Methodology

For reprint orders, please contact: reprints@future-science.com

Bioanalysis

A label-free platform for dopamine biosensing

Jesús Navarro[1], Javier Galbán*,[1] & Susana de Marcos[1]

[1]Analytical Biosensors Group, Analytical Chemistry Department, Faculty of Sciences, Aragón Institute of Nanoscience, University of Zaragoza, 50009-Zaragoza, Spain

* Author for correspondence: jgalban@unizar.es

Aim: This work presents a label-free platform for dopamine (DA) monitoring based on the spectroscopic properties of laccase. **Results:** Working in batch mode, DA ranging from 25 to 250 μM, can be determined without the interference of norepinephrine and epinephrine. Laccase immobilized in a polyacrylamide film is the basis of a platform for the label-free determination of DA. The linear range goes from 100 to 900 μM with an RSD of 5.3% and a film lifetime of more than 30 measurements. The biosensors also permit the DA + epinephrine + norepinephrine determination. **Conclusion:** The method permits the determination of DA and the total concentration of the three neurotransmitters, and could be used for DA monitoring in urine samples.

First draft submitted: 27 July 2017; Accepted for publication: 20 October 2017; Published online: 15 December 2017

Keywords: dopamine • laccase • optical biosensor

Optical biosensors continue to represent a challenging field of research [1]. This is largely due to two important factors: the rapid development of new antibodies, aptamers and others biomolecules, which can be used as bioreceptors with the appropriate capabilities of selectivity and stability (after integration in a solid support); and the development of new transduction schemes giving suitable sensitivity and concentration response ranges.

However, to date most of these biosensors have been devised as disposable systems and can only be used once. If the purpose of the research is the design of a biosensor for the continuous monitoring of an analyte (especially in clinical or pharmaceutical samples), bioreagents need to have two additional properties [2]: reversibility (after interacting with the analyte) and autoindicating capacity, in other words, optical properties that reversibly change during the recognition event. Biosensors having these properties are usually known as label free (or reagentless). These requirements greatly limit the number of available bioreagents or the analytical techniques to be used.

Enzymes may be suitable for this purpose [2]. First, as catalizers they are reversible in nature. Second, some of them have spectroscopic autoindicating properties (intrinsic or acquired after chemical modification) and can be used as label-free systems. Third, in some types of enzymatic reactions, the necessary cosubstrate can be supplied by the sample itself, as with hydrolytic enzymes (H_2O) and oxidase-type enzymes (O_2).

All enzymes have intrinsic absorption or fluorescence properties due to their amino-acids (especially tyrosine and tryptophan) [3], but these properties can only be used for label-free systems in very few cases. Additional optical properties may come from the coenzyme of which three different families can be mentioned, those belonging to the flavoenzymes group containing a flavine derivative (as FAD) [4,5], heme-proteins [6,7] and copper-containing proteins [8], these last being the subject of this paper.

Copper-containing proteins comprise a very important group of enzymes involved in oxidation–reduction reactions [9]. In these proteins, copper atoms are coordinated to two or more aminoacid-forming sites, which are given different names (T types) depending on the geometric and electronic configuration of the site: T1 (one Cu atom coordinated to two histidines, one cysteine and another one or two ligands), T2 (one Cu atom coordinated to two histidines or glutamines) and T3 (two copper atoms, each of which is coordinated to two histidines). T1 gives rise to an absorption band at about 600 nm conferring a blue color to the protein (blue proteins). T3 produces an absorption band at about 330 nm and also fluorescence at about 450 nm [10]. No spectroscopic properties have been found for T2. Laccase (Lac) is a copper-containing protein (usually extracted from many types of fungus and

newlands press

bacteria), which catalyzes the oxidation of different families of compounds by O_2 [11]. It is a multicenter copper protein containing four copper atoms (in T1, T2 and T3 sites). During the reaction, the substrate is oxidized in the T1, and then the electrons are transferred to the T2/T3 where oxygen is reduced to water. The maximum at 600 nm could be used for label-free systems, but it has been scarcely used. This is because the enzyme is usually applied in electroanalytical biosensors. Lac is prone to present several isoenzymes, some of which lack the blue color (they are called yellow or white Lac) [12]. The reason for this remains unclear, but the kinetic behavior of the enzyme does not change (it may even be more active than the blue one). Tyrosinase is another well-known copper-containing protein which is also involved in oxidation–polymerization reactions of monophenols and catechols, but only containing a T3 site.

Dopamine (DA) is an important natural catecholamine found in the CNS of mammals. It plays a critical role as a neurotransmitter (Ner) in the cardiovascular, renal and hormonal systems [13]. DA acts on the transmission of messages to different parts of the brain to coordinate body movements. Changes in the concentration of DA can involve serious brain diseases such as schizophrenia and Parkinson's [14]. Consequently, DA levels may be a marker of vital importance for biomedical diagnostics.

Analytical methods for DA and catecholamines have been frequently reviewed [10,15–16]. DA has interesting intrinsic spectroscopic (absorption and fluorescence) [17] and electrochemical [18] properties, however, considering the low sensitivity and selectivity usually required it is necessary: combining the detection with separation techniques, especially liquid chromatography [19,20]; the use of derivatization methods for fluorescence [21]; an adequately modified electrode [22] for amperometry; and especially by coupling the analyte to enzymatic reactions [10] using tyrosinase [23] and Lac [24]. In fact, the enzymatic reaction with Lac has been defined as a methodology for avoiding interferences in the determination of catecholamine by electrochemical biosensors [25].

During the last few years, several optical sensors approaches for DA determination have been proposed, most of them based on nanomaterials [26,27], specially graphene quantum dots [28–30], carbon dots [31] or gold nanomaterials [32–34]. These sensors present, in general, very good sensitivity. Nevertheless, in most of them, the sensing principle (charge transfer quenching, ionic interaction) do not include a molecular recognition event (as it occurs if bioreagents are used), so the selectivity could be compromised. Moreover, the most of these systems are not actually label free (because they require additional reagents for sensing) and, as far as we know, none of them are reversible, which limit their use as continuous monitoring systems.

