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Conjugated polymers nanostructured as smart interfaces for controlling the catalytic properties of enzymes

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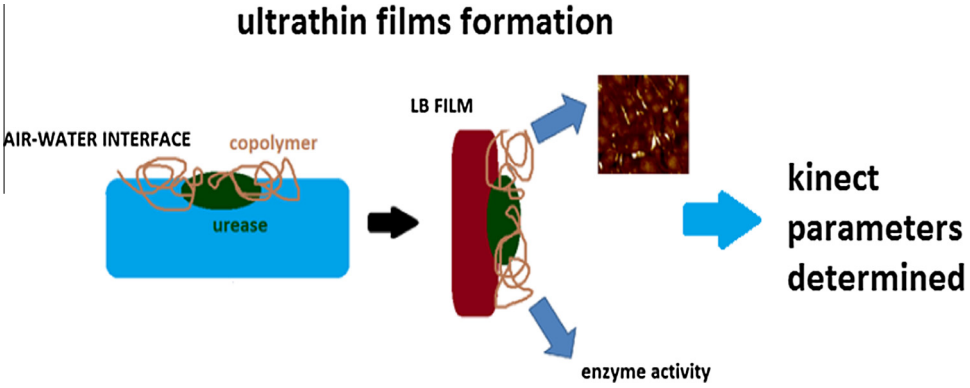
GRAPHICAL ABSTRACT



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ABSTRACT

The search for new molecular architectures to improve the efficiency of enzymes entrapped in ultrathin films is useful to enhance the effectiveness of biosensors. In this present work, conjugated polymers, based on thiophene and fluorine, were investigated to verify their suitability as matrices for the immobilization of urease. The copolymer poly[(9,9-dioctylfluorene)-*co*-thiophene], PDOF-*co*-Th was spread on the air-water interface forming stable Langmuir monolayers as determined by surface pressure-area isotherms, polarization-modulation reflection-absorption infrared spectroscopy (PM-IRRAS), and Brewster angle microscopy (BAM). Urease was incorporated in the floating monolayers being further transferred to solid supports as mixed Langmuir-Blodgett (LB) films. These films were then characterized with transfer ratio, fluorescence spectroscopy, PM-IRRAS and atomic force microscopy, confirming the co-transfer of the enzyme as well as its structuring in β-sheets. The catalytic activity was detected for urease, with a lower reaction rate than that encountered for the homogeneous environment. This was attributed to conformational constraints imposed to the biomacromolecule entrapped in the polymeric matrix.

1. Introduction

Conjugated polymers (CPs) are widely used in the field of biosensors because of their electric, electronic, magnetic and optical properties [1,2]. The immobilization of biomolecules in CPs matrices has been extensively explored in order to obtain an effective contact between these two elements, maintaining the activity and stability of the molecules, as well as ensuring the identification of the analyte of interest [3].

Polythiophenes are a class of conjugated polymers that have been exploited for the manufacture of biosensors because of their

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good solubility, processability, electrochemical stability, and the possibility to assembly new structures to adjust their physical properties [4,5]. However, polythiophenes have low photoluminescence quantum efficiency [6]. The combination of thiophene and fluorene groups, which have a high quantum yield, enables the formation of stable copolymers whose optical and electrical properties are useful for the application in optoelectronic devices and sensors [7–9].

The structuring of these polymers in nanostructured films is a suitable strategy to control the molecular architecture allowing for easy manipulation of the device properties. Among the kinds of procedures to form ultrathin films, Langmuir-Blodgett (LB) films not only allow for the formation of structures with control over the thickness and molecular architecture, but also serve to immobilize enzymes for the production of biosensors. For that, the enhancement of the activity properties of the immobilized biomolecule is desirable [10–12].

Some studies report the immobilization of the enzyme urease in LB films [13–17] and in conjugated polymer matrices [18–22]. Park et al. [18] developed a voltammetric biosensor immobilizing urease in poly(3-methylthiophene)-coated platinum electrodes by the chronoamperometric method. Other studies report the electrochemical immobilization of urease onto the PANi-Nafion/Au/ceramic plate composite film by the casting method, obtaining a sensor with high sensitivity and selectivity [19]. Furthermore, it is reported the covalent immobilization of this enzyme in a pyrrole copolymer film, producing a biosensor with a linear response to urea [20]. Vieira et al. developed a biosensor based on field-effect by means of films of polyaniline/urease constructed through the layer-by-layer technique [21]. Films of polypyrrole and urease were also fabricated by electropolymerization, and the effect of different dopant anions could be investigated [22].

In these works, it is clear that not only the structure of conjugated polymers may enhance the electrical and optical signals given by the enzymatic reaction, but also that these polymers serve as matrices for the immobilization of enzymes. The function and catalytic activity of the enzyme may depend on its conformation, which may be influenced by its interaction with the polymeric matrix. The structure of the matrices reported in these papers presents different levels of molecular organization, but none of them presents urease immobilized in structures layered as LB films.

