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Evaluation of a simple and low cost potentiometric biosensor for pharmaceutical and *in vivo* adrenaline determination

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ABSTRACT

In this work the polyphenol oxidase (PPO) was the main component of a biosensor for adrenaline determination. The activity of this enzyme was measured in several vegetables. Banana (*Musa* sp.) extracts presented better results with 974 UA (units of activity). The biosensor was constructed with a polyethylene tube (0.8 mm i.d.) filled with: carbon paste containing 50 UA of the PPO in phosphate buffer (pH = 7.00) solution and vaseline as agglutinant. When the biosensor was applied in medicine samples it provided a linear range from 8.00×10^{-9} to 8.00×10^{-4} mol L^{-1}; the results obtained with the proposed method and the Brazilian Pharmacopoeia method were in agreement (*t*-test). When it was applied in blood samples, the matrix-matching calibration was used, and the linear range was from 8.00×10^{-7} to 8.00×10^{-3} mol L^{-1}. *In vivo* studies were also done. The obtained results for those electrodes, which were inserted in the jugular vein of Wistar rats, were very promising.

1. Introduction

Catecholamines are organic compounds with an alkylamine chain bonded to a benzenic ring with two hydroxyl groups. An example of this class of substances is adrenaline, which is an important neurotransmitter. Adrenaline is a potent agonist of cardiac α and β-receptors, which are biosynthesized in the adrenergic medulla and sympathetic nerve terminals, and secreted by the suprarenal gland, along with noradrenaline (Felix et al., 2006).

Although a large portion of plasma adrenaline arises from the adrenal medulla, several investigations have suggested that local cardiac adrenaline storage and synthesis also play important roles in regulating cardiac function (Kawada et al., 1998), for example, restoring the cardiac rhythm in cardiac patients (Goodman and Gilman, 2006). Thus, adrenaline determinations, mainly *in vivo*, can be very useful for physiological studies.

Generally, adrenaline is synthesized and secreted at a basal rate in individuals at physical and emotional rest (Kildegaard et al., 2007). Larger quantities are released in situations involving physical or emotional stress. These situations include hypoglycemia (Robertson et al., 2003), exercise (Watt et al., 2001), acute pain (Wortsman, 2002), and other situations involving mental excitement. Adrenaline basal levels are approximately 0.2–0.4 nmol L^{-1} (35–70 pg mL^{-1}) in arterial plasma and 0.1–0.2 nmol L^{-1} (17.5–35 pg mL^{-1}) in venous plasma (Hjemdahl, 1993). Besides the adrenaline, other catecholamines are present in the blood, such as noradrenaline and dopamine with physiologic concentrations around 1.70 μg L^{-1} and 30.0 ng L^{-1}, respectively.

The determination of catecholamines in biological matrices requires analytical procedures capable of low detection limits, since they occur in low concentrations (nmol L^{-1} or μg L^{-1} magnitude). Therefore, the use of fluorimetry (Guo et al., 2002), spectrophotometry (Medina et al., 2000), cyclic voltametry (Liu et al., 2005), FIA with flourimetric detection (Torres et al., 1998), electrochemiluminescence (Li et al., 2002), potentiometric (Moreira et al., 2005), amperometric (Felix et al., 2006) and voltametric biosensors (Caruso et al., 1999), CLAE (fluorescence detector) (Hansen et al., 1999), chromatography and electrochemical methods, often using modified electrodes, is common (Garrido et al., 1997).

In this paper, a potentiometric biosensor for the adrenaline determination in pharmaceutical interest samples was proposed. An electrochemical biosensor is defined as "a self contained integrated device which is capable of providing quantitative or semi-quantitative information using biological recognition elements retained in direct spatial contact with an electrochemical

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transduction element" (Thevenot et al., 2001). Biosensors based on electrochemical transducers are the most common and more frequently mentioned in the literature. The available commercial biosensors usually are employed with amperometric technique, meantime in this work we opted for the potentiometry. The potentiometry consists of the measurement of the potential difference among two electrodes when the current of the cell is zero. The two electrodes are known as indicator and reference. The indicator electrode develops a variable potential depending on the activity or concentration of the analyte in the solution. That potential is measured in relation to a reference electrode. The change in the potential is related to the concentration in a logarithmic way (Eggins, 1996). Several biosensor examples exist using potentiometric detectors (Fernandes et al., 1999; Kormos et al., 2000; Rajesh et al., 2005; Reddy et al., 2003; Reher et al., 2002; Rotariu et al., 2004; Saurina et al., 1998).