DA levels in blood or urine are usually very low; however, after L-dopa pharmaceuticals administration for Parkinson treatment, the concentration in urine reach very high values and biosensors for the accurate monitoring elimination of DA are needed [35,36]. This paper proposes an optical biosensor for DA based in the label-free capability of yellow Lac.

Materials & methods

Apparatus

Absorption measurements were carried out with an Agilent 8453 UV–visible photodiode array spectrophotometer (1 nm slit; Agilent, CA, USA). Fluorescence measurement and contour plot (3D) spectral measurements were carried out with a Photon Technology International (PTI) Time Master fluorescence spectrometer (TM-12/2003).

Reagents

Laccase from *Trametes versiocolor* (12.9 $U{\cdot}mg^{-1}$, EC 1.10.3.2) (Lac), tyrosinase from mushroom 3130 $U{\cdot}mg^{-1}$, EC 1.11.18.1), dopamine hydrochloride (DA), epinephrine hydrochloride (Epi), norepinephrine hydrochloride (NorEpi), acrylamide and N,N′′-methylenebisacrylamide, were purchased from Sigma-Aldrich [37] (MI, USA) and used as received. N,N,N′′,N′′-tetramethylethylenediamine (TEMED) was purchased from BioRad (CA, USA) [38]. All other reagents and organic solvents were of analytical grade and used as received.

Absorbance measurements

Lac solution in phosphate buffer (pH 6.0; 0.1 M) or acetate buffer (pH 4.5; 0.1 M; 2 ml) were added to a quartz cell (1-cm path length), and the absorbance at the working wavelengths (400 and 480 nm) began to be monitored. Then 20 µl of the corresponding DA solution was added.

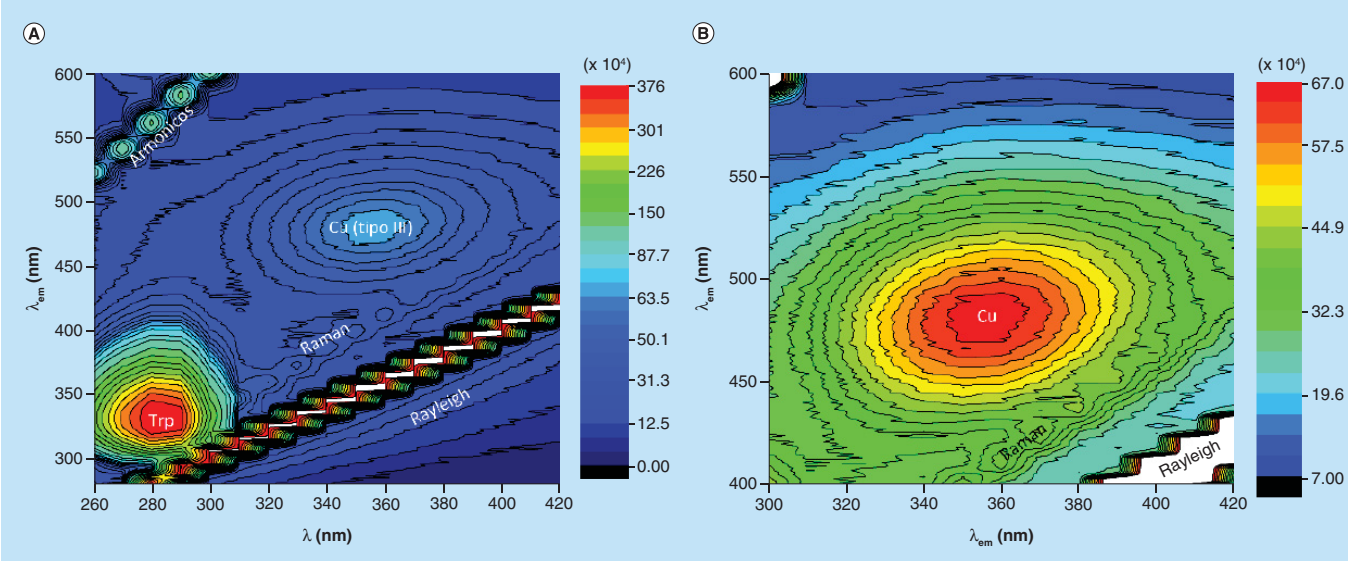

Figure 1. 3D fluorescence spectrum obtained with a 150 Uml^{-1} tyrosinase solution (pH = 6, phosphate buffer). The graph **(A)** shows the whole spectrum, indicating the fluorescence due to the aminoacids, especially Trp and that of the T3 copper center. The graph **(B)** shows the section corresponding to the T3 fluorescence enlarged.
Trp: Tryptophan.

Sensor film preparation (label-free platform) & measurements

A polyacrylamide film containing immobilized Lac was prepared following a previously described procedure [13] after optimizing the enzyme concentration (27.1 mg of Lac – 350 U – were dissolved in 800 µl of acetate solution) and located in a flow cell designed in the laboratory (Supplementary Figure 1). This flow cell is placed in the cuvette compartment of the spectrophotometer. The acetate buffer solution (pH 4.5; 0.1 M) was used as the carrier flow and 1.5 ml of DA of different concentrations was injected.

Results & discussion

Enzyme selection

At first, two copper enzymes were considered, Lac and tyrosinase. Both enzymes catalyze the oxidation–polymerization reaction of catechols, yielding polycatechols which strongly absorb in the range of 450–500 nm. DA, as a catechol, also gives this type of compound, so the absorption of the polydopamine (PDop) can be used as the analytical signals for this analyte determination. We were especially interested in developing a sensor for DA determination based on the intrinsic spectroscopic properties of the bioreagent, so we tested the intrinsic properties of both enzymes. However, we also tested the capabilities of the PDop absorption.

