

Synthesis of polyaniline catalyzed by soybean peroxidase immobilized in halloysite nanotubes

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ABSTRACT

Polyaniline is one of the most studied conducting polymers because of its low cost, high conductivity and excellent environmental stability. PANi synthesis can be carried by several methods, being chemically the most widely reported. However, this method generates by-products that are contaminants and interfere with the doping process. Recently, a versatile approach has been addressed to polymerize aniline using less harmful and drastic reaction media. One of them is the enzymatic polymerization, using well-known oxidative enzymes such as horseradish and soybean peroxidase, which can be activated in an aqueous medium by common oxidizing agents. Halloysite nanotubes, are natural porous inorganic nanostructures that have been used as a nano-reactors for the immobilization of a good number of enzymes. Here, we report the polymerization of aniline using SBP immobilized in HNTs. The obtained material was characterized by several techniques such as UV-vis, FTIR spectroscopy, TGA, TEM microscopy and XPS.

Keywords: polyaniline, soybean peroxidase, halloysite nanotubes, enzymatic.

1 INTRODUCTION

Conducting polymers can be prepared using chemical and/or electrochemical methods of polymerization. Among these, polyaniline (PANi) is one of the most studied polymers because its' easy synthesis, low cost, great versatility and environmental stability. The chemical polymerization of PANi usually is carried out in acid media in the presence of strong oxidant agents [i.e. Fe (ClO₄) and persulphate salts]. On such conditions anionic species and ammonium sulphate are formed and they interfere with the doping process. In resume, the chemical synthesis implies harsh conditions that impact the electrical conductivity as well as being an environmentally harmful process [1]. Alternative approaches have merge to polymerize aniline trying to use catalyst that work under less drastic reaction media, one of those alternatives is the use of oxidative enzymes [2]. Soybean peroxidase has been reported as a template-free catalyst to polymerize aniline in aqueous medium [3]. On the other hand, halloysite nanotubes (HNT) have been used as nanoreactors for the immobilization of several kinds of enzymes. Here we

report the use of halloysite to immobilize soybean peroxidase to be used as a catalyst in the polyaniline synthesis [4-5].

2 EXPERIMENTAL

2.1 Materials

Halloysite nanotubes, soybean peroxidase, hydrogen peroxide (30% wt), aniline, p-toluensulfonic acid, N-pyrrolidone (NMP), deionized water.

2.2 Enzymatic polymerization

Polymerization of aniline was carried out using soybean peroxidase (SBP) previously immobilized in halloysite nanotubes. The immobilization of soybean peroxidase was carried out by dissolving 40 mg of the enzyme in 100 mL of deionized water. The solution was kept in agitation for two hours, after that 200 mg of halloysite nanotubes were added, maintaining the suspension under agitation for 17 more hours at 4 °C. Once the enzyme was immobilized, 400 microliters of aniline were incorporated into the halloysite nanotubes. All the components in suspension were maintained under agitation for another 24 hours in order to allow the complete incorporation of this monomer. Before starting the polyaniline synthesis 3 g of paratoluenesulfonic acid (PTSA) were added to adjust the pH to 3, maintaining the mix reaction at 0 °C. The polyaniline synthesis started by adding step-wise 335 μL of H₂O₂ (30% solution). At the end of the reaction, the polymer was centrifuged and precipitated and then washed with methanol to eliminate the presence of possible residual monomer and oligomers. Finally, the material was lyophilized and stored.

2.3 Characterization techniques

UV-vis spectra of doped and undoped PANi in aqueous dispersion were collected on a Shimadzu UV-2401PC in absorbance mode, in the range 300-800 nm using quartz cells with NMP as baseline. FTIR spectra of PANi was obtained using a Nicolet iS5 spectrophotometer in the range 500-3500 cm⁻¹ on transmittance mode with and 8.0 cm⁻¹ resolution. Thermogravimetric analysis were carried out in a TA Instruments TGAQ500 equipment under the following method: equilibrate at 25 °C, ramp 20 °C/min to 600 °C,

select gas 2 (nitrogen) and a final ramp of 20 °C/min to 750 °C. TEM analysis of PANi was carried out in a TITAN 2080 electron microscope operating at 120 kV, the lacey carbon film was prepared by submerging it in a dispersion of approximately 2 mg of lyophilized sample in ethanol placed in an ultrasonic bath for 5 minutes. XPS experiments were performed in a PHI 5000 VersaProbe II X-ray photoelectron spectrometer (XPS), the X-ray source used was a monochromatic Al anode (1486.6 eV), the surveys were obtained with 117.5 eV pass energy, the analysis region was 1400 to 0 eV. The HRXPS signal was obtained with 11.5 eV pass energy and a dual beam charge neutralization system (PHI's patented) was used to compensate for charging during XPS data acquisition. All measurements were made in an ultra-high vacuum (UHV) chamber at a pressure around 3×10^{-8} mbar, in the fittings Gaussian functions were used (after a Shirley background correction) where the FWHM of all the peaks were constrained while the peak positions and areas were set free.