The present work aims to study if a copolymer of thiophene and fluorene immobilized in solid supports as LB films is appropriate as matrix for the incorporation of urease. The relative enzyme activity and the catalytic constants were determined, proving the feasibility of such assemblies for the construction of biosensors for urea in a proof-of-concept experiment.

2. Materials and methods

2.1. Copolymer synthesis

The copolymer poly[(9,9-dioctylfluorene)-*co*-thiophene], denominated here as PDOF-*co*-Th, was synthesized according to the literature [23,24], using the Suzuki reaction method, as seen in Scheme 1. In a reflux system without oxygen, 2.62 mmol of 9,9-dioctylfluorene-2,7-diboronic acid (Aldrich, 96%), and 2.12 mmol of 2,5-dibromothiophene (Aldrich, 95%) were added, and 0.013 mmol of tetrakis-(triphenylphosphine) palladium (P(Ph3)4Pd) (Aldrich, 99%) was employed as catalyst. Also, 120 mL of xylene, and 10 mL of 2 mol/L of potassium carbonate were added. This solution was stirred for 72 h at 100 °C under nitrogen flow (step 1). Thereafter, 0.5 mmol of 2-bromothiophene (Aldrich, 98%) was added and then kept under reflux for more 24 h (step 2). After this period, 20 mL of hydrogen peroxide were slowly added with the mixture kept at room temperature. The polymer was extracted with toluene, and recrystallized with methanol (yield 97%). Purity was near 100% as characterized with ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.64, 7.61, 7.55, 7.35, 7.28, 7.19, 7.05, 2.55, 2.25, 2.00, 1.51, 1.18, 1.03, 0.73. ^{13}C NMR (400 MHz, $CDCl_3$, δ, ppm): 124.64, 123.92, 120.15, 119.82, 40.49, 31.81, 30.04, 29.23, 23.78, 22.61, 14.09. ^{13}C NMR (400 MHz, solid state) δ: 152.9, 145.3, 142.1, 134.6, 125.4, 122.8, 56.9, 42.8, 31.6, 24.6 and 15.82.

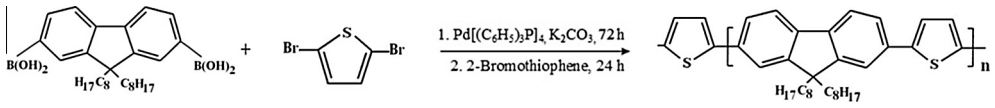
2.2. Characterization of PDOF-co-Th

UV–vis absorption spectra were collected by using a Shimadzu 1501 spectrophotometer. The measurements were performed in a solution using chloroform as solvent into 10 mm square quartz cells, in the λ = 200–800 nm range. The emission spectra (fluorescence) of the solutions in chloroform solution were obtained by using a Shimadzu 5301PC spectrophotometer with a slit width of 5 mm. The wavelengths of the maximum absorption according to the UV–vis spectra were used as excitation wavelengths.

2.3. Langmuir films

Ultrapure water was supplied by a Millipore system (resistivity of 18.2 MΩ cm, pH of 5.5–6.0, and surface tension of 72.5 mN/m at 25 °C) and employed as the subphase for the monolayers. PDOF-*co*-Th was dissolved in chloroform to yield a concentration of 20 mg/mL. Urease was dissolved in phosphate buffer solution (pH 7.5) to yield a concentration of 0.5 mg/mL. Aliquots of 25 μL of PDOF-*co*-Th were spread carefully, drop by drop, on the surface of the aqueous subphase. Aliquots of 25 μL of urease were inserted in the aqueous subphase close to the air-water interface and at least 30 min was allowed for enzyme adsorption and lateral diffusion. Longer waiting times were also employed and no significant difference was observed for the isotherms. The amount of polymer or enzyme incorporated at the air-water interface was low enough to initialize the curve in surface pressures close to zero. For the mixed monolayer, the surface pressure stabilized at 1.3 mN/m and then the interface was compressed.

The compression of the air-water interface was carried out with two movable barriers at a rate of 25 cm^2/min, using a mini-model Langmuir trough (KSV instruments). The surface pressure was measured during the compression using a Wilhelmy plate. All monolayers were produced at a constant temperature of 25 ± 1 °C. The Langmuir monolayers were characterized using surface pressure-area isotherms, Brewster angle microscopy (micro-BAM, KSV Instruments) and polarization-modulation infrared reflection absorption spectroscopy (PM-IRRAS, KSV Instruments). BAM presented a resolution of 12 μm, and a light source power of 50 mW and a wavelength of 659 nm. The incident beam was p-polarized and reached the air-water interface at a fixed angle



Scheme 1. Synthesis of poly[(9,9-dioctylfluorene)-*co*-thiophene] (PDOF-*co*-Th).

of 53°. The image processing used a USB camera providing 640 × 480 pixels, 30 fps, adjustable exposure time and gain. PM-IRRAS used a fixed incident angle of 85 °C, and modulation for the p and s fractions of the electric component of the light. BAM and PM-IRRAS data were obtained when the monolayer was allowed to relax after attained a given surface pressure.