Tyrosinase was assayed first. The molecular absorption of the free enzyme did not show any recognizable maximum in the UV–visible region (apart from those of the aminoacids). After the reaction with DA, absorption bands at 300 and 480 nm due to PDop were observed, but there were no additional changes due to the enzyme (Supplementary Figure 2). By measuring the absorbance of PDop at 480 nm, the DA was determined in the concentration range from 1×10^{-5} to 2.5×10^{-4} M (Supplementary Figure 3) with a $K_m = 1.15$ mM. Also, the fluorescence properties of the tyrosinase were tested. An emission maximum at 450 nm (excitation maximum at 355 nm) was observed (Figure 1). During the enzymatic reaction with DA, this fluorescence decreased, but this was not due to the intrinsic fluorescence variation of the enzyme but to the inner filter effect caused by the PDop. These fluorescence changes also permitted the DA determination in a concentration range from 1×10^{-5} up to, at least, 1×10^{-4} M (Supplementary Figure 4).

The molecular absorption spectra obtained for Lac corresponded to a yellow copper-containing enzyme. This showed the common aminoacids absorption in the UV region (200 and 280 nm) and a maximum at 400 nm due to the metal center of Cu [8]. In this case, fluorescence was not observed. During the enzymatic reaction with DA, the appearance of the maximum at 480 nm due to the PDop formation was significant but interestingly the

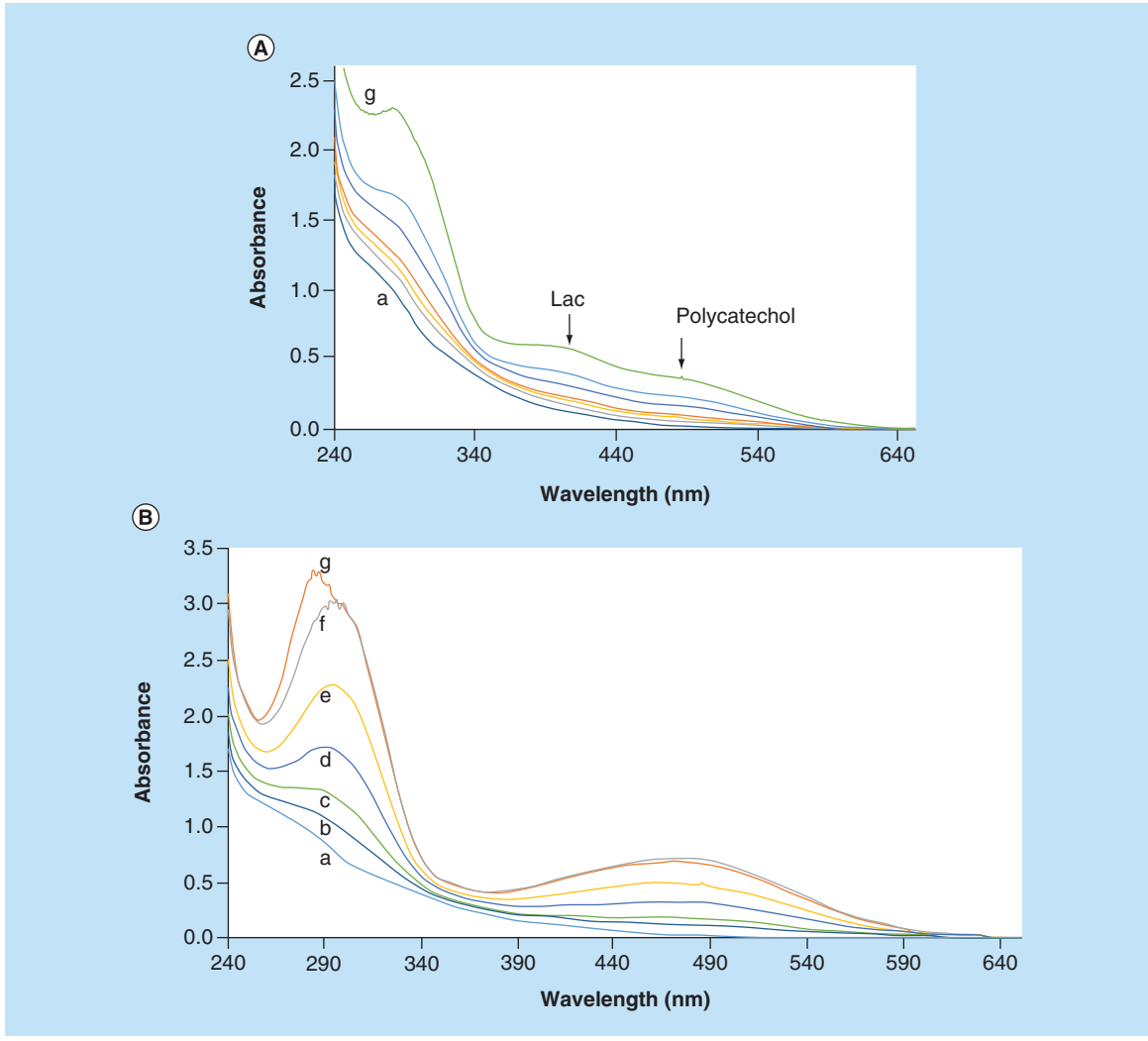

Figure 2. Molecular absorption spectra obtained, after reaching the equilibrium, during dopamine calibration studies. The maxima at 400 (Lac) and 480 nm (polycatechols) are marked. **(A)** The conditions used were as follows: pH = 4.5 (0.1 M acetate buffer) and 16.4 U ml^{-1} Lac. DA concentrations: a (lowest) 0 μM; 25 μM; 50 μM; 75 μM; 150 μM; 250 μM; g (highest) 400 μM. **(B)** The conditions used were: pH = 6.0 (0.1 M $H_2PO_4^-/HPO_4^{-2}$ buffer) and 16.4 U ml^{-1} Lac. DA concentrations: (a) 0 μM; (b) 25 μM; (c) 52 μM; (d) 76 μM; (e) 150 μM; (f) 250 μM; (g) 400 μM. DA: Dopamine; Lac: Laccase.

absorbance at 400 nm also changed, due to the formation of the reduced form of Lac, indicating that the intrinsic absorbance of this enzyme could be used for monitoring the enzymatic reaction (Figure 2).

