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Communications

First Enzymatic Synthesis of Water-Soluble Conducting Poly(3,4-ethylenedioxythiophene)

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The enzymatically catalyzed polymerization of 3,4-ethylenedioxythiophene in the presence of polystyrenesulfonate is introduced. This is the first time that an enzymatically catalyzed poly(3,4-ethylenedioxythiophene) (PEDOT) is reported. Horseradish peroxidase enzyme was used as a catalyst for the polymerization process leading to a water-soluble PEDOT that was characterized by UV−vis spectra, Fourier transform infrared, and electrical conductivity measurements. Water-soluble PEDOT showed excellent film formation ability as confirmed by atomic force microscopy images.

The field of intrinsically conducting polymers has attracted high attention due to their interesting electrical and optical properties. Since their discovery in 1977, they have been investigated for many technological applications, such as organic lightweight batteries, microelectronics, optical displays, antistatic coatings, and electromagnetic shielding.[1] These highly promising materials have traditionally been synthesized by monomer oxidation in the presence of a strong oxidant, and they usually show high insolubility and intractability, once synthesized.[2] The use of enzymes as biocatalysts for the synthesis of conducting polymers has been studied in recent years as a "green synthesis process" alternative.[3] Enzymes can offer environmentally benign reaction conditions,[4] a high yield of polymerization, and higher control in regioregularity and stereochemistry,[5] consequently resulting in soluble and processable conducting polymers.[6] In most cases, the research was focused on the synthesis of polyaniline (PANI). In these biocatalysis reactions, a peroxidase enzyme, such as horseradish peroxidase (HRP) or others such as palm tree peroxidase or laccasse, are used as catalysts for aniline polymerization in an aqueous buffer. Different synthetic methodologies such as micellar media,[7] reverse micelles,[8] interfacial polymerizations,[9] or polymeric stabilizer templates[10] have been investigated.

In spite of the great potential applications that the biocatalysis shows, this green synthesis process has not yet been applied to other technologically interesting polymers such as polypyrrole and polythiophenes.[11] Of particular interest, poly(3,4-ethylenedioxithiophene) (PEDOT) is becoming the most successful for commercial applications. This is clearly stated in a very recent paper by Bruno et al.,[12] where this lack is said to be probably due to the high oxidation potential of monomers such as 3,4-ethylenedioxythiophene (EDOT) and pyrrole (Py), compared to that of the oxidant HRP/H_2O_2, concluding that these monomers are inappropriate for this enzymatic synthesis process. Contrary to these statements, the goal of this communication is to report the first enzymatic polymerization of EDOT.

In a typical experiment, HRP catalyst, H_2O_2 oxidant, EDOT monomer, and polystyrenesulfonate (PSS) template were mixed in an acidic aqueous phase (Scheme 1). The reaction was carried out at 4 °C, and a blue-colored polymer solution was obtained that clearly indicated the formation of PEDOT. It is well-known that an acidic media is suitable to increase the rate of polymerization. The protonic acids and a variety of Lewis acids catalyze the equilibrium reaction of EDOT to the corresponding dimeric and trimeric compounds without further oxidation or reaction.[13] The necessity of an acidic media was also verified

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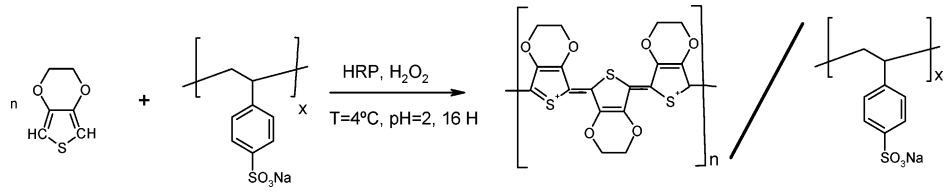
Scheme 1. Enzymatic Polymerization Mechanism for EDOT



in previous research on biocatalysis.[10,11,14] In our case, three different pH values were studied (pH = 2, pH = 4, and pH = 6) to establish the most optimum one for the adequate synthesis of EDOT. Figure 1 shows the UV−vis spectra for these three reactions. As can be observed, the only spectra showing the presence of a bipolaron absorption band at 800 nm associated with the PEDOT polymer was the one obtained at pH = 2.