Each data regarding isotherms, PM-IRRAS spectra or images obtained by BAM was repeated at least three times in three independent measurements. Data show representative results assuring the reproducibility of the measurements.

2.4. Langmuir–Blodgett (LB) films

Glass and quartz slides were used as solid supports for the assembly of LB films and were cleaned with a solution 5% in mass of KOH in ethanol in an ultrasonic bath for 5 min. The transfer of the polymeric films from the air–water interface to the solid supports was performed with a 5 mm/min dipping rate and a constant surface pressure of 30 mN/m; the first layer was obtained by lifting the solid support from the aqueous subphase. This value of surface pressure was chosen because it provides compacted films permitting the transfer to solid substrates through the LB technique. For multilayer LB films, an interval of 20 min was elapsed before the subsequent dipping with the plate at the most upward position for drying.

The LB films were characterized firstly by transfer ratio, and then by UV–vis (Shimadzu 1501 spectrophotometer), fluorescence (Shimadzu RF-301PC), and PM-IRRAS (KSV Instruments) spectroscopies. All LB films were assembled at a constant temperature of 25 ± 1 °C. Atomic Force Microscopy (AFM) was also employed for further characterization, and the images were obtained in the tapping mode, employing a resonance frequency of approximately 300 kHz, a scan rate of 1.0 Hz, and scanned areas of 5.0 × 5.0 μm. For these measurements, a Digital AFM-Nanoscope IIIA instrument and a tip made of silicon were employed. The mass of the deposited material on the solid substrate was determined according to the Sauerbrey equation [25], using a quartz crystal microbalance (SRS – Stanford Research Systems model QCM200).

2.5. Enzymatic catalysis and Michaelis-Menten constant (Km)

In order to check the catalytic activity of urease in the presence of the copolymer, when both are transferred to solid supports, films prepared with 5, 7 and 9 layers were immersed in an indicator solution containing urea (25 mmol/L, bromocresol purple (0.015 mmol/L), EDTA (0.2 mmol/L) in a cuvette with total volume of 2 mL. All the components of this solution were purchased from Sigma-Aldrich. The pH of the solution was adjusted to 5.8 with NaOH (Sigma-Aldrich). Urease catalyzes the hydrolysis of urea, forming ammonia. The consequent increase in the pH values changes the color of the solution due to bromocresol purple. The absorption at 588 nm was monitored using a UV–vis spectrophotomer.

Aiming at determining the Michaelis-Mentem constant (K_m), 5 LB films with 9 layers composed of copolymer and enzyme were prepared and each film was immersed in the pH indicator solution described above with growing concentrations of urea (20, 25, 35, 50 and 100 mmol/L). The catalytic activity of urease was estimated by measuring the absorption of the solution at 588 nm. By means of the calibration curve for ammonium, the initial velocity of the reaction for each concentration was determined and plotted in function of the concentration of urea. Using the Lineweaver and Burk double reciprocal plot, the value of K_m was calculated. The same procedure was performed to measure the values of K_m of the free enzyme in solution, using the following substrate concentrations: 0.1, 0.15, 0.25, 0.4, and 0.5 mmol/L. Each value of activity is an average of at least 3 measurements with 3 different films from 3 independent experiments with standard deviation always lower than 5%. Nanogravimmetry by means of a quartz crystal microbalance (SRS – Stanford Research Systems model QCM200) was employed to estimate the mass of film deposited on a surface bounded by gold electrodes in the thin disk of quartz as substrate. The mass of the deposited film was determined according to the Sauerbrey equation [25]. Each value of mass is an average of at least 3 measurements with 3 different films from 3 independent experiments with standard deviation always lower than 5%.

3. Results and discussion

3.1. Langmuir monolayers

Fig. 1 shows the surface pressure–area isotherms for the copolymer and urease spread alone at the interface, as well as their mixture. For the copolymer, the isotherm shows that the surface pressure lifts off at the area of 200 Å^2/polymer unity. With subsequent compression, the surface pressure continues to increase until a maximum value of 60 mN/m approximately.

Although urease is soluble in water, when inserted in the air-water interface, its molecules are distributed between the subphase and the air-water interface, forming a Gibbs monolayer. When this monolayer is compressed, a maximum surface pressure of 10 mN/m is attained. For the mixed copolymer-enzyme monolayer, the film expands when the enzyme is introduced. This means that the isotherm presents areas for a given surface pressure higher than expected, caused by the incorporation of the enzyme between the copolymer molecules, reflecting in changes in the supramolecular structure at the interface.

