PVA cryogels obtained by biomimetic synthesis of polyaniline using hematin supported on halloysite nanotubes as catalyst

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ABSTRACT

We studied the biomimetic synthesis of polyaniline as an alternate route to prepare electrical conductive cryogels (ECC), unique structures that combine intrinsic conductivity and hydrogel properties. For that purpose, hematin a natural porphyrin with peroxidase-like activity was supported in the lumen of halloysite nanotubes to be used as a biomimetic catalyst to polymerize aniline on its' conductive form, both the catalyst and the final product were characterized by TEM and XPS. Experiments using this catalyst were carried out to probe the *in situ* and *ex situ* synthesis of PANI in the presence of PVA to further obtain cryogels. TGA, FTIR, swelling behavior, conductivity and resistivity were used to characterize ECC. Both the *in situ* and *ex situ* biomimetic synthesis of polyaniline allowed the cryogel structure formation. The best result of swelling behavior was obtained when PANI was synthesized *in situ*. It is expected that such materials could be innocuous and free of any toxic contaminant, due to the synthesis method.

Keywords: PVA cryogels, polyaniline, biomimetic polymerization, halloysite nanotubes, hematin.

1 INTRODUCTION

Halloysite has received great attention due to its nanotube shape, it is a very interesting aluminosilicate. In this work, these nanostructures were used to support hematin, which is a natural porphyrin with peroxidase-like activity. In a previous report, we demonstrate that hematin supported in halloysite nanotubes catalyzed the biomimetic synthesis of aniline yielding polyaniline with high electrical conductivity in very stable aqueous solution. This catalyst is much more resistant to inactivation by low pH or high temperatures than soybean and horseradish peroxidases [1].

Over the last several decades, hydrogels have been a focus of research because of its excellent properties for biological, medical and technological applications. These materials have the capacity of absorbing high amounts of water, swelling and increasing its size considerably, while keeping its shape [2].

Poly(vinyl alcohol) is a cheap, semi-crystalline, hydrophilic polymer that is able to generate cryogels by alternating freeze-thawing cycles [3-4]. The freeze-thawing process causes the PVA mixture to cross-link through intra- and inter- hydrogen bonding formation, between hydroxyl groups of the PVA molecules. PVA cryogels feature thermic stability in a wide range of temperature, low density and excellent durability.

2 EXPERIMENTAL

2.1 Materials

Halloysite nanotubes, hematin porcine, sodium carbonate and bicarbonate, hydrogen peroxide (30% wt.), aniline, N-methyl-2 pyrrolidone (NMP), p-toluensulfonic acid (PTSA), deionized water, PVA (Mw 60,000), sodium phosphate dibasic and monobasic.

2.2 Immobilization of hematin

20 mg of hematin (Hem) were dissolved in 50 mL of carbonate buffer pH 9.5 and stirred for 10 hours, then 1 g of halloysite nanotubes (HNT) was added and the reaction was stirred for another 24 hours. The modified nanotubes were centrifuged and washed 3 times with acid solution pH 5, frozen and lyophilized for further characterization.

2.3 Biomimetic polymerization

100 mg of Hem/HNTs and 200 µL of aniline were added to 50 mL of deionized water, 3 g of PTSA were added to reach pH 3, temperature was kept at 0 °C and 440 µL of hydrogen peroxide were step-wise incorporated (10 µL per minute). The reaction took place under a nitrogen atmosphere, after the addition of peroxide it was stirred for one hour. The product PANI was washed 2 times with methanol and 1 with acid solution, to obtain the doped form. Finally, the obtained material was frozen and lyophilized.

