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A novel support for laccase immobilization: Cellulose acetate modified with ionic liquid and application in biosensor for methyldopa detection

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ABSTRACT

A material based on cellulose acetate (CA) and the room temperature ionic liquid 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide (BMI·N(Tf)$_2$) was developed and characterized by scanning electron microscopy, electron dispersive spectroscopy and infrared analysis. Laccase (Lac) from *Aspergillus oryzae* was immobilized in this material to investigate the behavior of methyldopa by square-wave voltammetry. Under optimized conditions, the Lac biosensor based on CA/BMI·N(Tf)$_2$ exhibited an excellent electrocatalytic performance: the analytical curve showed good linear range for methyldopa concentrations from 34.8 to 370.3 μM with a detection limit of 5.5 μM. This sensor demonstrated acceptable stability (ca. 60 days; at least 350 determinations), good repeatability and reproducibility (relative standard deviations of 1.5 and 4.3%, respectively). The recovery study of methyldopa in pharmaceutical formulations ranged from 94.1 to 105.9%. The determination of this substance using the biosensor compared favorably with that using a spectrophotometry procedure at the 95% confidence level, and indicated potential application to methyldopa determination in pharmaceutical samples.

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1. Introduction

Cellulose is among the most abundant renewable organic resources and is also easily biodegradable, thus representing a relatively low contamination risk to the environment. Cellulose derivatives, such as cellulose nitrate, cellulose acetate (CA), and carboxymethyl cellulose exhibit an excellent biocompatibility and these promising materials are thus suitable for immobilization of biological compounds (Wu et al., 2009). The ideal support for enzymes, for example, should be inert, stable and resistant to mechanical force. Other properties should also be considered: shape, distribution, pore size and expandability. Often the increased stability, selectivity and activity of the enzyme are obtained by combining immobilization techniques and the proper selection of the support. Many studies have been carried out on the use of cellulose matrices for adsorption and covalent bond immobilization (Ikeda et al., 2002; Dalla-Vecchia et al., 2004; Wu et al., 2009; Wan et al., 2010).

The immobilization of enzymes is an indispensable part of the development of biosensors. In this study, the enzyme laccase (Lac) was immobilized on CA modified with the ionic liquid (IL) 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide BMI·N(Tf)$_2$. ILs have been considered as a group of solvents with unique properties, such as negligible vapor pressure, thermal and chemical stability, recyclability, and nonflammability. They have also attracted considerable attention among scientists because they are versatile cations, anions, or combinations of the two, which make their properties designable according to different requirements (Xing et al., 2010).

Currently in polymer science, ILs are not only used as the media for the polymerization process but also in the preparation of functional materials. These materials can be prepared by the covalent attachment of an IL to the support surface, which is usually silica or a polymer with the main characteristic being its porous structure (Xing et al., 2010). Moreover, the unique structure of the modified material may have other additional advantages such as the immobilization of more active forms of the species with the establishment of a porous contact region between the phases within the structure (Xu et al., 2005).

Lac (EC 1.10.3.1, *p*-benzenediol: oxygen oxidoreductase) is one of the most important enzymes in terms of applicability and versatility in industry. It belongs to a family of multicopper oxidases that catalyze the oxidation of a range of inorganic and aromatic compounds (principally phenols) with the concomitant reduction of molecular oxygen to water (Mayer and Staples, 2002; Durán et al., 2002). Several applications of Lac have been reported, such as in the food, textile, pulp and paper, and cosmetics industries, as well as in the areas of nanobiotechnology, synthetic chemistry and the construction of biosensors, in particular for the determination

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of several phenolic compounds (Couto and Herrera, 2006; Mousty et al., 2007; Fernandes et al., 2008; Franzoi et al., 2009a,b, 2010).

Methyldopa (2-amino-3-(3,4-dihydroxyphenyl)-2-methyl-propanoic acid) is a catechol derivative (catecholamine) widely used as an antihypertensive agent. It can be converted to 1-methyl dopamine and 1-methyl norepinephrine (Talebpour et al., 2004). It is a centrally acting α-2-adrenoceptor agonist, which reduces the sympathetic tone of muscles and produces a fall in blood pressure. This catecholamine contains a chiral centre and can therefore occur either as an S- or R-isomer, and has antihypertensive activity due to the S-isomer (Gadkariem et al., 2009).