In general, it can be stated that the isotherm for the mixed monolayer shows a profile that is hybrid between the two components, copolymer and urease. A degenerate first-order transition can be observed in surface pressures near 10 mN/m, and can be attributed to a molecular rearrangement at the air-water interface. It is possible to note that the maximum surface pressures attained for the monolayer were higher for the mixture, reaching values as high as 64 mN/m, at which the monolayer collapses. This indicates a cooperative behavior between copolymer and urease, forming an interface that is more resistant to compression. It is important to

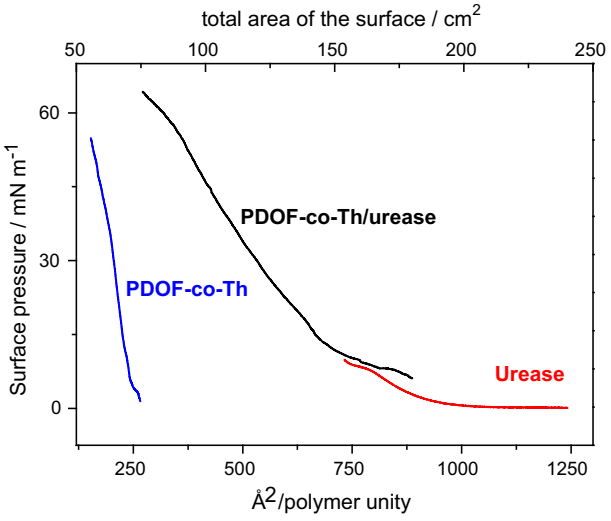


Fig. 1. π-A isotherms for the first cycle of compression obtained from the solutions of 5 mg/mL of PDOF-*co*-Th in chloroform and 20 mg/mL of urease in a phosphate pH buffer and their mixture. Area of the curve for urease must be seen only as "total area of the surface".

emphasize that the monolayers are usually compressed beyond the equilibrium surface pressure, typically between 25 and 35 mN/m, and therefore the collapse is attained when the monolayer is in a metastable state.

To check the reversibility of the isotherm of the mixed film, three cycles of monolayer compression-decompression were performed (Fig. 2). Analyzing the cycles, it is possible to notice that the curves do not coincide in terms of compression and decompression for the same cycle. Also, the first cycle is not coincident with the second and third ones. In general, the decompression curves of the monolayer present lower values of surface pressure in a given area. This result suggests the formation of kinetically irreversible structures during the compression, which are not easily unformed during the decompression. After the second compression of the monolayer, slight differences are observed when the first and the second cycles are compared. However, the isotherm for the third cycle tends to be coincident with the second one, which means that a reversible monolayer in terms of surface rheology was reached. Therefore, it is important to mention that since the isotherms are not reversible for the two first cycles, data on the monolayer structure of the films are obtained in the equilibrium (or in the steady state) only if the monolayer is left to relax after attained a given surface pressure.

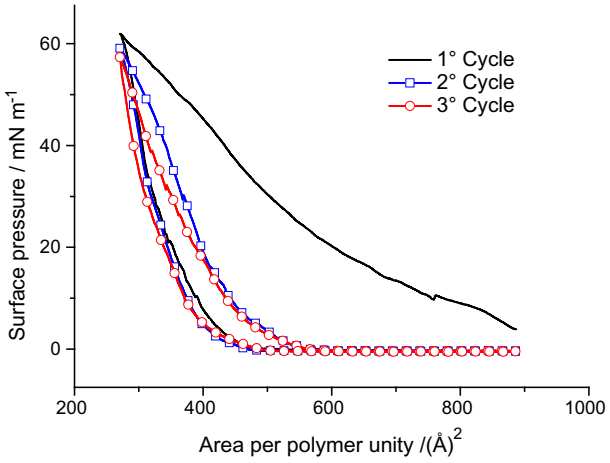


Fig. 2. Cycles of compression–decompression for a PDOF-*co*-Th/urease film at the air–water subphase.

In Fig. 3, the images obtained by Brewster angle microscopy (BAM) show the structures formed at the air-water interface. The film with the pure co-polymer at 30 mN/m presents a pattern that is relative homogenous, with only some isolated brilliant spots. Decompressing the film, its homogeneity is broken, being observed plates clustered in large brocks separated by "valleys" with low brightness. This kind of hysteresis is related to the fact that the large polymer domains formed during compression are not as easily deformed as they are formed; corroborating the hypothesis inferred from the compression/decompression curves. This may be related to possible molecular arrangements of the polymer attained due to the compression of the air-water interface. In fact, the pristine chain of the polymer is not rigid and the formation of the layer is not uniform due to the possible overlapping of layers when the barriers compress the material, not returning to the initial conditions after decompression. With urease, the monolayer also presents a similar profile during the compression of the interface. However, some differences in the morphology due to the presence of the enzyme may be depicted. At 30 mN/m, some fractures of the main domains are observed, and during the decompression, the large plates are relatively less homogenous in terms of degree of brightness, presenting some clearer regions with small domains. It is probable that the presence of small amounts of urease (which does not present contrast of domains for its Gibbs monolayers) may affect the manner how the polymer aggregates and desegregates during the process of compression/decompression.