Lac studies in batch

It was ascertained from the bibliography that Lac works effectively at a pH ranging from 4 to 7. Previous assays indicated that the optimum working conditions were obtained at pH 6.0 phosphate buffer (0.1 M) and pH 4.5 acetate buffer (0.1 M), the main difference between both pHs is the kinetic rate. Figure 3 compares the kinetic records at these two pHs and measuring at two wavelengths, 400 (Lac intrinsic absorption) and 480 nm (PDop absorption). It can be seen how at pH 6.0 both, the PDop is quickly formed and the increase in the absorbance at 480 nm prevents the absorbance at 400 nm being observed. However, at pH 4.5, the PDop is formed very slowly, so the changes due to the enzyme are observed and the determination can be carried out at both wavelengths. This difference in the progress of the reaction has led to the study of two methodologies for the determination of DA, based on PDop formation (480 nm) or Lac intrinsic (400 nm) absorbance.

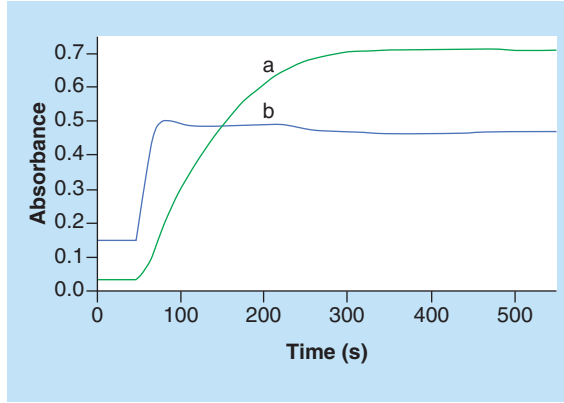

Figure 3. Variation of absorbance of laccase (16.4 U ml^{-1}) with time during the enzymatic reaction with dopamine (2.52 x 10^{-4} M). (a) reaction at 400 nm and pH = 4.5; (b) reaction at 480 nm and pH = 6.0.

Table 1. Variation of the analytical signal Δ Abs$_{400}$ (Abs$_{max,400}$ - Abs$_{min,400}$) during the reaction, with the concentration of laccase (acetate buffer pH 4.5, 0.1 M and dopamine 0.25 mM).

Lac concentration (U ml^{-1})	Δ Abs$_{400\ nm}$	Time† (s)
4	0.163	850
8	0.153	300
16	0.162	120
32	0.169	70
64	0.173	40

†Time necessary to reach the maximum.
Lac: Laccase.

Table 2. Analytical figures of merit for the dopamine determination at 400 (intrinsic absorbance) and 480 nm (polydopamine absorbance).

Figures of merit	pH 6, 480 nm	pH 4.5, 480 nm	pH 4.5, 400 nm
Calibration line	2728x† + 0.0184	832x† + 0.0038	1128x† + 0.000
Lineal range	25–250 μM	25–150 μM	25–250 μM
RSD (n = 5)	3.4%	4.1%	4.2%

† 'x' being dopamine concentration in M, in the calibration line.

When working at pH = 4.5, the variation of the intrinsic absorbance of the Lac at 400 nm can be followed. Before determining the analytical capabilities of the method, the effect of the enzyme concentration was first optimized. Table 1 shows the variation of the analytical signal ΔAbs$_{400}$ (Abs$_{max,400}$ - Abs$_{min,400}$) during the reaction, with the concentration of Lac ranging from 4.0 to 63.0 U ml^{-1}. From these results, a concentration of 16.4 U ml^{-1} was selected as the optimum (for working in equilibrium methods) because the changes at 400 nm are sufficiently fast and the changes at 480 nm do not cause interference. The effect of the buffer composition (acetate, phosphate, etc.) and its concentration (0.1 M, 0.01 M) was also studied. However, no differences were observed in any case. The analytical characteristics of the enzymatic method based on the intrinsic absorption of Lac in the optimal conditions are summarized in Table 2. The spectra obtained for the calibration points are shown in Figure 2A.

Working at pH = 4.5 also permits the determination of DA, but measuring in this case the PDop absorption (at 480 nm). However, if this alternative is to be used, better results are obtained by working at pH = 6.0. The analytical capabilities of this method using the previously optimized Lac concentration (16.4 U ml^{-1}) are also shown in Table 2 and the spectra of the calibration study in Figure 2B. The Michaelis–Menten constant obtained for Lac ($K_{m,Lac}$) in these conditions was 1.94 mM (Supplementary Figure 5), which is comparable to that obtained by other authors in similar experimental conditions [39]. As can be seen, working at pH = 6 the sensitivity is higher than at pH 4.5 and it could be an interesting alternative for DA determination in batch.

As stated above, DA is a Ner of the CNS where other catechol Ner such as Epi and NorEpi may also appear. For many biological applications, the total concentration of Ner is more important than the individual concentrations.

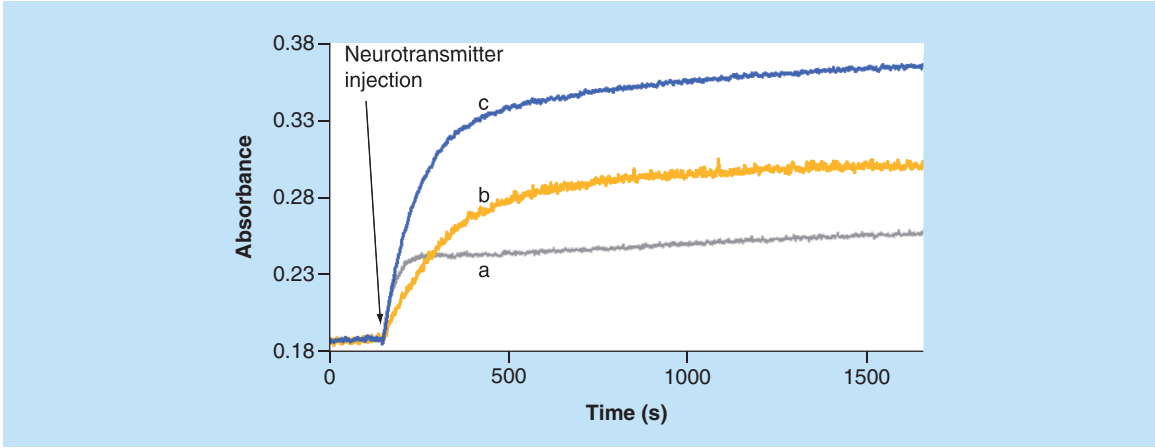

Figure 4. Effect of norepinephrine and epinephrine on the dopamine signal. Variation of absorbance of Lac (16.4 U ml^{-1} in acetate buffer [pH 4.5; 0.1 M]) at 400 nm after the addition of **(A)** DA 50 μM; **(B)** NorEpi 50 μM and Epi 50 μM; **(C)** DA 50 μM, NorEpi 50 μM and Epi 50 μM.
DA: Dopamine; Epi: Epinephrine; Lac: Laccase; NorEpi: Norepinephrine.

