimization

The pH influences of the proposed microbial biosensor was studied in the range of 5-8, but the optimum pH was found to be pH 6.6-6.8 where the *Bacillus subtilis* cells present a maximum microbiological pathway.

Analytical Applications

The EMa biosensor proved useful for the assay of the enalapril maleate in pharmaceutical preparations, RENITEC® tablets (5, 10, 20 mg *enalapril* maleate/tablet). The results presented in Table 1 were in good agreement with the spectrophotometric method (λ=208-213 nm). As shown in Table 1, a high

precision was attainable. However, the proposed biosensor method is faster than the spectrophotometric method. The response of the proposed biosensor is not affected by uric acid, ascorbic acid, Ca^{+2}, Mg^{+2} ions which are considered some of the inhibitors which are commonly present in biological fluids.

CONCLUSIONS

Enalapril microbial biosensor electrode was developed by using *Bacillus subtilis* cells, which exhibited useful analytical characteristics for the determination of enalapril in its pharmaceutical tablet formulations. The biosensor showed reproducible response characteristics and also was faster in the assay of the active constituent.

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REFERENCES

1. P. H. Vlasses, G. E. Latijiani, D. P. Conner, Clin. Pharm., **4**, 27-40 (1985).

2. R. O. Davies, J. D. Irvin, D. K. J. Kramsch, Clin. Pharmacol., **18**, 215-295 (1984).

3. M. Oklobdzija, J. Kuftinec, M. Hohnjec, F. Kajfez, Acta Pharm. Jugsl., **38**, 167-183 (1988).

4. X. Z. Qim, D. P. Ip, E. W. Tsai, J. Chromatogr., **626**, 251-258 (1992).

5. H. Shioya, M. Shimojo, Y. Kawahara, Biomed. Chromatogr., **6**, 59-62 (1992).

6. R. T. Sane, A. J. Vaidya, J. K. Ghadge, Indian Drugs, **29**, 244 -245 (1992).

7. J. Salamoun, K. Slais, J. Chromatogr., **537**, 249-257 (1991).

8. F. F. Orlando, A. S. Ahmad, G. G. Guilbault, Anal. Chem., **60**, 2397-2399 (1988).

9. G. Rechnitz, Chem. Eng. News, **24**, (1988).

10. G. G. Guilbault, J. H. Luong, Chimia, **42**, 267 (1988).

11. S. F. Chou, Analyst, **121**, 71-73 (1996).

12. Y. Kitagawa, M. Ameyama, K. Nakashima, E. Tamiya, Analyst, **112**, 1747 (1987).

13. K. Riedel, R. Rennegerg, F. Scheller, Appl. Microbiol. Biotechol., **28**, 272-275 (1988).

14. A. Ciucu, V. Magearu, S. Fleschin, I. Lucaciu, Anal. Letters, **24**, 267-280 (1991).

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