In this work, the biological recognition element utilized for the biosensor are the polyphenol oxidases (PPO), a family of enzymes with a dinuclear copper center, widely distributed in the plant and animal tissues (Rocha and Morais, 2001; Sanchez-Ferrer et al., 1988; Ünal, 2007). The structure of the active site of the enzymes, in which copper is bounded by six or seven histidine residues and a single cysteine residue, is highly conserved. In the presence of molecular oxygen, two distinct reactions are catalyzed involving phenolic compounds: the o-hydroxilation of monophenols to o-diphenols (monophenolase activity) and oxidation of o-diphenols to o-quinones (diphenolase activity). This reaction involving the oxidation of a number of phenolic compounds to the corresponding quinones easily undergoes secondary reactions with amino acids, proteins or other phenols to form melanin pigments. Enzymatic browning impairs the sensory properties and marketability of the product, and also lowers their nutritional value (Goulart et al., 2003; Mayer, 2006; Matuschek and Svanberg, 2005; Rapeanu et al., 2006; Ünal, 2007).

The aim of this work was to construct a mini-potentiometric biosensor for adrenaline determination and to apply it in samples of medicines, blood and also for *in vivo* measurements.

2. Experimental

2.1. Reagents and solutions

All solutions were prepared with distilled and deionized water (MilliQ Academic System, Millipore). Adrenaline, noradrenaline, dopamine and the bovine soroalbumine were purchased from Sigma. Potassium chloride and nitric acid were purchased from Merck. L-ascorbic acid, uric acid and urea were purchased from Vetec.

Polyvinilpirrolydone (Henrifarma) and potassium dihydrogenphosphate (Synth) were used for the crude extract preparation. The total proteins concentration was measured using the Biuret method (Summer and Mirbäck, 1951), employing copper sulfate (Vetec) and sodium/potassium tartrate (Mallinkrodt). Carbon powder (Synth) and vaseline (Rioquimica) were used for the construction of the carbon paste biosensor.

2.2. Obtention of the banana (Musa sp.) crude extract

A 25 g of the peeled fruit was homogenized in a blender with 100 mL of 0.1 mL L^{-1} phosphate buffer, pH 7.0, containing 2.5 g of the protection agent polyvinilpirrolidone-Polyclar K-30, for 3 min. The homogenate was filtered through cheesecloth and centrifuged at 5000 rpm for 30 min at 25 °C. The resulting supernatant was stored at 4 °C in a refrigerator, and used as enzymatic source (Fatibello-Filho and Vieira, 2002; Moreira et al., 2005).

2.3. Measurement of the enzymatic activity and total protein concentration

The enzymatic activity was measured using a Shimadzu® UV 2401 spectrophotometer. As previously described (Mataveli et al., 2007), the polyphenol oxidase activity was determined in triplicate by measuring the absorbance at the wavelength of 410 nm of the adrenalinequinone produced in the reaction of four different amounts of the crude extract, from 0.05 to 0.20 mL (increments of 0.05 mL), 2.4 mL of adrenaline in 0.1 mol L^{-1} phosphate buffer. The same buffer was used to complete 3 mL in the cuvette. The enzymatic activity (UA – unit of activity) is numerically equal to the angular coefficient of the graphic of the velocity *vs* enzyme quantity multiplied by 1000 (Moreira et al., 2005; Harper, 1973).

2.4. Biosensor preparation

A volume containing 50 UA of polyphenol oxidase, obtained from the banana crude extract, and 375 mg of graphite powder were mixed in a mortar with a pestle for 20 min. Subsequently, 125 mg of vaseline (Rioquimica) was added to the powder and mixed for 20 min to obtain the final paste. The modified carbon paste was packed into the tip of a polyethylene tube (0.8 mm i.d.) and a copper wire was used to provide the electric contact.

2.5. Samples

Medicine samples – 1.000 mg L^{-1} adrenaline aqueous solutions were used only diluted in a 25 mL volumetric flask with 0.001 mol L^{-1} HCl. The samples were acquired in the Odontological Clinic of the Universidade Federal de Alfenas.