Catecholamines are present in relatively large amounts in pharmaceutical formulations and much effort has been devoted to the development of simple, rapid, accurate and precise analytical procedures. Several analytical methods have been reported for the quantitative determination of methyldopa. These methods include titrimetry (USP, 2000; Amin, 1986), fluorimetry (Salem, 1993), kinetics measurements (Martinez-Lozano et al., 1991; Matthieu et al., 2006), gas chromatography (Sharma et al., 1996), high-performance liquid chromatography (Benza et al., 1996; Muzzi et al., 2008), spectrophotometry (Vieira and Fatibello-Filho, 1998; Madrakian et al., 2006) and reflectance spectroscopy (Ribeiro et al., 2006). Some of these methods are not simple and others are time consuming or involve procedures with rigorous control of the experimental conditions or suffer interference from the tablet matrix and consequently are not suitable for routine analysis. Biosensors offer an alternative means to determine methyldopa and have the advantage of low cost, high sensitivity, easy operation and the possibility for the construction of simple portable devices for fast screening purposes (Badawy et al., 1996; Mohammadi et al., 2008; Tortolini et al., 2010).

In this paper, a novel polymeric support, based on CA and BMI·N(Tf_2) IL, was used for Lac immobilization and was incorporated into a carbon paste electrode (CPE). Herein, the synergistic effect of the CA/BMI·N$(Tf)_2$ combination and Lac enzyme was used to improve the electrochemical performance of the biosensor. Scanning electron microscopy (SEM) and electron dispersive spectroscopy (EDS) analysis were used to characterize the support with BMI·N$(Tf)_2$. Infrared (IR) analysis was carried out to compare the pure CA and CA/BMI·N$(Tf)_2$. The effects of the various operational parameters on the sensor response were investigated in detail to obtain the best performance of the proposed sensor, which was then applied to methyldopa determination in pharmaceutical samples by square-wave voltammetry (SWV).

2. Experimental

2.1. Chemicals and solutions

The halide-free IL BMI·N$(Tf)_2$ was prepared according to a known procedure and dried over molecular sieves (4 Å) (Cassol et al., 2006). CA (Sigma–Aldrich, 39.8 wt% of acetylation content) and acetone (Merck, 99.8%) were used to prepare the support, according to the method described in the literature (Gelesky et al., 2009).

Lac from *Aspergillus oryzae* was purchased from Novozymes (Denilite® II BASE). The carbon paste was prepared using graphite powder (Acheson 38, Fisher Scientific) and high purity Nujol purchased from Sigma–Aldrich. Methyldopa, citric acid, sodium chloride, sucrose, glucose, fructose, lactose, magnesium stearate and starch and 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) were obtained from Sigma–Aldrich. A stock solution of methyldopa (0.2 mM) was prepared daily in acetate buffer solution (0.1 M, pH 5.5) with the aid of ultrasonic agitation and this buffer was used as the supporting electrolyte throughout the experiments. Reagents were of analytical grade and used as received. All solutions were prepared with ultrapure water (18.2 MΩ cm) obtained from a Milli-Q purification system.

2.2. Preparation of the support CA/BMI·N$(Tf)_2$

CA (10 g) was added to a reaction flask containing 90 mL of acetone, and the mixture was allowed to sit for 24 h at room temperature under a dry nitrogen atmosphere. After a viscous syrup was formed, 1.0 g of BMI·N$(Tf)_2$ was added to 5.0 g of the syrup. The mixtures were magnetically stirred until a homogeneous phase was obtained. The support was prepared by spreading the homogeneous phase over a glass plate, calcined at 300 °C and triturated to obtain the powder.

2.3. Characterization of CA/BMI·N$(Tf)_2$: scanning electron microscopy (SEM), electron dispersive spectroscopy (EDS) and infrared (IR) analysis

The morphology of the polymeric supports CA and CA/BMI·N$(Tf)_2$ was observed through SEM (Philips, model XL30 microscope) with an accelerating voltage of 15 kV and EDS elemental analysis was performed using a JEOL model JSM 5800 microscope with 10 and 20 kV and magnifications of 100× and 500×. The IR analysis of the materials was carried out using a Shimadzu FTIR spectrometer, model 8300. The spectra were obtained at room temperature with a resolution of 4 cm^{-1} and 100 cumulative scans.