Our results demonstrate that the higher oxidation potential of the monomer may not be a reason for the lack of polymerization in previous failed attempts. Water-soluble PEDOT can be easily obtained if appropriate working conditions (pH = 2) are used. Furthermore, a polymerization time as long as 16 h was required before the formation of well-defined PEDOT. However, as it is well-known, the activity of the HRP enzyme decreases abruptly in acidic media (at pH = 4, the HRP activity is around 0 after 1 h[10b]), but surprisingly, even if the enzymatic activity should normally be finished sometime after the first 60 min, the reaction was observed to proceed uninterrupted throughout the 16 h. These unexpected results led us to think that under these strong acidic conditions the enzyme could be deactivated and the polymerization process could be triggered only by H_2O_2. Thus, a control experiment was performed employing the same described conditions with no HRP enzyme. Since, in this case, no polymerization was observed, the HRP enzyme was assumed to be the catalyst of the polymerization reaction. A first possible explanation for the enzyme activity could be the temperature. The low temperature could decrease the degradation of the HRP enzyme. To verify this assumption, a new reaction was carried out following the same procedure but, this time, at room temperature. In this case, the reaction proceeded throughout 16 h, and a blue-colored ink was obtained. The properties of this PEDOT were not, however, as optimal as those obtained at low temperature: Its conductivity was 1×10^{-5} S/cm, 2 orders of magnitude lower than that obtained with PEDOT synthesized at 4 °C. An even more convincing explanation for the enhanced enzyme activity proposed by the authors is the presence of an excess of EDOT in the media. As the solubility of the monomer is very poor in water, the reaction takes place in a biphasic media, where the HRP is localized more preferentially in the monomer phase. To prove this statement, a mixture of EDOT, template, and enzyme was prepared in acidic water (pH = 2). This solution was kept under magnetic stirring for 24 h. Then, a solution of H_2O_2 was added. The reaction proceeded as usual, and a conducting water-soluble PEDOT was obtained after an additional 16 h. Thus, the EDOT monomer droplets would act as (a) enzyme protectors against deactivation as well as (b) a monomer feed to keep a constant EDOT concentration in the reaction media.

Conducting polymer films were obtained by simple casting the PEDOT solutions. The chemical nature of the polymer was

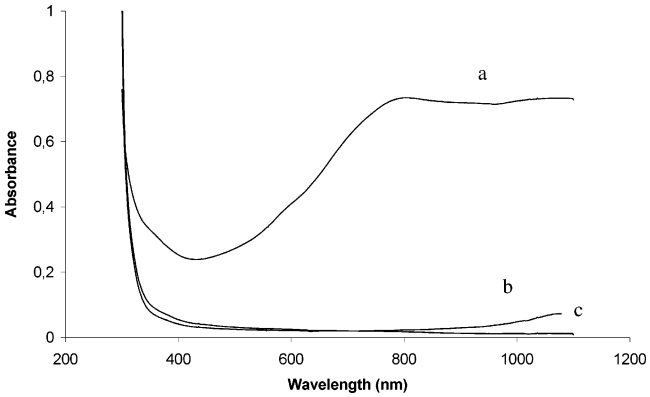


Figure 1. UV−vis spectra for PEDOT complexes synthesized at different pH values for 16 h: (a) pH = 2, (b) pH = 4, (c) pH = 6.

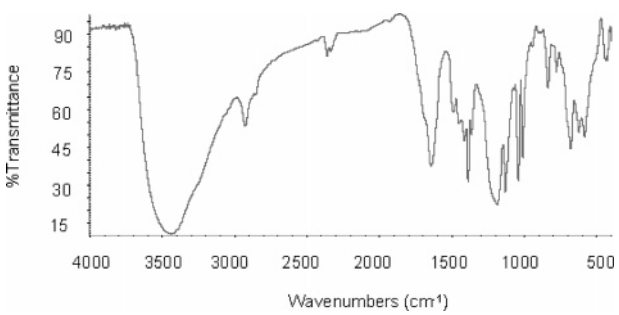


Figure 2. FTIR spectra for an enzymatic PEDOT/PSS film synthesized at a EDOT/PSS ratio of 1:1.