The spectrophotometric method established in the Brazilian Pharmacopoeia (1977) was used to check the accuracy of the results obtained with the proposed method. It was realized a spectrophotometric scanning between 230 and 350 nm, where the adrenaline (in solution of 1.00×10^{-2} mol L^{-1} HCl) presents maximum absorption in 280 nm. For the analytic curve, adrenaline solutions were used with concentrations of 8.00×10^{-5}; 1.60×10^{-4}; 2.40×10^{-4}; 3.10×10^{-4} mol L^{-1}, and a Shimadzu® UV 2401 spectrophotometer was used for this determination.

Rat blood samples were collected using two drops of a commercial heparin solution as anticoagulant for each 5 mL of the sample. The samples were diluted 1:10 with normal saline solution for the standards preparation. The range of the analytical curve was from 8.00×10^{-12} to 8.00×10^{-3} mol L^{-1} in a total of 10 standards.

In vivo measurements – 3 Wistar male rats were used, with ages varying from 60 to 75 days, and approximately 350 g each one. All of them were anesthetized with ketamine cloridrate. The biosensor and the Ag/AgCl reference electrode were introduced in the jugular vein of the animals, where the difference of potential between the electrodes was measured.

To verify the biosensor response, adrenaline aliquots were injected in the animals. After the introduction of the electrodes, 15 min were necessary to achieve a stable baseline. The animals had approximately 16 mL of blood (Felix et al., 2006), thus the amount of adrenaline injected was calculated to have a final concentration of 1.23×10^{-6} mol L^{-1} in the animal blood stream. The change in the electrode potential was monitored through all the duration of the anesthesia (approximately 2 h).

3. Results and discussion

3.1. Protein activity determinations

PPO activity and total protein concentration determinations were performed by spectrophotometry. The PPO activity was

Table 1
Optimization of biosensor parameters.

Biosensor parameter	Range studied	Optimal value
Enzyme units	25–300	50
pH	6.0–8.0	7.0
i.d. of the polyethylene tube (mm)	0.4–0.8	0.4
Time for analysis (min)	0–20	10

i.d., internal diameter.

measured for 7 days successively and varied from 864.0 to 952.8 UA.

3.2. Evaluation of the biosensor

To verify if the PPO was responsible for the signals measured, electrodes without the enzyme were made, and no potentiometric signals were obtained.

To obtain optimum response conditions, some parameters were studied: Table 1 summarizes the range over each variable was investigated, and the optimal values.

Good results were obtained using the biosensor constructed using UA and the phosphate buffer pH 7.0, similar to the pH blood value. It may be mentioned that working pH can be dependent of the enzyme source and the subtracts. In this context, Ünal (2007) using banana (*Musa cavendishii*) as enzyme source and cathecol as subtract, obtained two pH optima values: 5.5 and 7.0.

For *in situ* measurements, signals were stabilized after 10 min of measurements, as shown in Fig. 1. Thus, to improve the return time

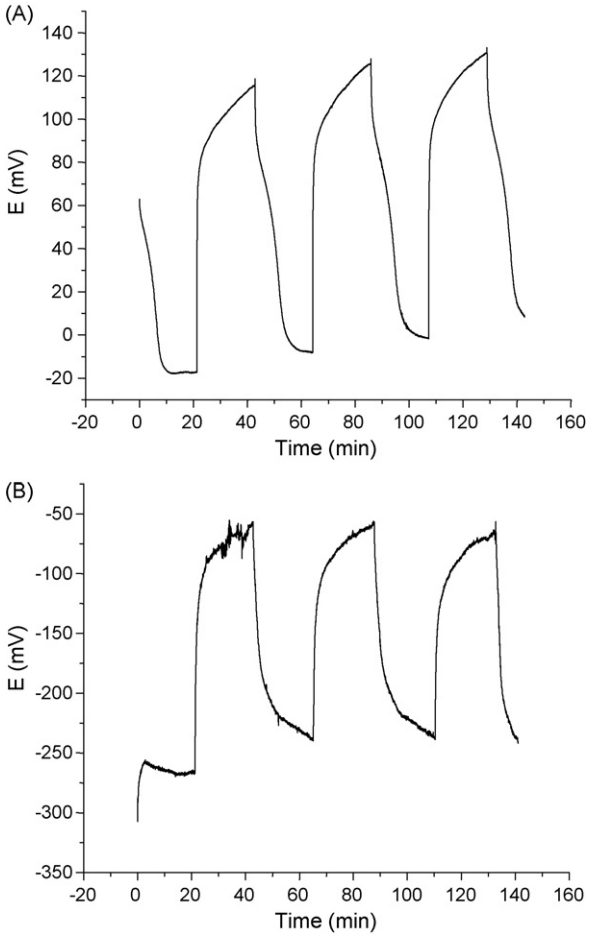

Fig. 1. Signals obtained for samples containing 8×10^{-5} mol L^{-1} of adrenaline in: (A), phosphate buffer pH 7.00; (B), rat blood sample.