The floating monolayers at 30 mN m^{-1} were also characterized using PM-IRRAS (Fig. 4). The spectra of the mixed films differed from the spectra of the pure polymer and urease at the air–water interface in terms of peak positions. In the spectra obtained for urease, pure or mixed with the conjugated polymer, it was possible to identify the typical bands of the polypeptide structure of the enzyme. The bands centered at 1205, 1303 and 1324 cm^{-1} are

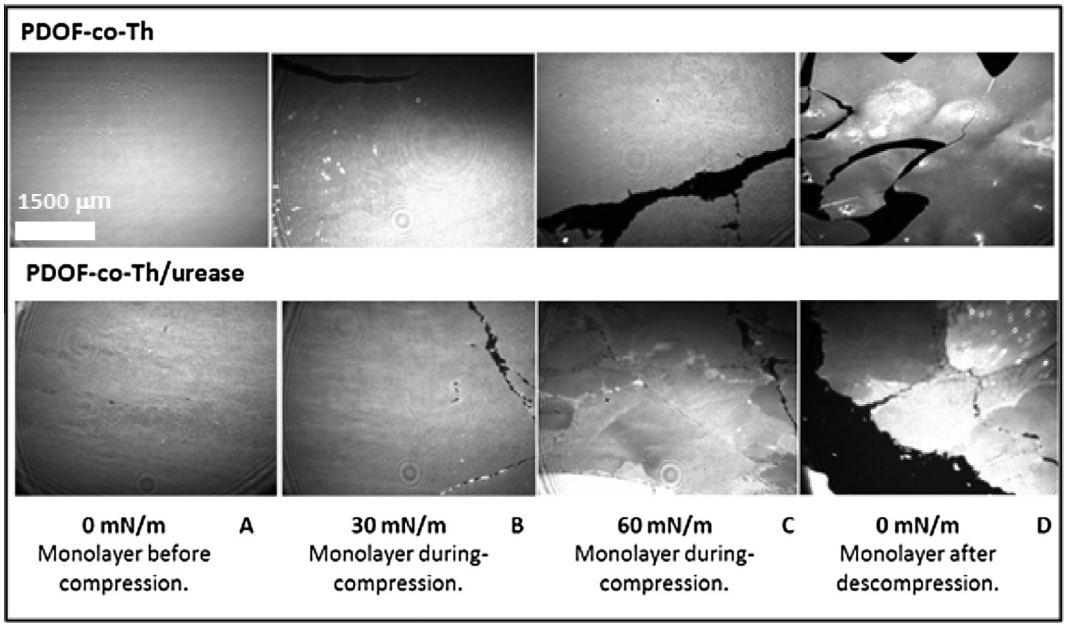


Fig. 3. BAM images (3600 × 4000 µm) of Langmuir compression of PDOF-*co*-Th films: (A) before compression, (B) at 30 mN/m, (C) at 60 mN/m and (D) after the film decompression.

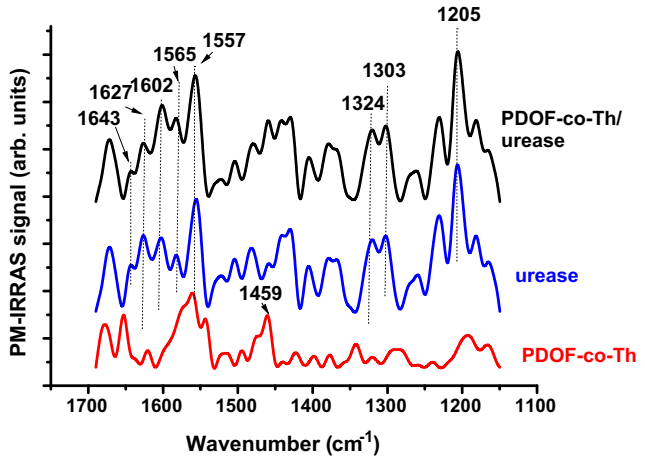


Fig. 4. PM-IRRAS spectra for PDOF-*co*-Th, urease and PDOF-*co*-Th/urease at the air–water subfase (30 mN/m).

related to the in-phase combination of N—H in plane bending and C—N stretching vibrations (amide III); the stretching band for C=O (amide I) is present at 1643, 1627 and 1602 cm^{-1}; a band related to the C—N stretching vibrations in combination with N—H bonding is located at 1565 cm^{-1} (amide II). For the films containing PDOF-*co*-Th, without and with the enzyme, it is possible to identify bands centered at 1557 and 1459 cm^{-1}, which is related to the thiophene group.

It is important to emphasize that the characterization of the mixed monolayer with surface pressure-area isotherms, PM-IRRAS and BAM indicates the cooperative interaction between the copolymer and the enzyme. The presence of urease in the copolymer monolayer expanded the monolayer and increased the surface pressure of maximum compression, indicating the incorporation of the enzyme in the film. Also, it is clear that urease changed the rheological properties of the monolayer since the film became more compressible at high surface pressures for the mixed monolayer. PM-IRRAS also showed changes in the bands assigned for the copolymer, showing specific interactions with urease, which reveals changes in the conformation of the macromolecules present at the interface. However, BAM images did not show significant alterations in the morphology structure of the copolymer monolayer due to the incorporation of urease, revealing that the copolymer is the main component able to form the pattern of heterogeneity observed.

3.2. Langmuir-Blodgett films

PDOF-*co*-Th/urease films were transferred from the air–water interface to solid supports at a constant surface pressure of 30 mN/m. Analyzing the values of transfer ratio obtained for the depositions, it is possible to affirm that the films that were prepared have a Z-type architecture, given that successful coatings of the surface occur only during the substrate removals.