2.4. Measurement of Lac activity and its immobilization

Lac activity was calculated as the quantity of enzyme which oxidizes 1.0 μmol of substrate per minute at 420 nm. The enzymatic activity was determined in triplicate by measuring of the absorbance at 420 nm of the product formed from the reaction between 2.8 mL of 0.5 mM ABTS solution and 0.2 mL of Lac in 0.1 M acetate buffer (pH 5.0) at 25 °C.

Aliquots of the homogenate containing 0.3–0.9 U mL^{-1} of Lac were added to 20 mg of the CA/BMI·N$(Tf)_2$ support. The resulting material was dried at room temperature and used for construction of the biosensor.

2.5. Biosensor construction

CA/BMI·N$(Tf)_2$ (20 mg) containing 0.5 U mL^{-1} of immobilized Lac (11%, w/w) was hand mixed with 100 mg of graphite powder (56%, w/w) in a mortar for 20 min to ensure a uniform mixture. Subsequently, 60 mg of Nujol (33%, w/w) were added and mixed for at least 20 min to form a homogeneous final paste. The resulting modified carbon paste was tightly packed into a plastic syringe (1.0 mm internal diameter) and a copper wire was inserted to obtain the external electrical contact. The biosensors with unmodified CA and free Lac were prepared in a similar way.

2.6. Instrumentation, and electrochemical and UV measurements

Electrochemical measurements (SWV) were performed using an Autolab PGSTAT12 potentiostat/galvanostat (Eco Chemie, The Netherlands) connected to data processing software (GPES, software version 4.9.006, Eco Chemie). A conventional three-electrode system was used with a biosensor as the working electrode, a platinum wire as the counter electrode and Ag/AgCl (3.0 M KCl) as the reference electrode.

SWV measurements were carried out in an unstirred, non deaerated acetate buffer solution (0.1 M pH 5.5) at room temperature (25.0 ± 0.5 °C) and all potentials were measured and reported *vs.*

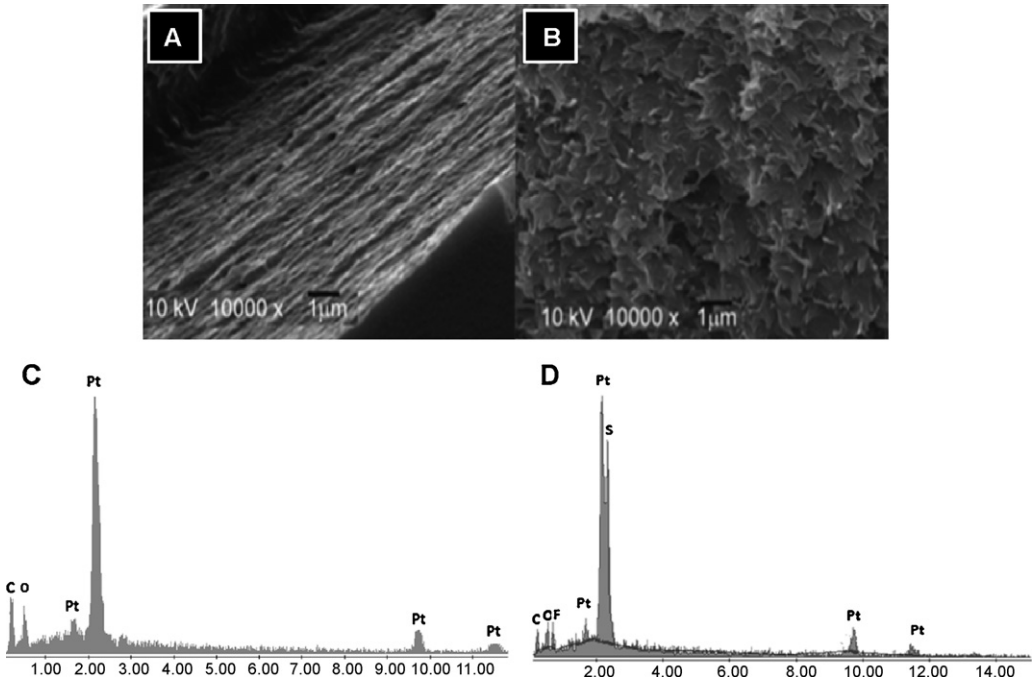

Fig. 1. SEM micrographs of the (A) pure CA and (B) CA/BMI·N(Tf)$_2$ and EDS spectra of the (C) pure CA and (D) CA/BMI·N(Tf)$_2$ polymeric materials.