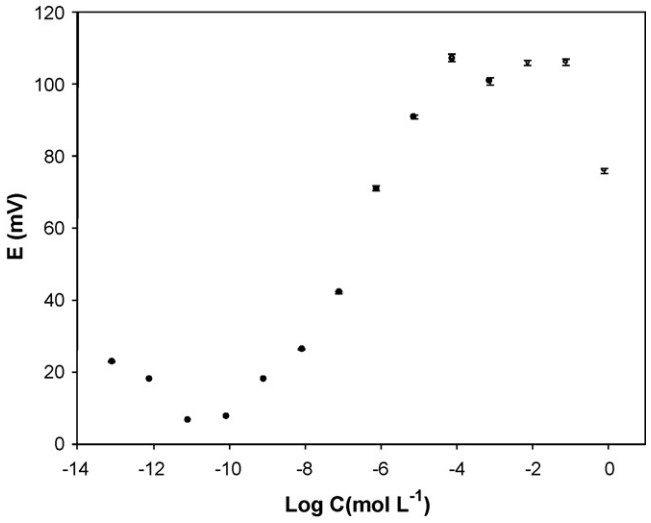

Fig. 2. Analytical curve obtained for the biosensor in phosphate buffer with standard deviation error bar.

of baseline, hydrogen peroxide, in a concentration of 0.163 mol L^{-1}, was used. The hydrogen peroxide caused oxidation and desorption of the adrenaline from the surface of the electrode, becoming the PPO available for following measurements. The use of hydrogen peroxide was based on Zhao et al. (2009) which determined uric acid monitoring the hydrogen peroxide that was catalytically produced by the reducing reaction. It is believed in the present work the hydrogen peroxide is acting in adrenaline oxidation and producing desorption of adrenaline from biosensor surface. This hypothesis was empirically confirmed, because after using the H_2O_2 the signal returned to base line and the measurements presented good accuracy.

3.3. Analytical characteristics of the biosensor

For the medicine samples determination, an analytical curve prepared in phosphate buffer (pH 7.0) was used. For blood samples and *in vivo* measurements matrix-matching calibration was employed.

The analytical curve obtained with phosphate buffer (pH 7.0) can be seen in Fig. 2. The Limit of detection (LOD) obtained was 8×10^{-9} mol L^{-1} and the linear response was from 8×10^{-9} to 8×10^{-4} mol L^{-1}. Therefore, the potentiometric biosensor is more sensitive than others described in the literature (Caruso et al., 1999; Felix et al., 2006; Moreira et al., 2005).

The analytical curve obtained for blood was done employing a matrix-matching calibration (Buck and Lindner, 1994) and is showed in Fig. 3. The obtained LOD was 8.0×10^{-7} mol L^{-1}, the linear range was from 8.00×10^{-7} to 8.00×10^{-3} mol L^{-1}. This LOD is above the basal adrenaline level (order of nmol L^{-1}) (Hjemdahl, 1993). However, upon stress conditions (hypoglycemia, exercises or mental activity) the adrenaline level can reach values up to 1.2×10^{-6} mol L^{-1} (Robertson et al., 2003; Watt et al., 2001), being inside the linear range of proposed biosensor.

The precision of the measurements were checked by the variation coefficient and it was always lower than 0.94% and 7.42% for buffer and blood, respectively. The similarity of the angular coefficients (sensibility) of the analytical curves for both matrices is one indication that this biosensor is not susceptible to matrix interferences.

Considering that the oxidant reaction of the adrenaline intermediated by the PPO involves two electrons (Chen and Peng, 2003), the response of the biosensor was sub-Nerstinian.