Films with 1, 3, 5, 7 and 9 monolayers were analyzed using the PM-IRRAS technique as shown in Fig. 5. It is possible to note the presence bands related to vibrational transitions of chemical groups of PDOF-*co*-Th and urease. The mains bands are centered at 1677 cm^{-1} (amide I), 1627 cm^{-1} (amide II) 1557 cm^{-1} (amide II), and 1459 cm^{-1} (thiophene ring), being consistent with the spectra obtained for the films at the air-water interface. Also, a new amide II band at 1523 cm^{-1} was found. The slight differences in relation to the air-water interface reflect changes in the supramolecular structure when the film is supported on a solid support and when it is supported on a liquid interface. Variations in terms of relative intensity and general shape of the bands are

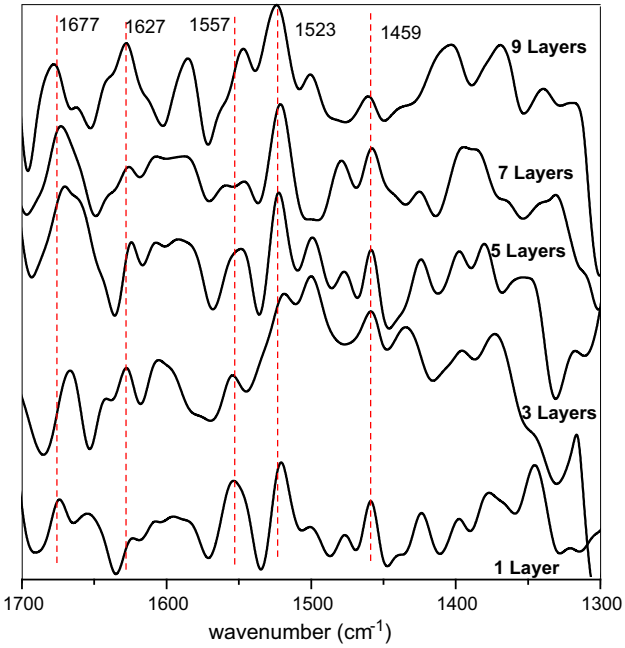


Fig. 5. PM-IRRAS spectra of PDOF-*co*-Th/urease Langmuir–Blodgett films.

also observed when films with different number of layers are compared, being related to changes in terms of conformation of the polymer and enzyme when the molecular arrangement becomes thicker.

The surface morphology of the films was investigated by AFM (Fig. 6). For better clarity, we present the films with the lowest and the highest number of layers (1 and 9, respectively). It may be noted that the films contain aggregates when supported in solid substrates. Also, by increasing the thickness of the film, the surface becomes more homogeneous, and some defects of the surface morphology as ridges, protuberances and holes can be noted. This is an effect of an expected pattern of micro- heterogeneity of the surface, common when flexible macromolecules cover a solid support. Some domains with 25–30 nm high peaks can be observed for the 1-layer LB film. For the 7-layer film, domains as high as 35 nm approximately are found. Also the thickest film presents some domains with deeper valleys, reaching values as low as 24 nm. The 7-layer film also presents a lower roughness in comparison to the 1-layer film. This fact can be explained by the overlapping of layers, which may correct irregularities on the surface caused by the polymer.

3.3. Optical proprieties of Langmuir–Blodgett films

PDOF-*co*-Th is easily transferred from the liquid interface to the solid support, and acts as a matrix that enables the co-transfer of urease. Fig. 7 shows the absorption and emission spectrum of the copolymer in solution and of the mixed enzyme-copolymer 9-layer film. The absorption spectra becomes wider in solid phase, but better defined when the enzyme is inserted. However the edge, the beginning of the absorption, was not changed, suggesting that urease has no interference in the polymer transition (approximately 470 nm, −2.6 eV).

The emission spectra obtained for the PDOF-*co*-Th LB films, pure or mixed with urease, present a shift to the blue region compared to the spectrum obtained for the copolymer in solution. The lower wavelengths (in absorption and fluorescence) may result from steric interactions and conformational changes, leading to a non-planar conformation, decreasing the conjugation length [26,27].

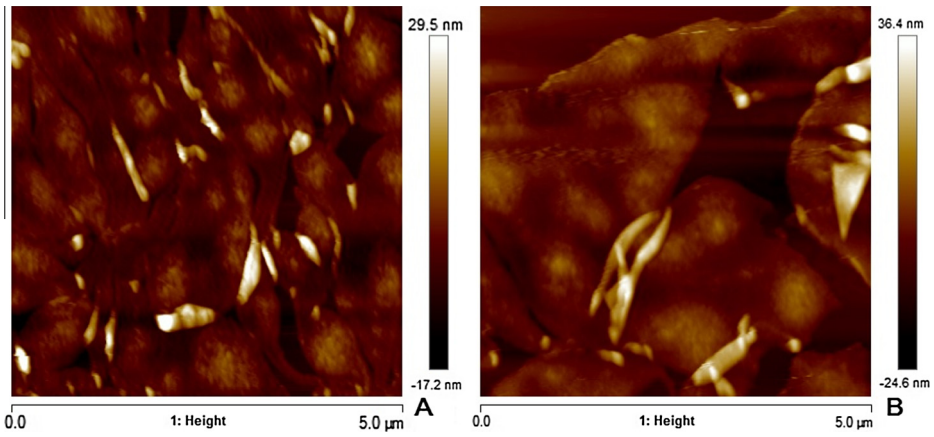


Fig. 6. AFM images for PDOF-*co*-Th/urease LB film. (A) 1 layer; (B) 9 layers.