3 RESULTS AND DISCUSSION

The polymerization of aniline starts immediately after the addition of the hydrogen peroxide and it's characterized by the change of color, which is indicative of the presence of emeraldine salt. The formation of this polymer was confirmed by UV-vis spectroscopy of the PANi dissolved in N-methylpyrrolidone. The spectrum shows a band at 330 nm, which is associated to the $\pi-\pi^*$ transition of the benzenoid rings and the presence of a second band around 600 nm that has been assigned to a benzenoid to quinoid excitonic transition.

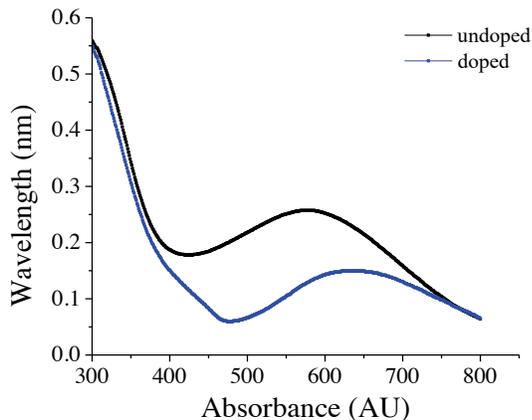


Figure 1: UV-Vis absorption spectra of doped and undoped PANi.

In the FTIR analysis, the characteristic peaks of PANi were observed. The signals corresponding to the stretches of the quinoid rings (1660 cm^{-1}) and benzenoids (1490 cm^{-1}), in addition to signals at 1150 cm^{-1} can be seen due to a symmetrical stretch characteristic of the amino group and at 1310 cm^{-1} is attributed to the stretching of C-N groups. The peak at wavenumber 1030 cm^{-1} is a characteristic signal of the SiO groups.

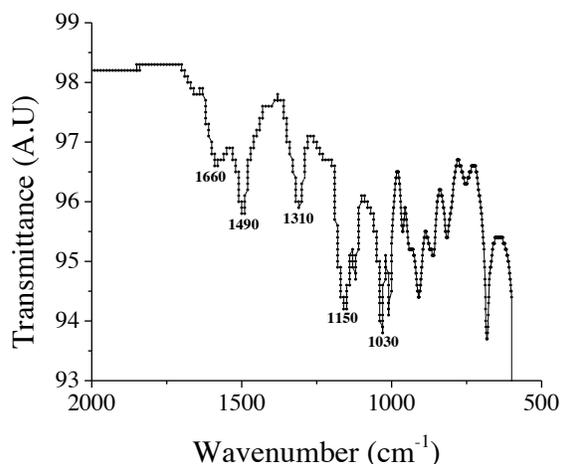


Figure 2: FTIR spectra of PANi.

The as-synthesized PANi shows a good thermal stability. In the TGA analysis, the initial weight loss at temperature below 150 °C can be attributed to residual water retained in the sample after drying.

The second loss at 229 °C may correspond to the thermal degradation of the peroxidase, the major weight loss occurred above 375 °C and it was attributed to the beginning of thermal decomposition of PANi and finally at the temperature beyond 500 °C is associated to the complete degradation of the polymeric chains of this polymer.

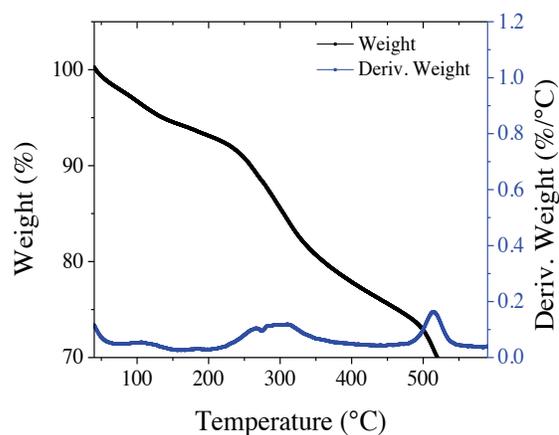


Figure 3: Thermogravimetric curves of PANi.

It is important to highlight that during TEM analysis an interesting morphology was observed. Such morphology is a quasi-spherical nanostructure along the nanotube body. The origin of the formation of those nanostructures deserve more studies that are underway. The dark appearance inside the halloysite nanotubes could be indicative of the immobilized SBP.

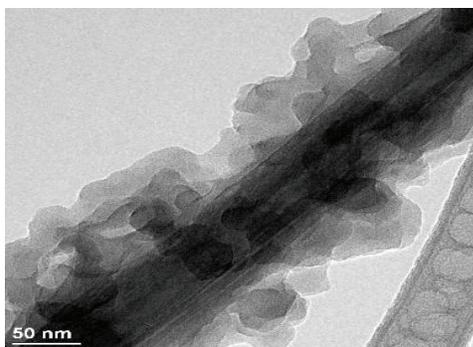


Figure 4: TEM image of PANI/HNT

The XPS analysis of PANi mainly confirms the presence of carbon (C1s), oxygen (O1s) and nitrogen (N1s).