2.4 In situ fabrication of cryogels

A 10% PVA solution was prepared by dissolving 5 g in 50 mL of hot water, it was stirred for 2 hours at 80 °C, 100 mg of Hem/HNTs and 200 µL of aniline were added, the mix was kept in agitation for 10 hours. All the aqueous suspension was placed in an ultrasonic bath for 35 min at 45 °C in order to disperse Hem/HNTs. The pH was adjusted to 3 by adding 3 g PTSA approximately. The reaction was carried out at 0 °C and was started by adding 440 µL of H_2O_2. The reaction mix was maintained in agitation for one more hour under a nitrogen atmosphere. At the end of the reaction the product was poured into plastic containers and

then submitted to four cycles of freeze-thawing of 24 h each one.

2.5 Ex situ fabrication of cryogels

A 10% PVA solution was prepared and 100 mg of doped PANI were added, the mix was stirred for 1 hour and put in an ultrasonic bath for 35 min at 45 °C, it followed the same procedure of freeze-thawing.

2.6 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. TEM analyses of HNT, Hem/HNTs and PANI were carried out in a TITAN 2080 electron microscope operating at 120 kV, all lacey carbon films were prepared by submerging it in a dispersion of approximately 2 mg of each 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 and 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 3x10-8 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. Thermogravimetric analyses 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. FTIR spectra of the cryogels were obtained using a Nicolet iS5 spectrophotometer in the range 1400-4000 cm^{-1} on transmittance mode with and 8.0 cm^{-1} resolution. Volumetric conductivity of doped PANI and PVA PANI cryogels was recorded on a Keithley 2400 SourceMeter, based on ASTM D4496 and the CABOT method. Swelling degree was calculated by submerging dry films of each cryogel in 80 mL of phosphate buffer pH 4 and 7 for 24 hours and weighting them after certain time. For the cryogels' characterizations samples were cut in films and lyophilized.

3 RESULTS AND DISCUSSION

Halloysite nanotubes changed color from clear to dark green, due to the hematin adsorption and after the addition of H_2O_2 the polymeric reaction also presented a change of color, suggesting the oxidation of aniline. UV-vis spectra indicate that PANI can revert from doped state to undoped on a NH_4OH dispersion.

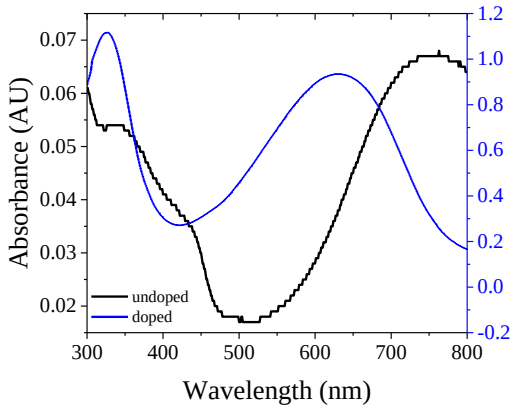

Figure 1: UV-vis spectra of doped and undoped PANI.

TEM microscopies show that: a) HNTs have a cylindrical shape with a longitudinal channel, b) HemHNT do not show major differences against original ones and c) PANI shows an uniform coating as well as a complete filling of tubular lumens. It has been reported that HNTs have typical dimensions of 10-50 nm in outer diameter, 5-20 nm in inner diameter and 2-40 mm in length.

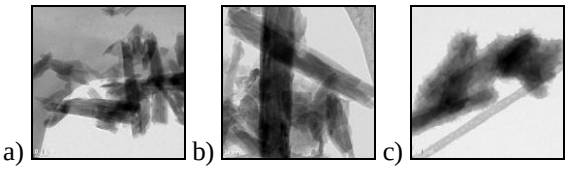

Figure 2: TEM microscopies of a) HNTs, b) hematin immobilized on HNTs and c) PANI supported on HemHNT

The chemical nature and state of the samples were analyzed by XPS, Figure 3 shows the survey of PANI in this spectrum.