Ag/AgCl. In a typical run, 10 mL of the supporting electrolyte was transferred to a clean, dry cell and the required volumes of the methyldopa and a pharmaceutical sample were added by micropipette. The SWV measurements were performed applying a sweep potential of between +0.6 and −0.5 V, at a frequency of 10–120 Hz, pulse amplitude of 10–100 mV and scan increment of 0.5–10.0 mV, after successive additions of the analyte. After a stirring period of 60 s to homogenize the solution, square-wave voltammograms were recorded.

A FEMTO UV–vis spectrophotometer, model 800XI, with a quartz cell (optical path of 1.0 cm) was used for the determination of methyldopa by comparative method. The concentration was determined by recording the absorbance and comparing the results with the calibration graph.

2.7. Preparation and analysis of samples

Two samples of pharmaceuticals containing methyldopa (I – Medley and II – EMS) were acquired from a local drugstore (Florianópolis, Santa Catarina, Brazil) and analyzed using the proposed biosensor. One tablet of each sample was dissolved in 100 mL of 0.1 M hydrochloric acid. Aliquots of pharmaceutical samples were transferred to the cell and quantified using SWV, after successive additions of methyldopa standard solution. All measurements were performed in triplicate. A spectrophotometric method for methyldopa determination available in European Pharmacopoeia (EP, 2007) was used for the comparison of the analytical results with those obtained using the proposed biosensor.

3. Results and discussion

3.1. Structure characterization of CA/BMI·N(Tf)$_2$

The combination of IL with cellulose derivatives is an alternative that can generate new and versatile catalytic materials for efficient enzyme immobilization. The supports CA and CA/BMI·N(Tf)$_2$ were characterized based on the SEM, EDS and IR analysis.

The SEM images obtained for the pure CA and CA/BMI·N(Tf)$_2$ are shown in Fig. 1A and B, respectively. It can be clearly observed that the morphological structure of the materials changes with or without the presence of IL. In particular, the CA (Fig. 1A) seems to have a scaled and non-homogeneous structure. In contrast, the addition of the IL (Fig. 1B) seems to cause an increase in the micro-structural order and a greater homogeneity was observed for CA/BMI·N(Tf)$_2$. This result is in agreement with the general concept that imidazolium ILs tend to act as entropic drivers for the formation of nanostructured materials. The surface area of the pure cellulose acetate film containing 0.5 g of BMI·N(Tf)$_2$ was 38 m^2/g (±10%) and that of the film with 1.0 g was 24 m^2/g (±10%), demonstrating a reduction in the superficial area when the amount of IL was doubled. This result suggests that BMI·N(Tf)$_2$ produces an occupation of the free porous arrangement in the polymeric material and the introduction of BMI·N(Tf)$_2$ IL in the CA gel probably causes an increase in the separation between the cellulose macromolecules that results in a higher flexibility, lower viscosity and better formability of the polymeric material (Cassol et al., 2006; Gelesky et al., 2009).

The EDS spectra of the CA and CA/BMI·N(Tf)$_2$ are shown in Fig. 1C and D. EDS analysis of the polymeric support CA/BMI·N(Tf)$_2$ (Fig. 1D) showed S and F signals, indicating the presence of the BMI·N(Tf)$_2$ in the powder obtained.

The IR spectra of the pure CA and CA/BMI·N(Tf)$_2$ are shown in Fig. 2. The presence of the BMI·N(Tf)$_2$ IL in the support was confirmed by the stretching band at 3170 cm^{-1} which is due to the presence of aromatic C–H groups. The addition of the BMI·N(Tf)$_2$ IL in the polymeric material demonstrated significant decrease in the intensity of the band at 3400 cm^{-1}, indicating participation of the –OH group in the interaction electrostatic with the anion of the BMI·N(Tf)$_2$ IL. Also it is possible that the bis(trifluoromethane sulfonyl)imide anion of the IL strongly interacts with the hydrogen bond networks formed in the cellulose acetate chains through the nitrogen atom (Zhu et al., 2006). These results are corroborated by the infrared spectra of the cellulose acetate membrane dispersed in IL BMI·N(Tf)$_2$.