Table 3. Selective determination of dopamine in synthetic samples of urine containing the three neurotransmitters in the molar ratio found in biological fluids in patients with normal and abnormal contents.

Figures of merit	DA, standard	Normal samples	Abnormal samples
Calibration line	400.5x + 0.0063	398.6x + 0.0073	410.1x + 0.016
Lineal range	25–250 μM	25–250 μM	30–250 μM
RSD (n = 5)	4.2%	4.3%	4.2%

DA is determined measuring dAbs400nm (x being dopamine concentrations in M, in the calibration lines).
DA: Dopamine; Lac: Laccase; Ner: Neurotransmitter.

Table 4. Total neurotransmitters determination in synthetic samples of urine containing the three neurotransmitters in the molar ratio found in biological fluids in patients with normal and abnormal contents

Figures of merit	Ner, standard	Normal samples	Abnormal samples
Calibration line	2728x + 0.018	2756x + 0.002	2804x + 0.025
Lineal range	25–250 μM	25–250 μM	25–250 μM
RSD (n = 5)	3.4%	3.8%	3.8%

Ner are determined measuring the δAbs480 (polydopamine absorbance) when the equilibrium is reached.
Ner: Neurotransmitter.

For this reason, the response of these compounds to Lac was also studied. To do this, the variation of $Abs_{400\ nm}$ with the two Ner was obtained in a concentration of around 0.05 mM (which is within the linear range of Lac-DA). As can be seen in Figure 4, either Epi or NorEpi react with Lac but at a reaction rate much lower than DA. We also studied the response of Lac with different solutions containing NorEpi, Epi and DA at different relationships. To maximize the DA response against the two other catecholamines, measurements were made in kinetic mode. It was found that it is possible to determine DA in the presence of the two other Ner measuring the reaction changes at 400 nm during the first 30 s ($dAbs_{400} = \Delta Abs_{400}/\Delta t_{30}$), if the DA/(NorEpi + Epi) ratio is 1 or higher. Moreover, the total Ner concentration can also be calculated from the total absorbance variation at 480 nm ΔAbs_{480} ($\Delta Abs_{480} = Abs_{480,max} - Abs_{480,0}$) observed during the reaction.

Two calibration studies were performed using synthetic urine samples containing normal (2.5) and pathological (1.5) DA/(NorEpi + Epi) ratios usually found in this type of fluid (e.g., ClinCheck® [40]), and the results were compared with those obtained using synthetic samples containing the same concentration of DA alone (Tables 3 & 4). The slopes of the calibration lines obtained measuring $dAbs_{400}$ (Table 3) were statistically similar (95%) for DA and the synthetic urine samples containing Ner, indicating that DA determination can be carried out without the

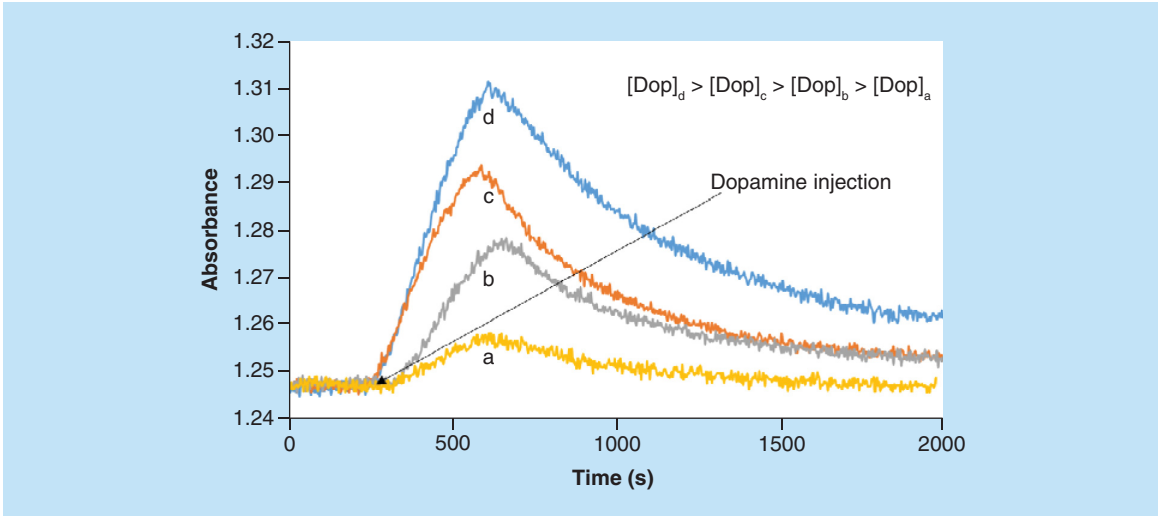

Figure 5. Variation of absorbance (at 400 nm) of a polyacrylamide laccase film (350 U) at different concentrations of dopamine. Flow of 0.5 ml min^{-1} and 3 min injection in acetate buffer (pH 4.5; 0.1 M). DA concentrations: (a) 120 μM; (b) 360 μM; (c) 600 μM; (d) 840 μM.
DA: Dopamine.

interference of the Epi and NorEpi. Moreover, the slopes of the calibration lines obtained using ΔAbs_{480} (Table 4) were also statistically similar (95%), which permits using this parameter for the total Ner determination.