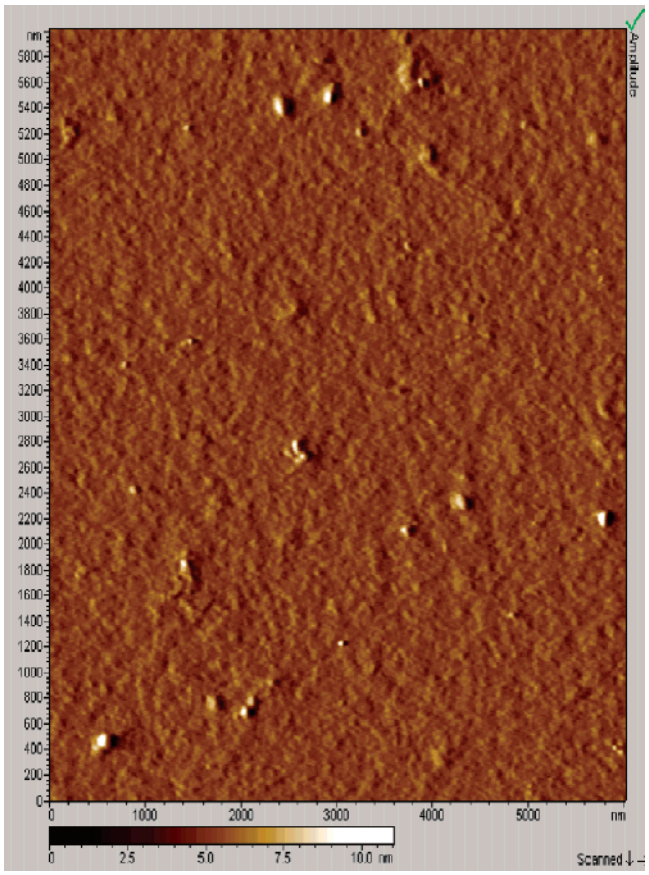


Figure 3. Atomic force microscopy pictures obtained from a PEDOT/PSS film (6 μm × 6 μm) synthesized at a EDOT/PSS ratio of 1:1.

studied by Fourier transform infrared (FTIR) spectroscopy. Figure 2 shows a FTIR spectrum where the classical bands for PEDOT at 1698, 1381, 1066, and 940−875 cm^{-1} and for the PSS stabilizers at 1009 and 1128 cm^{-1} can be observed. Furthermore, the electrical conductivity of the films was verified by the four-point probe. Typical values for PEDOT of 2×10^{-3} S/cm were measured, further confirming the success of our synthetic route.

Finally, it is worth noting that the film formation capacity was surprisingly good, not only at a macroscopic level but also at a microscopic level as can be observed in the surface picture of the film obtained by atomic force microscopy (Figure 3). A high homogeneity with slight differences in surface roughness were observed even at a 6 μm × 6 μm scale.

In summary, for the first time in the literature, the enzymatically catalyzed polymerization of EDOT in presence of PSS was successfully performed. Specific synthesis conditions, such as an acidic water solution with a pH of 2, required for this successful polymerization were stated. UV−vis spectra, FTIR, electrical conductivity values, and microscopy pictures for PEDOT obtained confirm the formation of a highly water-soluble conducting polymer with an interesting film formation ability. Our present research is focused on the encapsulation of the enzyme into solid supports for easily recovering the enzyme from the aqueous phase and to reuse it in several polymerizations allowing a high mass scale production.

Experimental Section. *Materials.* Horseradish peroxidase (HRP, EC 1.11.1.7, type II, 150−200 units/mg solid) and EDOT (99%) were purchased from Sigma-Aldrich Chemicals S.A. Poly(sodium 4-styrenesulfonate) (PSS) was purchased from Sigma-Aldrich Chemicals S.A. Hydrogen peroxide (H_2O_2, 30 wt %) and hydrochloric acid (HCl, 1 N) were obtained from Quimibacter S.L. The concentration of the HRP stock solution was 3 mg/mL.

Enzymatic Polymerization of EDOT. In a typical polymerization reaction, equimolar quantities (typically 0.055 M) of the template (PSS) and EDOT were dissolved into 20 mL of distilled water at room temperature. Next, the pH was adjusted to 2 with addition of HCl. A monomer/template complex was formed spontaneously. Approximately 2 mL of HRP solution (3 mg in 1 mL of distilled water) and an equimolar amount (typically 0.055 M) of hydrogen peroxide solution were added under magnetic stirring. The reaction was carried out at 4 °C for 16 h, and a characteristic blue-colored PEDOT solution was obtained.

Characterization Methods. UV−vis spectra were recorded on a UV-1603 Shimadzu spectrometer. In each measurement distilled water was used as the control. FTIR spectra were recorded on a spectrophotometer (AVATAR 360 FTIR) using KBr pellets. The electrical conductivity of the samples was measured with a homemade four-probe instrument. The surface morphology was obtained by a Pico Plus atomic force microscope operating in acoustic mode.

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