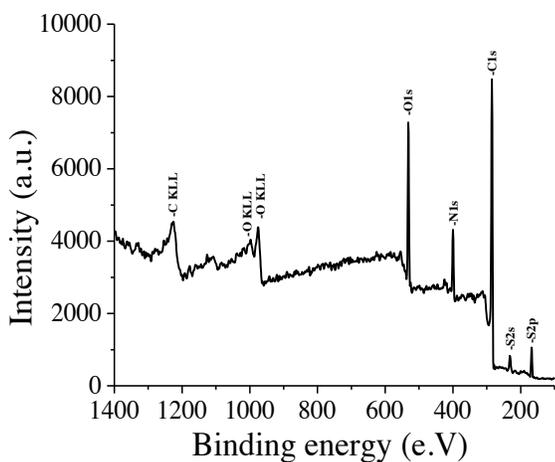


Figure 5: XPS of PANi synthesized on halloysite nanotubes.

Fig. 5 shows the XPS survey of PANi obtained enzymatically on halloysite nanotubes modified with soybean peroxidase, it was found that the atomic percentages of our final product are 73.0, 15.0, 9.3 and 2.6 % for C, O, N and S respectively.

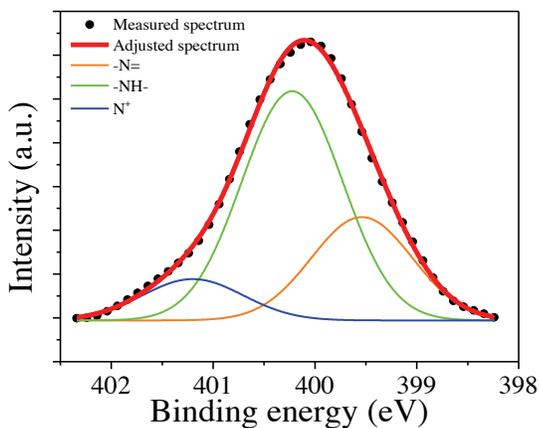


Figure 6: XPS spectra (N1s) of PANi

Sample	N1s		
	-N=	-NH-	N+
PANI	0.26	0.63	0.09

Table 1: Percentages of area N1s of PANi in XPS.

Fig. 6 shows the presence of three peaks on the N1s deconvolution curve which are attributed to -N= (399.5 eV), -NH (400.2 eV) and N+ (401.2 eV). The percentages showed in Table 1 indicate that PANi is found in the emeraldine salt oxidative state. The remaining Ns are attributed to the N+ formation.

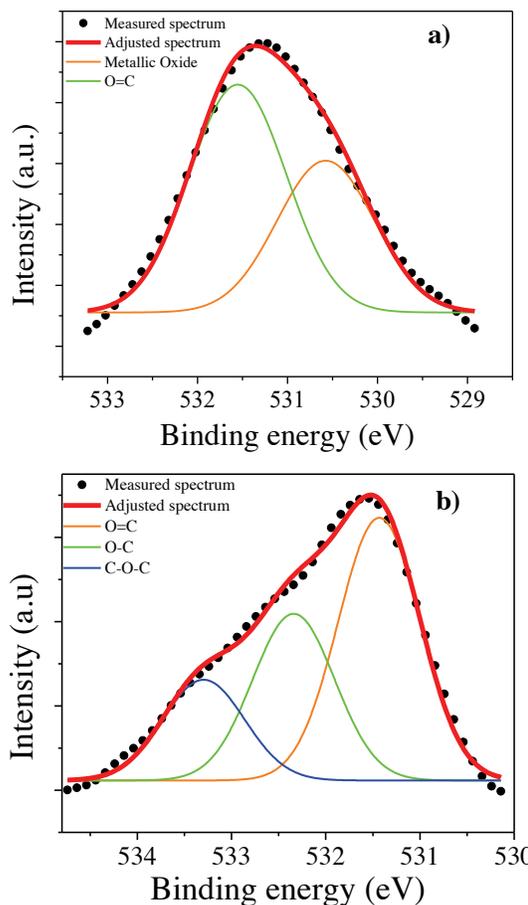


Figure 7: XPS spectra O1s of a) HNT and b) PANi.

Sample	O1s			
	Metallic oxide	O=C	O-C	C-O-C
HNT	0.34	0.51	---	---
PANI	---	0.48	0.31	0.21

Table 2: Percentages of area O1s of PANi and HNT in XPS.

Furthermore, the O1s deconvolution analyses of HNT and PANi (shown in Fig. 7 in subsections a) and b) respectively) indicate that the absence of metallic oxide in PANi is related

to the efficient modification process of the nanotubes, also the results obtained indicate that it disappears to give place to other types of oxygen bonds.

4 CONCLUSIONS

PANi synthesis was achieved and results obtained by UV-visible and FTIR spectra reveal the formation of the emeraldine salt with a good thermal stability and TEM microscopies showed an interesting morphology. It was corroborated by XPS that the PANi synthesis on modified halloysite nanotubes was successful. The reaction was done under contaminant-free and very moderate conditions.

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