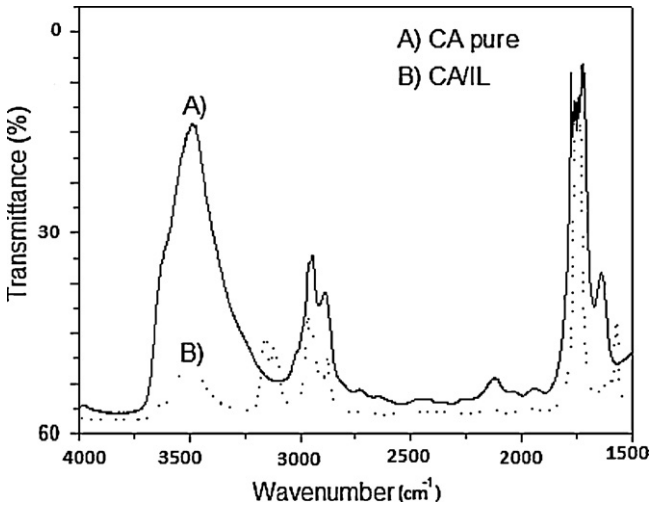

Fig. 2. Infrared spectra of the (A) pure CA and (B) CA/BMI·N(Tf)$_2$.

3.2. Lac immobilization and enzymatic reaction

A key factor in the construction of a biosensor is the need to achieve adequate and effective enzyme immobilization. The Lac enzyme has been successfully immobilized in a variety of supports, including chitosan and cyclodextin, using several methods (Fernandes et al., 2008; Franzoi et al., 2009b, 2010). In this study, a new material with excellent properties composed of CA and BMI·N(Tf)$_2$ IL was developed and used as a support for Lac immobilization.

The plasticizer effect of IL reduces the intermolecular forces that are usually present in the CA. This allows the $N(Tf)_2^-$ anion of the IL to strongly interact with the hydrogen bond networks formed in the CA chains through the nitrogen atom. Therefore, the introduction of the IL probably causes an increase in the distance between the cellulose macromolecules which results in greater flexibility, lower viscosity, and better formability of the cellulose material (Gelesky et al., 2009). The Lac and CA/BMI·N(Tf)$_2$ combination exhibits an excellent synergistic effect that enhances the activity and stability of the biosensor when used for methyldopa determination.

Thus, the Lac enzyme is entrapped within the interstitial space of the CA/BMI·N(Tf)$_2$ and this system tries to mimic its natural environment which can result in considerable stabilization of the enzyme structure, hence increasing its activity. In addition, ILs may be responsible for the stabilization of the supported enzyme, which offers a favorable microenvironment for the enzymatic reaction, improving the biosensor performance (Yang and Pan, 2005). Fig. 3A shows the construction steps of the Lac biosensor based on CA/BMI·N(Tf)$_2$, used for methyldopa determination.

Lac biosensors have been employed for the determination of a broad range of polyphenols (Fernandes et al., 2008; Franzoi et al., 2009a,b, 2010). Here, Lac catalyzed the oxidation of methyldopa to quinone and this product was electrochemically reduced to methyldopa on the biosensor surface at a potential of +0.07 V *vs.* Ag/AgCl, as illustrated in Fig. 3B.

3.3. Electrochemical response of Lac biosensors to methyldopa

SEM, EDS and IR analysis confirmed the presence of BMI·N(Tf)$_2$ within the CA polymeric matrix. To show the importance of the Lac enzyme adsorbed on the CA/BMI·N(Tf)$_2$ support, the electrochemical behavior of methyldopa using different sensors was investigated by SWV in the potential range of +0.6 and −0.5 V *vs.* Ag/AgCl. Fig. 4 shows the voltammograms obtained using the (a) bare CPE, (b) biosensor (free Lac), (c) biosensor//CA and (d) biosensor//CA/BMI·N(Tf)$_2$ in 0.2 mM methyldopa in 0.1 M acetate buffer solution (pH 5.5), with optimized parameters. The cathodic current of *o*-quinone reduction to methyldopa was used as the analytical response (cathodic current values are shown in the inset of Fig. 4).