Label-free platform

Lac was immobilized in polyacrylamide films as the basis of the biosensor platform. Lac was entrapped during the polymerization of acrylamide and bis-acrylamide following the procedure described above [7] (see 'Sensor film preparation' section). The Lac film was placed in a homemade flowcell (Supplementary Figure 1) and the changes in absorbance at 400 nm with time were measured, using the acetate buffer (pH 4.5; 0.1 M) as the carrier solution at a flow rate of 0.5 ml min^{-1}.

Figure 5 shows the absorbance changes at the chosen wavelength (400 nm) obtained during a calibration study. The lower sensitivity of this continuous methodology (compared with the method in solution) was expected due to the measurement procedure and the immobilization of the enzyme. The reversibility of the enzyme and thus the continuous monitoring of the reactions were achieved thanks to the re-oxidation of the enzyme with the oxygen dissolved in solution.

The analytical characteristics of the methodology were studied by injecting 1.5 ml of the desired solution in the carrier solution. This showed a linear range for DA, which goes from 100 to up least 900 μM, and an RSD of 5.3% when measuring both the peak height (H) and peak area (S) of the transient signal. The K_m obtained for Lac in the solid phase was 8.04 mM (Supplementary Figure 6), which is, at the light of the experimental results, higher than that in solution. The lifetime of the sensor film was about 1 week; during this time, 32 measurements were carried out using solutions containing 560 μM DA, and a relative standard deviation (RSD) of about 8% was obtained ($n = 32$). From this measurement a diminution in sensitivity was observed which was statistically different from those previously obtained.

The response of the biosensor film in the presence of NorEpi and Epi in ratios similar to those of the urine synthetic samples, described in the previous section, was also studied (with the Ner ratio found in normal or abnormal biological fluids) and compared with those of solutions containing only DA. Figure 6 shows the variation in $Abs_{400\ nm}$ with time of the biosensor film for a solution containing only DA and another solution with the synthetic sample (in both cases, the concentration of DA is the same). It can be observed that the changes in absorbance during the first seconds and until the signal reaches the maximum values (H) are the same in both cases. However, some differences were observed in the regeneration process (S). These differences could be assumed to be due to the different reaction rates of Lac with these Ner. As has been seen in the batch measurements, the DA reaction is faster and therefore the initial change can be assumed to be due only to this compound. Subsequently,

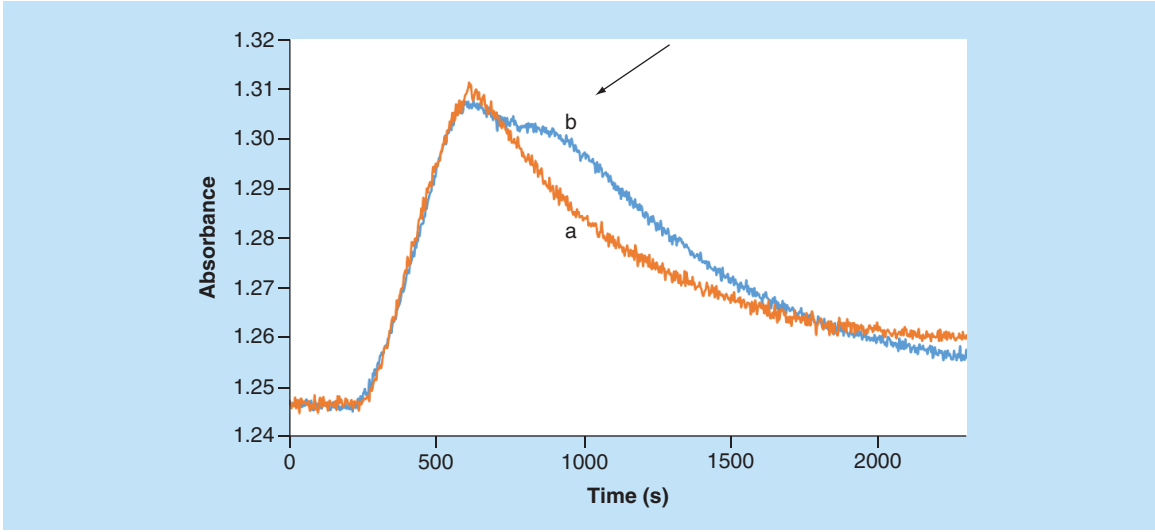

Figure 6. Effect of norepinephrine and epinephidrine on dopamine signal in the optical sensor. Variation of absorbance (at 400 nm) of a polyacrylamide Lac film (350 U) with (a) DA 840 μM; (b) DA 840 μM, NorEpi 500 μM and Epi 100 μM (flow rate 0.5 ml min^{-1}, 3 min injection in acetate buffer [pH 4.5; 0.1 M]).
DA: Dopamine; Epi: Epinephrine; Lac: Laccase; NorEpi: Norepinephrine.

Table 5. Comparison of the analytical characteristics of the label-free platform for the determination of dopamine (by measuring Δ Abs_{400}) and the total neurotransmitter (by measuring area) in standard and synthetic urine samples containing abnormal concentrations (x being DA or neurotransmitter concentrations in M).

Figures of merit	H, DA	H, Ner	S, DA	S, Ner
Calibration line	76.5x + 0.00	73.0x + 0.00	54826x - 0.5	56454x - 6
Lineal range	100–900 μM	100–900 μM	100–900 μM	100–900 μM
RSD (n = 5)	5.3%	5.8%	4.7%	5.4%

DA: Dopamine; Ner: Neurotransmitter.

the NorEpi or Epi reacts and leads to a second oxidation process, as can be deduced from the shape of the signal. The changes in the initial signal were evaluated and related to the concentration of DA in the sample.

These results suggest that both DA alone and total Ner can be determined from the analytical signal. According to this, the H and the S of the signal should be dependent on the DA concentration, and the total DA + NorEpi + Epi concentrations, respectively. In order to test this, calibration studies were performed using solutions containing DA alone and solutions containing the three Ner in proportions similar to that of the pathological Ner/DA ratio. The results are summarized in Table 5. As can be seen, no significant differences ($p = 0.05$) were obtained in the slope of the calibration lines obtained using H, which allows the DA determination in the presence of NorEpi and Epi. Moreover, neither were significant differences observed between the slope of the calibration lines using S for DA and DA + NorEpi + Epi, indicating that the S could be used for the total Ner determination. In both cases the RSD values are lower than 6%.