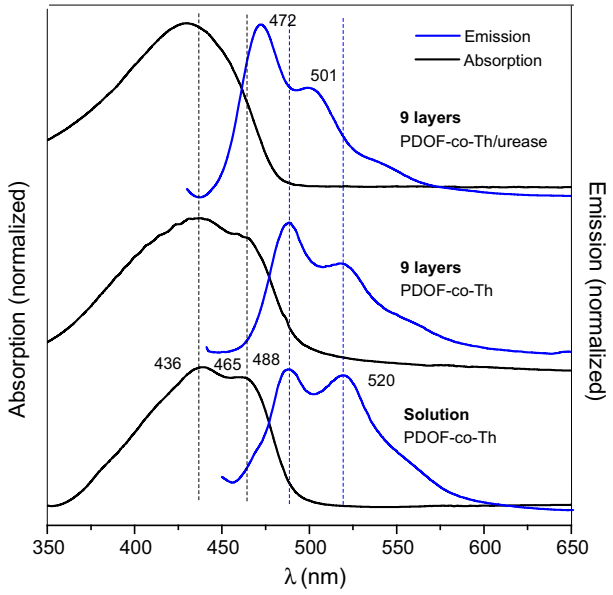


Fig. 7. Normalized absorption and emission spectra (excitation at 430 nm) for PDOF-*co*-Th solution (chloroform, 5.6×10^{-6} g mL^{-1}), PDOF-*co*-Th and PDOF-*co*-Th/urease 9 layers films.

3.4. Enzymatic catalysis and Michaelis-Menten constant (Km)

The results obtained for the enzymatic analysis of the PDOF-*co*-Th/urease films with 9, 7 and 5 monolayers are shown in Fig. 8a. It is important to emphasize that thinner films (1 or 3 layers) did not present enough enzyme activity to be detected, probably because of the quantity of urease immobilized together the polymer. The films were immersed into the indicator solution and the absorption at 588 nm was monitored with time. It is possible to notice that the films with higher number of layers present higher values of enzymatic activity. Up to 5 h of analysis, a linear relationship between time and absorption of the 9-layer film is observed (Fig. 8b).

The kinetic parameters of maximum velocity (Vm) and the Michaelis-Menten constant (K_m), obtained through the Lineweaver-Burk double reciprocal plot, were obtained for the enzyme in solution and for the 9-layer film (Fig. 9). This modeling of enzymatic catalysis considers the reaction according to the following mechanism:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \overset{k_2}{\rightarrow} P + E$$

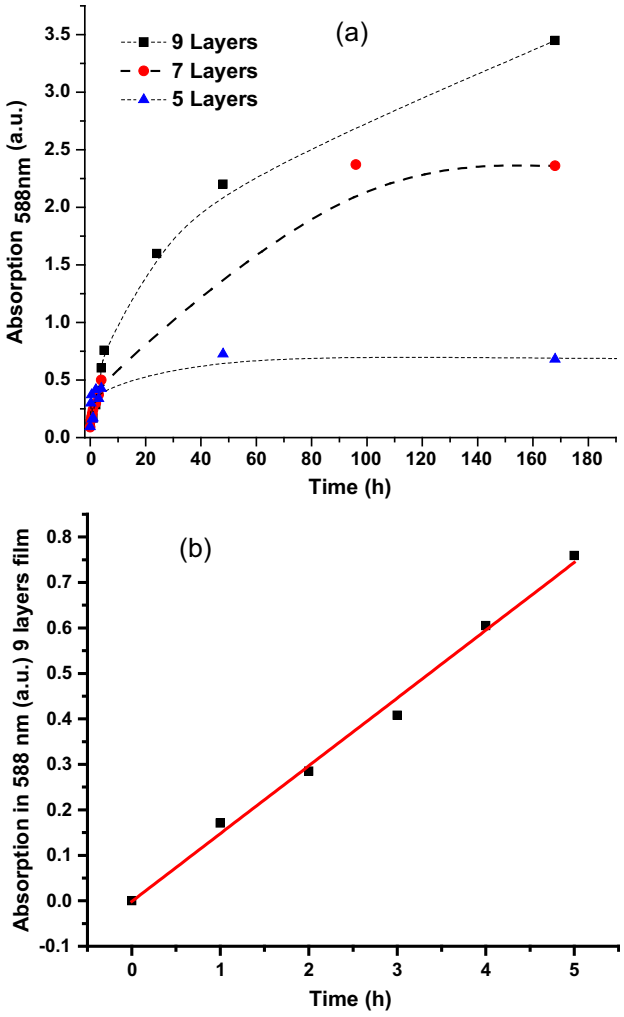


Fig. 8. (a) Absorption at 588 nm of urea solution (25 mM contained in 2 mL cuvette) as a function of time during the enzymatic analysis. (b) Absorption (588 nm) versus time during the first 5 h of the enzymatic analysis of a 9-layers film of PDOF-*co*-Th/urease.

where E is the enzyme, S is the catalytic substrate, and P is the final product. The values of k_1, k_{-1} and k_2 represent the rate constant for each step.