The Lac enzyme can catalyze the oxidation reaction of methyldopa and thus its incorporation in the electrode (curve b) causes

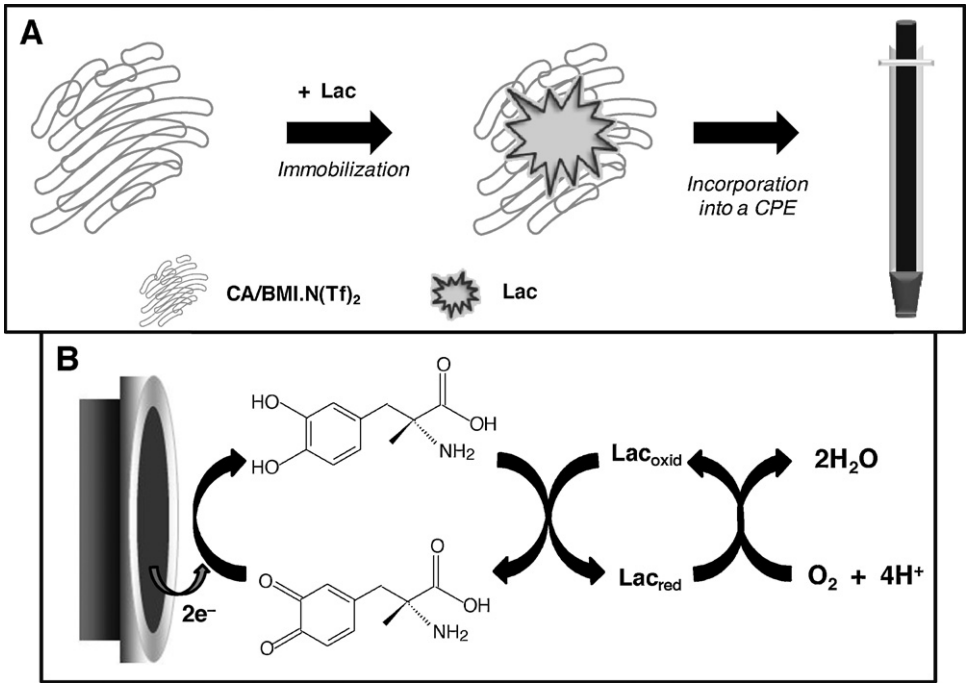

Fig. 3. (A) Schematic representation of construction of Lac biosensor based on CA/BMI·N(Tf)$_2$ and (B) Lac-catalyzed oxidation of methyldopa with its subsequent electrochemical reduction on the biosensor surface.

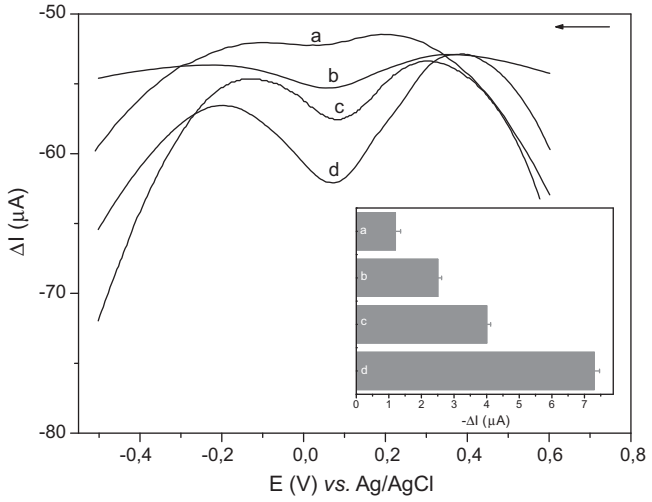

Fig. 4. Square-wave voltammograms obtained using (a) bare CPE, (b) biosensor (free Lac), (c) biosensor//CA and (d) biosensor//CA/BMI·N(Tf)$_2$ in 0.2 mM methyldopa in 0.1 M acetate buffer solution (pH 5.5); frequency 90 Hz, pulse amplitude 100 mV and scan increment 4.0 mV. Inset: cathodic peak current values for bare CPE and different biosensors.