Conclusion

A label-free platform for DA determination has been developed based on the intrinsic properties of the enzyme Lac during its enzymatic reaction. The method was first optimized in batch and afterward the enzyme was entrapped into a polyacrylamide film, the basis of the label-free platform. The response is only dependent on the analytical signal of the enzyme involved in the reaction. This platform present two important advantages compared with other previously reported optical sensor for DA: it is based on a real label-free bioreceptor and it can be used in continuous mode. The method has been optimized for DA determination in the presence of other Ner such as NorEpi and Epi, two compounds that are usually found in the same samples as DA, with good results. Moreover, it has also been possible to determine the total concentration of the three Ner.

Future perspective

From the results given, the biosensor described in this paper can be applied for DA monitoring in urine samples coming from patients being treated with L-dopa. According to the results, the device is able to give a measurement in about 15 min, during about 7.5 h (considering that 32 measurements could be done with the same film). Alternatively, the system permits making a measurement every 60 min during more than 1 day, which is the time usually programmed for drug elimination studies. This could be one of the most interesting applications of this methodology.

In our opinion, the field of biosensors is going to develop toward systems which are able to continuously monitor the analyte concentration, instead of single-use systems, and this type of biosensor is only possible if the biological receptor can be used several times without the necessity of a regeneration step. The use of the intrinsic spectroscopic properties of enzymes is a very interesting possibility. However, this requires having reliable information about the kinetic of the enzyme to be used as a basis of the system.

Executive summary

- The possibilities offered by the use of the intrinsic spectroscopic properties of tyrosinase and laccase as label-free systems for dopamine (DA) determination without the interference of other neurotransmitters have been studied. Laccase gives better analytical capabilities.
- Using this enzyme, DA can be determined in a batch mode, working at 400 nm and after setting the pH to 4.5. However, the best possibilities of the system are obtained after immobilizing the enzyme into a polyacrylamide film (label-free platform).
- The transient analytical signals obtained with this label-free platform are reversible, at least, during 32 cycles. From each transient signal, it is possible to determine both DA (from the peak height of the signal) and the DA + epinephrine + norepinephrine (from the area of the signal) concentrations in the same measurement.
- The analytical capabilities of this system permit its use for pharmacokinetics studies of DA elimination through urine.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.future-science/doi/suppl/10.4155/bio-2017-0161

Financial & competing interests disclosure

This work was supported by the MINECO of Spain (projects CTQ2012-34774 and 2016-76846R); the Gobierno de Aragón (DGA-FEDER) (Research Group E-74): the Gobierno de Aragón and the Fondo Social Europeo (grant J Navarro). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Wang X, Wolfbeis OS. Fiber-optic chemical sensors and biosensors (2013–2015). *Anal. Chem.* 88, 203–227 (2016).
2. Galbán J, Sanz-Vicente I, Ortega E, del Barrio M, de Marcos S. Reagentless fluorescent biosensors based on proteins for continuous monitoring systems. *Anal. Bioanal. Chem.* 402, 3039–3054 (2012).

•• **Represents an update of the different biochemical reagents which have been used for label-free biosensor. The analytical figures of merit and the most interesting applications of continuous monitoring systems based on these type of receptors are also reviewed.**

3. Permyakov EA. *Luminescent Spectroscopy of Proteins.* CRC Press, FL, USA (1993).

• **A very interesting introductory book for studying the intrinsic fluorescence of the enzymes (in the UV zone) and their biochemical applications.**

4. Trettnak W, Wolfbeis OS. A fully reversible fiber optic glucose biosensor based on the intrinsic fluorescence of glucose oxidase. *Anal. Chim. Acta* 221, 195–203 (1989).

• **It is the seminal paper dealing with the utilization of the FAD fluorescence properties of flavoproteins for optical biosensors development.**

5. Galban J, Sanz-Vicente I, Navarro J, de Marcos S. The intrinsic fluorescence of FAD and its application in analytical chemistry: a review. *Methods Appl. Fluoresc.* 4, 042005 (2016).

6. Astuti Y, Topoglidis E, Cass AG, Durrant JR. Direct spectroelectrochemistry of peroxidases immobilised on mesoporous metal oxide electrodes: towards reagentless hydrogen peroxide sensing. *Anal. Chim. Acta* 648, 2–6 (2009).

• **From many papers dealing with the optical properties of the different peroxidase species, this paper undoubtedly relates their electrochemistry and the spectrophotometric properties.**

7. Sanz V, de Marcos S, Galbán J. A reagentless optical biosensor based on the intrinsic absorption properties of peroxidase. *Anal. Chim. Acta* 502, 7–13 (2004).

•• **Details the procedure for the preparation of a label-free platform similar to that used in this paper.**

8. Sanz J, de Marcos S, Galbán J. Autoindicating optical properties of laccase as the base of an optical biosensor film for phenol determination. *Anal. Bioanal. Chem.* 404, 351–359 (2012).

9. Solomon EI, Sundaram UM, Machonkin TE. Multicopper oxidases and oxygenases. *Chem. Rev.* 96, 2563–2605 (1996).

10. Ribeiro JA, Fernandes PMV, Pereira CM, Silva F. Electrochemical sensors and biosensors for determination of catecholamine neurotransmitters: a review. *Talanta* 160, 653–679 (2016).

11. Giardina P, Faraco V, Pezzella C, Piscitelli A, Vanhulle S, Sannia G. Laccases: a never ending story. *Cell. Mol. Life. Sci.* 67, 369–385 (2010).

•• **Compiles important aspects related with the scientific and industrial aspects of the laccase, including information about different families of this enzyme.**

12. Xu F. Recent progress in laccase study: properties, enzymology, production, and applications. In: *Bioprocess Technology: Fermentation, Biocatalysis and Bioseparation.* Flickinger MC, Drew SW (Eds). John Wiley & Sons, NY, USA, 1545–1554 (1999).