Considering $K_m = [(k_{-1} + k_2)/k_2]$ and $V_m = k_2[E]_o$, where $[E]_o$ is the initial concentration of enzyme, the reaction rate can be estimated as:

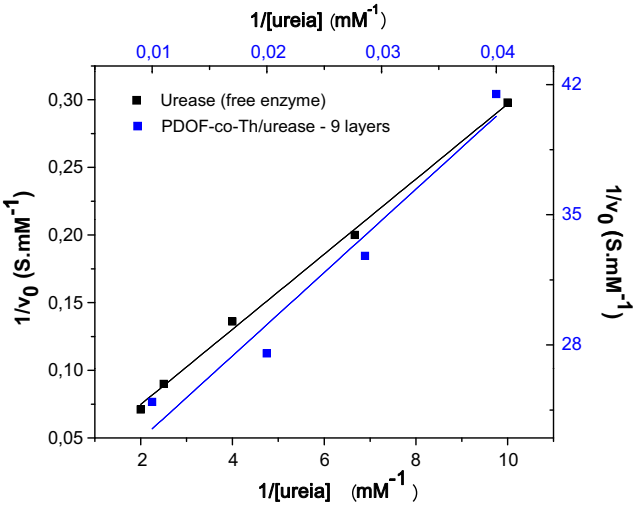


Fig. 9. Lineweaver-Burk linearization of the urease in solution (black) and of the 9-layer LB PDOF-*co*-Th/urease film (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

$$v = \frac{V_m[S]}{K_m + [S]}$$

As a result, the parameters for the enzyme in solution were determined: V_m is 14 mM s^{-1} and K_m is 1.45 mM. For the 9-layer film, V_m is 0.04 mM s^{-1} and K_m is 53 mM. The value of K_m found for the film is close to the values reported in the literature for other immobilized enzymes [28]. Since the enzyme is immobilized on solid supports, it is expected conformational changes in its tertiary structure. Also, as the substrate (urea) strives for accessing the catalytic site of the enzyme, the value of K_m increases and the maximum velocity decreases. The loss of catalytic activity for enzymes immobilized onto solid supports is frequently reported [17,29], being attributed essentially to restrictions in terms of mobility for the polypeptide that forms the core of the enzyme. This loss of activity can be also attributed to possible conformation changes as enzymes strive for achieving a lower surface energy when transferred from a liquid interface to a solid support. In this case, the enzyme may also not expose adequately their catalytic sites. Exceptions are reported, especially when the catalytic site of the enzyme is sensitive to hydrophobic environments, as when it is immobilized in micelles [30] or lipid Langmuir-Blodgett films [31].

Even though the enzyme activity of urease in the polymer LB films was considerably lower than in homogeneous environment, it is was sufficient for detecting urea as the absorbance varied expressively. Moreover, not only the response was fast, but also a linear dependence of the absorbance on time was observed for a long period of time, which may be even more important than obtaining a high activity, as in the latter the signal tends to saturate shortly. Another important aspect to be emphasized is the stability, since these films could be reused one month later, with insignificant loss of activity, which is therefore advantageous in producing biosensors.

4. Conclusions

In this work, we demonstrate that PDOF-*co*-Th is useful to be employed as a matrix for urease in mixed Langmuir and Langmuir-Blodgett films. The key findings of this paper were (i) urease expanded the copolymer monolayers, affecting the structuring and morphology of the films, as demonstrated with PM-IRRAS and BAM; (ii) cycles of compression-decompression associated with BAM images suggest the formation of irreversible domains, with large plates being separated in the decompression process leading to a heterogeneous pattern; (iii) the presence and structuring of urease could be detected in the transferred films, as well as the catalytic parameters; and (iv) the enzyme activity for the LB films was lower than that obtained in homogenous environment. The hypothesis for this lower enzyme activity is due to restrictions of the macromolecule in terms of mobility affecting the access of the substrate to the catalytic site of the enzyme. Although the decrease of enzyme activity is reported by various reports [17,29], the innovation of this work relies in the fact that the nanostructured film with controlled enzyme activity facilitates the determination of the kinetic parameters, which may have implications in using this approach for analytic or sensing purposes. We believe that this present work may contribute to understanding why certain molecular architectures exhibit superior performance and how synthetic polymers influence the properties of enzymes when they are entrapped in nano-organized structures.

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