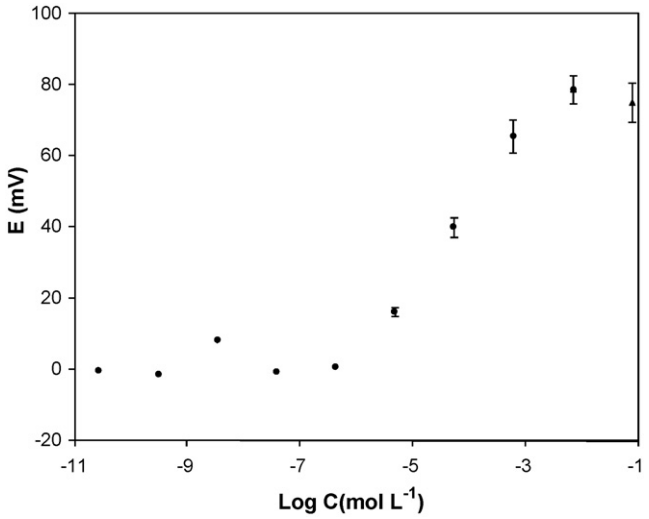

Fig. 3. Analytical curve obtained for the biosensor in blood with standard deviation error bar.

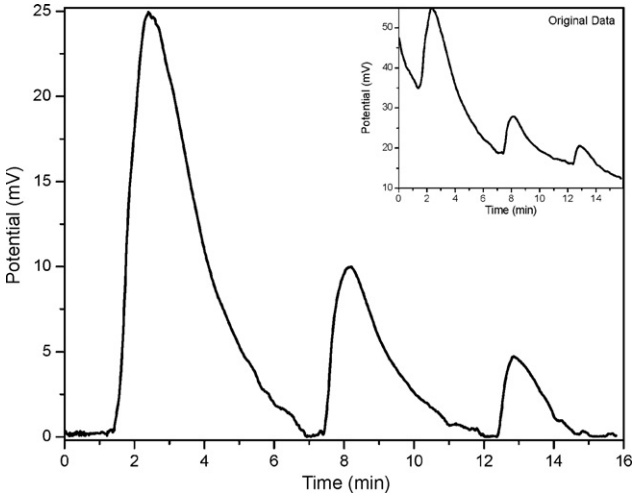

Fig. 4. *In vivo* measurements of adrenaline. The injections consisted of 0.300 mL of 6.56×10^{-5} mol L^{-1} of adrenaline. The baseline was adjusted using the Origin® program.

The interference studies were made using some compounds that are present in blood samples (Abdel-Hamid et al., 1995): uric acid, ascorbic acid, urea, dopamine, noradrenaline. All the compounds were tested in three different concentrations, the first one being the same concentration normally found in the blood, and the others are two and three times higher, respectively. The adrenaline concentration was maintained on 8.00×10^{-5} mol L^{-1}, since this concentration is in the linear response range. The main interference was caused by ascorbic acid (*ca.* 113%) on biosensor response; nevertheless, this interference occurred only for high concentrations (three times higher), what does not happen normally in blood. All other compounds did not interfere significantly (less than 10%).

3.4. Determination of adrenaline in the samples

Table 2 shows the results obtained for the analysis of three medicine formulations using the biosensor, one of which was compared to the official UV spectrophotometric method (Brazilian Pharmacopoeia, 1977). The results obtained with both methods are in agreement with 95% confidence level (*t*-test).

To check accuracy for blood sample determinations, an addition/recovery test was made. It was added 2.73×10^{-6} mol L^{-1} and recovered 2.44×10^{-6} mol L^{-1}. Considering the complexity of sample, the obtained recovery of 89.4% can be satisfactory.

3.5. In vivo measurements

The biosensor and the reference electrodes were inserted in the jugular veins of the rats (Wistar), and the obtained signals can be seen in Fig. 4.

It was not possible to wait on the stabilization of the baseline due to the duration time of the animal anesthesia. However the response time of the electrode was of approximately 4 min. Thus, a graphic treatment to adjust baseline was done.

Table 2
Determination of adrenaline by the official method (Brazilian Pharmacopoeia, 1977) and by the potentiometric biosensor.