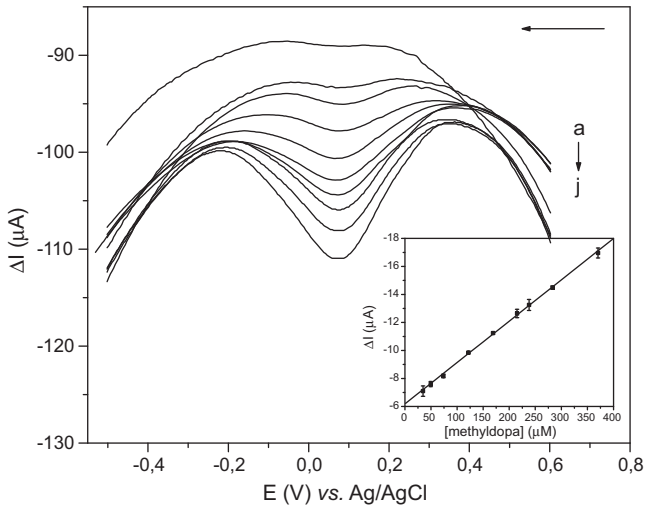

Fig. 5. Square-wave voltammograms obtained using Lac biosensor based on CA/BMI·N(Tf)$_2$ for (a) blank in acetate buffer solution (0.1 M; pH 5.5) and methyldopa solutions at the following concentrations: (b) 34.8; (c) 49.5; (d) 73.9; (e) 121.9; (f) 169.1; (g) 215.0; (h) 238.0; (i) 283.0 and (j) 370.3 μM; frequency 90 Hz, pulse amplitude 100 mV and scan increment 4.0 mV. Inset: calibration curves for methyldopa.

an increase in the cathodic current when compared with that obtained for the bare CPE (curve a). The incorporation of CA in the biosensor (curve c) increased the current value of *o*-quinone reduction to methyldopa, since CA offers a biocompatible environment for the enzyme. The greater response observed for the biosensor//CA/BMI.N(Tf)$_2$ (curve d) is attributed to the excellent immobilization process, resulting in a more sensitive electrode. The novel material based on CA and BMI.N(Tf)$_2$, which combined the biocompatibility of the former and the conductivity and enzymatic stabilization power of the latter, presents superior properties over unmodified CA. In addition, the comparison of the response obtained with biosensor//CA/BMI.N(Tf)$_2$ and biosensor//CA showed the formation of a matrix suitable for Lac immobilization.

3.4. Optimization of the biosensor construction and analytical conditions

To optimize the performance of the Lac biosensor based on CA/BMI·N(Tf)$_2$ for the determination of methyldopa, some parameters, such as Lac concentration, supporting electrolyte and pH, along with the frequency, pulse amplitude and scan increment used in the SWV, were investigated.

The effect of different Lac unit values (0.3–0.9 U mL^{-1}) on the biosensor response was evaluated. The analytical signals (cathodic peak currents) for 0.2 mM methyldopa in 0.1 M acetate buffer solution at pH 5.5 increased with the enzyme concentration up to 0.5 U mL^{-1}. Consequently, this enzyme concentration was selected for further studies.

The influence of different 0.1 M supporting electrolytes and pH values, including acetate buffer solution (pH 4.0–5.5) and phosphate buffer solution (pH 6.0–8.0), were investigated. The best voltammetric responses for methyldopa were obtained in acetate buffer solution at pH 5.5.

The SWV parameters were optimized using the best response of the biosensor to 0.2 mM methyldopa solutions. Frequency values in the range of 10–120 Hz, pulse amplitudes between 10 and 100 mV and scan increments from 0.5 to 10.0 mV were evaluated. The best resultant peak currents were obtained using values for frequency, pulse amplitude and scan increment of 90 Hz, 100 mV and 4.0 mV, respectively.

3.5. Analytical performance of biosensor in methyldopa determination

Under the optimal working conditions the analytical curves were obtained with the biosensor in the potential range of +0.6 to −0.5 V *vs.* Ag/AgCl. The electrochemical reduction to methyldopa was obtained at a potential of +0.07 V. Fig. 5 shows the square-wave voltammograms and analytical curve constructed from cathodic current peak *vs.* methyldopa concentration, obtained employing the proposed Lac biosensor based on CA/BMI·N(Tf)$_2$. As can be observed, the cathodic current increased with methyldopa concentration and the analytical curve was linear from 34.8 to 370.3 μM ($\Delta I = 0.262\ (\pm 0.041) - 3.486 \times 10^4\ (\pm 2.028 \times 10^2)$ [methyldopa]; $r^2 = 0.9998$); where ΔI is the cathodic peak current in μA and [methyldopa] is the concentration in μM. The detection limit (three times the signal blank/slope) using the biosensor was 5.5 μM. The satisfactory analytical performance of the proposed biosensor can be attributed to the efficiency of the immobilization of the Lac in the polymeric material – CA/BMI·N(Tf)$_2$ and the high conductivity of the ionic liquid facilitating electron transfer on the biosensor surface.