13. Schultz W. Multiple functions at different time courses. *Annu. Rev. Neurosci.* 30, 259–288 (2007).

14. Scatron B, Javoy-Agid F, Rouquier L, Dubois B, Agid Y. Reduction of cortical dopamine, noradrenaline, serotonin and their metabolites in Parkinson's disease. *Brain Res.* 275, 321–328 (1983).

15. Yusoff N, Pandikumar A, Ramaraj R, Lim HN, Huang NM. Gold nanoparticle based optical and electrochemical sensing of dopamine. *Microchim. Acta* 182, 2091–2114 (2015).

16. van Staden JF, van Staden RI. Flow-injection analysis systems with different detection devices and other related techniques for the *in vitro* and *in vivo* determination of dopamine as neurotransmitter. A review. *Talanta* 102, 33–43 (2012).

17. Wang HY, Sun Y, Tang B. Study on fluorescence property of dopamine and determination of dopamine by fluorimetry. *Talanta* 57, 899–907 (2002).

18. Chen SM, Peng KT. The electrochemical properties of dopamine, epinephrine, norepinephrine, and their electrocatalytic reactions on cobalt(II) hexacyanoferrate films. *J. Electroanal. Chem.* 547, 179–189 (2003).

19. van Schoorsa J, Viaeneb J, van Wanseelea Y *et al.* An improved microbore UHPLC method with electrochemical detection for the simultaneous determination of low monoamine levels in *in vivo* brain microdialysis samples. *J. Pharm. Biomed. Anal.* 127, 136–146 (2016).

20. de Benedetto GE, Fico D, Pennetta A *et al.* A rapid and simple method for the determination of 3,4-dihydroxyphenylacetic acid, norepinephrine, dopamine and serotonin in mouse brain homogenate by HPLC with fluorimetric detection. *J. Pharm. Biomed. Anal.* 98, 266–270 (2014).

21. Seto D, Maki T, Soh N, Nakano K, Ishimatsu R, Imato T. A simple and selective fluorometric assay for dopamine using calcein blue-$Fe2^{+}$ complex fluorophore. *Talanta* 94, 36–43 (2012).

22. Wang Y, Xiao Y. Glassy carbón electrode modified with poly(dibromofluorescein) for the selective determination of dopamine and uric acid in the presence of ascorbic acid. *Microchim. Acta* 178, 123–130 (2012).

23. Bujduveanu MR, Yao W, Le Goff A *et al.* Multiwalled carbon nanotube-$CaCO_3$ nanoparticle composites for the construction of a tyrosinase-based amperometric dopamine biosensor. *Electroanalysis* 25, 613–619 (2013).

24. Cesarino I, Galesco HV, Moraes FC, Lanza MRV, Machado SAS. Biosensor based on electrocodeposition of carbon nanotubes /polypyrrole/laccase for neurotransmitter detection. *Electroanalysis* 25, 394–400 (2013).

25. Quan D, Shin W. Amperometric detection of catechol and catecholamines by immobilized laccase from DeniLite. *Electroanalysis* 16, 1576–1582 (2004).

26. Ding L, Qin ZW, Xiang CL, Zhou G. Novel fluorescent organic nanoparticles as a label-free biosensor for dopamine in serum. *J. Mat. Chem. B* 5, 2750–2756 (2017).

27. Rasheed P, Lee JS. Recent advances in optical detection of dopamine using nanomaterials. *Microchim. Acta* 184, 1239–1266 (2017).

•• **Represents an update of the state of the art for dopamine determination using optical sensors based on nanomaterials.**

28. Chen XQ, Zheng N, Chen SF, Ma Q. Fluorescence detection of dopamine based on nitrogen-doped graphene quantum dots and visible paper-based test strips. *Anal. Methods* 9, 2246–2251 (2017).

29. Zhao JJ, Zhao LM, Lan CQ, Zhao SL. Graphene quantum dots as effective probes for label-free fluorescence detection of dopamine. *Sens. Actuators B* 223, 246–251 (2016).

30. Zhou X, Ma PP, Wang AQ *et al.* Dopamine fluorescent sensors based on polypyrrole/graphene quantum dots core/shell hybrids. *Biosens. Bioelectron.* 64, 404–410 (2015).

31. Qu KG, Wang JS, Ren JS, Qu XG. Carbon dots prepared by hydrothermal treatment of dopamine as an effective fluorescent sensing platform for the label-free detection of Iron(III) ions and dopamine. *Chem. Eur. J.* 19, 7243–7249 (2013).

32. Wang HB, Zhang HD, Chen Y, Huang KJ, Liu YM. A label-free and ultrasensitive fluorescent sensor for dopamine detection based on double-stranded DNA templated copper nanoparticles. *Sens. Actuators B* 220, 146–153 (2015).

33. Wang P, Xia M, Liang O *et al.* Label-free SERS selective detection of dopamine and serotonin using graphene-Au nanopyramid heterostructure. *Anal. Chem.* 87, 10255–10261 (2015).

34. Luo YH, Ma L, Zhang XH, Liang AH, Jiang ZL. SERS detection of dopamine using label-free acridine red as molecular probe in reduced graphene oxide/silver nanotriangle sol substrate. *Nano. Res. Lett.* 10, 230–234 (2015).

35. Davidson DF. Elevated urinary dopamine in adults and children. *Ann. Clin. Biochem.* 42, 200–207 (2005).

•• **This is a very important reference for understanding how the dopamine concentration levels in urine change after L-dopa ingestion in patients.**

36. Davidson DF, Grosset K, Grosset D. Parkinson's diseade: the effect of L-dopa therapy on urinary free catecholamines and metabolites. *Ann. Clin. Biochem.* 44, 364–368 (2007).

•• **This is a very important reference for understanding how the dopamine concentration levels in urine change after L-dopa ingestion in patients.**

37. Sigma Aldrich. www.sigmaaldrich.com

38. BioRad. www.bio-rad.com

39. Frasconi M, Favero G, Boer H, Koivula A, Mazzei F. Kinetic and biochemical properties of high and low redox potential laccases from fungal and plant origin. *Biochim. Biophys. Acta* 1804, 899–908 (2010).

40. Recipe. ClinChek urine controls information (Biogenic Amines section). www.recipe.de/en/products_qa_chek_cont.html