Sample	Label value (g L^{-1})	Pharmacopoeia (g L^{-1})	Biosensor (g L^{-1})
1	1.000	Not determined	0.920 ± 0.230
2	1.000	Not determined	1.190 ± 0.145
3	1.000	1.065 ± 0.001	1.026 ± 0.002

Using the known medium volume of blood in a rat (16 mL), the injected concentration of adrenaline was calculated as 1.23×10^{-6} mol L^{-1}. Three injections with the same adrenaline concentration were done, and the biosensor response were 20.5, 9.47 and 4.87 mV, respectively. The calculated adrenaline concentrations, using the equation of matrix-matching calibration curve, were 8.63×10^{-6}, 2.49×10^{-6} and 1.48×10^{-6} mol L^{-1} for injections 1, 2 and 3, respectively. These different values obtained for the same quantity of adrenaline injected can be explained due the metabolism of the animals. Generally, after 10 min of an intravenous injection of adrenaline, it is completely eliminated from the blood (Axelrold, 1959), during this time the adrenaline receptors are primed on cells surfaces. In this work, the intervals between injections were about six minutes, and after the second injection the receptors were already activated, thus increasing the adrenaline metabolism rate, and consequently decreasing the signal response. Therefore, considering the complexity of the sample, the results are considered promising. It also can be noted that the baseline was reestablished in the blood stream. This can be attributed to the presence of dissolved oxygen in the circulatory system, which has the same function as the hydrogen peroxide used in medicines samples. Another important fact is the rapid adrenaline metabolization that diminishes its concentration in approximately two min. It should be emphasized that the effect of most of the catecholamine is no longer than a few minutes (Bacq, 1949).

In the literature, there was no evidence for the *in vivo* adrenaline determination. However, it is evident that the monitoring of this analyte concentration is important during surgical interventions, which can be indicative of heart failure.

For comparison effect it can be mentioned some *in vivo* measurements with other sensors: Earl et al. (1998) that implanted electrodes (voltammetric measurements) for the catecholamine determination, in the sagui brain. The results showed that the cyclic voltammetry, in this case, was unable to detect the analyte in concentrations lower to 10^{-8} mol L^{-1}. Therefore, it cannot be used for monitoring dopamine in the absence of stress that increases its concentration. Studies accomplished for the biosensors for glucose are more advanced. Different animals are used, such as mice, dogs, chimpanzees and humans (Koschwanez and Reichert, 2007). These biosensors can be as much intravascular as subcutaneous, and certain complications exist for the intravascular implantation. When experiments of long duration are accomplished, such

complications as thromboembolisms and infections may be propagated.

Before exposure, it can be affirmed that the present work presents preliminary results, due to the small amount of evaluated samples. However the results are promising, once they point to the possibility of *in vivo* employment of a simple and low cost electrode.

4. Conclusions

The main characteristics of the developed biosensor are: easy to make, well-adjusted size for *in vivo* measurements, good performance in applied samples and low cost.

To the best of our knowledge *in vivo* determinations of adrenaline are not found in the literature, nevertheless are important during surgery procedures to predict a cardiac arrest. Finally, this work presents promising results, pointing to the possibility of the use of a simple and low cost biosensor for *in vivo* determinations of adrenaline in next future.