3.6. Repeatability, reproducibility and stability of the biosensor

The repeatability of the current response for the proposed biosensor in a solution containing 0.2 mM of methyldopa solution in acetate buffer solution (0.1 M; pH 5.5) was investigated. The relative standard deviation (RSD) was 1.5% for successive assays ($n = 6$).

Another important parameter that needs to be considered is the reproducibility of the biosensor which was investigated by detecting 0.2 mM methyldopa. Five biosensors were constructed using the same procedure and were independently used for detection of methyldopa under the optimized conditions described previously. The RSD was 4.3%, demonstrating that the biosensor has a good reproducibility.

Additional experiments were carried out to test the stability over a period of 60 days (every 5 days) at room temperature, in triplicate and using three concentrations of methyldopa. The stability of the Lac biosensor based on CA/BMI·N(Tf)$_2$ was measured by the cathodic current response and 90% of its initial response to

Table 1
Methyldopa determination in pharmaceutical formulations using the spectrophotometric method and the biosensor.

Sample[a]	Label value	Comparative method	Biosensor	R_{e1} (%)	R_{e2} (%)
I	278.4	281.4 ± 0.1	276.2 ± 0.2	−0.8	−1.8
II	278.4	279.6 ± 0.1	275.1 ± 0.3	−1.2	−1.6

R_{e1} = biosensor *vs.* label value; R_{e2} = biosensor *vs.* comparative method.
[a] mg tablet^{-1}; $n = 3$; confidence level of 95%.

methyldopa was maintained within the investigated period. Thus, the Lac activity was very efficiently retained in the novel polymeric material – CA/BMI·N(Tf)$_2$.

3.7. Interference and recovery tests and methyldopa analysis in pharmaceutical samples

The anti-interference ability is an important characteristic of sensors and describes the selectivity of the electrode toward the target compound in the presence of potentially interfering compounds. The effect of excipients commonly found together with methyldopa in pharmaceutical samples, such as citric acid, sodium chloride, sucrose, glucose, fructose, lactose, magnesium stearate and starch, were investigated and was considerate as interference when the current signal presents variation of ±5%. The ratios of methyldopa concentration to the excipients were fixed at 0.1 and 1.0 and the response of the biosensor in the presence of these substances was compared with that obtained using only methyldopa. The influence of each species tested under studied conditions was lower than 5%, indicating no interference in the methyldopa response obtained with the proposed biosensor.

Analytical recovery was investigated by the standard addition method, in triplicate, using two pharmaceutical samples (I and II). Different amounts of methyldopa (11.8, 23.4 and 34.7 mg L^{-1}) were added to the samples and the percentage recovery values were calculated by comparing the concentrations detected in the samples with and without the addition of known concentrations of the methyldopa standard solution. Methyldopa recoveries of 94.1–105.9% from these samples demonstrate the satisfactory accuracy of the proposed biosensor. It can be clearly observed that the recovery results obtained indicate an absence of matrix effects in these determinations.

To investigate the practical application of the new biosensor system, some pharmaceutical samples were examined using the proposed biosensor applying the standard additions method and the results were compared with those obtained by comparative method and label values provided by the manufacturers. As shown in Table 1, the relative error between the two methods and the label values was from −1.8 to ⁻0.8%, suggesting an acceptable agreement (95% confidence level). Thus, the proposed method for the direct determination of methyldopa concentration in pharmaceutical samples is highly feasible.

4. Conclusions

In this study, a novel polymeric material based on CA and BMI.N(Tf)$_2$ was successfully prepared for incorporation in a biosensor. This material was able to immobilize Lac, leading to highly efficient and robust biocatalysts. Under optimized conditions, the biosensor shows a wide linear range of 34.8–370.3 μM and a detection limit of 5.5 μM. It also offers good anti-interference ability, stability and reproducibility. Moreover, this sensor showed an acceptable level of accuracy for methyldopa determination in pharmaceutical samples.

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