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References

Abdel-Hamid, I., Atanasov, P., Wilkins, E., 1995. Anal. Chim. Acta 313, 45–54.
Axelrold, J., 1959. Phys. Rev. 39, 751–776.
Bacq, Z.M., 1949. Pharmacol. Rev. 01, 01–26.
Brazilian Pharmacopoeia, 1977. 3rd ed. São Paulo.
Buck, R.P., Lindner, P., 1994. Pure Appl. Chem. 66, 2527–2536.
Caruso, C.S., Vieira, I.C., Fatibello-Filho, O., 1999. Anal. Lett. 32, 39–50.
Chen, S.M., Peng, K.T., 2003. J. Electroanal. Chem. 547, 179–189.
Earl, C.D., Sautter, J., Xie, J., Kruk, Z.L., Kupsch, A., Oertel, W.H., 1998. J. Neurosci. Methods 85, 201–209.
Eggins, B.R., 1996. Biosensors: Introduction, 1st ed. Wiley Teubner, New York.
Fatibello-Filho, O., Vieira, I.C., 2002. Química Nova 25, 455–464.
Felix, E.S., Yamashita, M., Angnes, L., 2006. Biosens. Bioelectron. 21, 2283–2289.
Fernandes, J.C.B., Kubota, L.T., Neto, G.D.O., 1999. Anal. Chim. Acta 385, 3–12.
Garrido, E.M., Lima, J.L.F.C., Delerue-Matos, C., 1997. J. Pharm. Biomed. Anal. 15, 845–849.
Goodman, L.S., Gilman, A., 2006. As bases farmacológicas da terapêutica, 11th ed. McGraw-Hill Interamericana do Brasil, Rio de Janeiro.
Goulart, P.F.P., Alves, J.D., Magalhães, M.M., Lima, L.C.O., Meyer, L.E., 2003. Food Chem. 83, 7–11.
Guo, J.Z., Guo, F.Z., Hong, C.Y., 2002. Anal. Chim. Acta 463, 257–263.
Hansen, A.M., Kristiansen, J., Nielsen, J.L., Byrialsen, K., Christensen, J.M., 1999. Talanta 50, 367–379.
Harper, H.P., 1973. Manual de química fisiológica, 3rd ed. Atheneu, São Paulo.
Hjemdahl, P., 1993. Bailliers Clin. Endocrinol. Metab. 7, 307–353.
Kawada, T., Yamasaki, T., Akiyama, T., Sato, T., Shishido, M., Sugimachi, M., Inagaki, M., Alexander, J., Sunagawa, K., 1998. J. Chromatogr. B 714, 375–378.
Kildegaard, J., Christensen, T.F., Johansen, M.D., Randlov, J., Hejlesen, O.K., 2007. Diabetes Technol. Ther. 9, 501–507.
Kormos, F., Sziráki, L., Tarsiche, I., 2000. Lab. Rob. Autom. 12, 291–295.
Koschwanez, H.E., Reichert, W.M., 2007. Biomaterials 28, 3687–3703.
Li, F., Cui, H., Lin, X.Q., 2002. Anal. Chim. Acta 471, 187–194.
Liu, A., Honma, I., Zhou, H., 2005. Biosen. Bioelectron. 21, 809–816.
Mataveli, L.R.V., Antunes, N.J., Brigagão, M.R.P.L., Luccas, P.O., 2007. Infarma 19, 33–35.
Matuschek, E., Svanberg, U., 2005. Food Chem. 90, 765–771.
Mayer, A.M., 2006. Phytochemistry 67, 2318–2331.
Medina, A.R., De Córdova, M.L.F., Diaz, A.M., 2000. Mikrochim. Acta 134, 101–105.
Moreira, L.N., Magalhães, C.S., Luccas, P.O., 2005. Infarma 16, 59–62.
Rajesh, Bisht, V., Takashima, W., Kaneto, K., 2005. React. Funct. Polym. 62, 51–59.
Rapeanu, G., Loey, A.V., Sanout, C., Hendrickx, M., 2006. Food Chem. 94, 253–261.
Reddy, R.R.K., Basu, I., Bhattacharya, E., Chadha, A., 2003. Curr. Appl. Phys. 3, 155–161.
Reher, S., Lepka, Y., Schwedt, G., 2002. Michrochim. Acta 140, 15–20.
Robertson, R.P., Sutherland, D.E., Seaquist, E.R., Lanz, K.J., 2003. Dibetes, 1689–1694.
Rocha, A.M.C.N., Morais, A.M.M.B., 2001. Food Control 12, 85–90.
Rotariu, L., Bala, C., Magearu, V., 2004. Anal. Chim. Acta 513, 119–123.
Sanchez-Ferrer, A., Bru, R., Cabanes, J., Carcia-Carmona, F., 1988. Phytochemistry 27, 319–321.
Saurina, J., Hernández-Cassou, S., Fàbregas, E., Alegret, S., 1998. Anal. Chim. Acta 371, 49–56.
Summer, J.B., Mirbăck, K., 1951. The Enzymes–Chemistry and Mechanism of Action, 1st ed. Academic Press, New York.
Thevenot, D.R., Toth, K., Durst, R.A., Wilson, G.S., 2001. Biosens. Bioelectron. 16, 121–131.
Torres, C.A., Romero, M.A., Calatayud, M.J., 1998. Mikrochim. Acta 128, 187–190.
Ünal, M.U., 2007. Food Chem. 100, 909–913.
Wortsman, J., 2002. Endocrinol. Metab. Clin. North Am. 31, 79-+.
Watt, M.J., Howlett, K.F., Febbraio, M.A., Spriet, L.L., Hargreaves, M., 2001. J. Physiol. 534, 269–278.
Zhao, C., Wan, L., Wang, Q., Liu, S., Jiao, K., 2009. Anal. Sci. 25, 1013–1017.