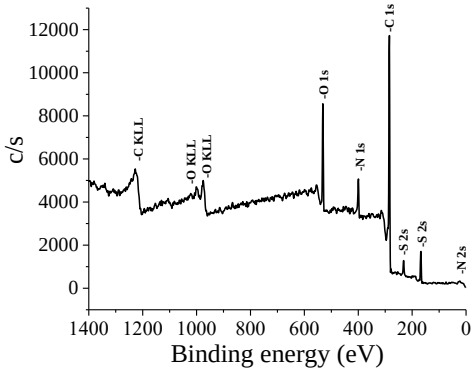

Figure 3: PANI spectrum by XPS

It has been possible to review the atomic composition of the sample (C1s, O1s, N1s, and S2p is 77.2, 13.5, 5.2 and 4.1% respectively). Furthermore, the high-resolution spectrum of O1s signals of each sample indicate the effective modification of the HNTs, the deconvolution processes allow identifications of the oxygen bindings. In original HNTs there is metallic oxide and after hematin immobilization and aniline polymerization, the metallic oxide percentage decreases and disappears to give rise to another type of oxygen bonds.

Sample	O 1s				
	Metallic oxide	**O=C**	**O=C-N**	**O-C / OH-C**	**C-O-C**
HNT	0.39	-	-	0.60	-
HemHNT	0.09	0.47	-	0.43	-
PANI	-	0.49	0.33	0.13	0.03

Table 1: Comparison between oxygen bonds.

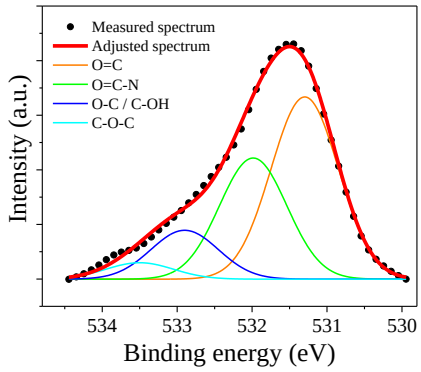

Figure 4: XPS high-resolution O1s deconvolution curve of PANI.

Doped form of PANI was confirmed by it being highly conductive, polyaniline conductivity on cryogels decreases but they still show semiconductive properties. The cryogel obtained via *in situ* presented higher conductivity than the *ex situ*, being the latter on an insulating/semiconductive range.

Sample	**Resistance (Ω)**	**Resistivity (Ω cm)**	**Conductivity (S/cm)**
PANI	9	8.1	$1.23x10^{-1}$
PVA PANI *in situ*	$10.7x10^{6}$	$4.28x10^{6}$	$2.34x10^{-7}$
PVA PANI *ex situ*	$64x10^{6}$	$3.20x10^{7}$	$3.13x10^{-8}$

Table 2: Volumetric conductivities of PANI and cryogels.

Both thermogravimetric curves show a minor weight loss at around 100 °C related to residual humidity, an important weight loss at 225 °C corresponding to the cross linked chains of PVA, another one between 430-450 °C which is attributed to the thermic degradation of byproducts in the PVA decomposition and the carbon main chain. PANI´s curve presents a loss at 250 °C associated to hematin and doping agent degradation, while the one at 460 °C is attributed to PANI´s chains degradation.

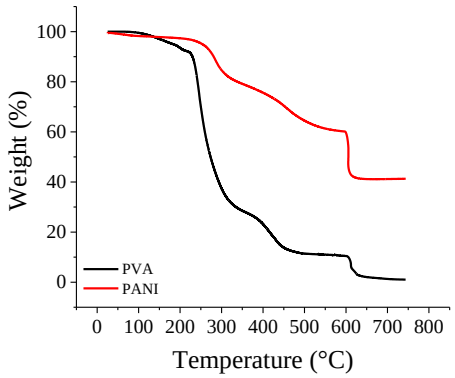

Figure 5: Thermogravimetric curves of PANI and PVA cryogel

FTIR analysis of the cryogels show the characteristic peaks related to hydroxyl and acetate groups. The long band at 3332 cm^{-1} is attributed to intra and intermolecular O-H stretching, the vibration band at 2938 cm^{-1} to the C-H stretching of alkyl groups and the peaks at 1723 cm^{-1} and 1416 cm^{-1} to the C=O and C-O stretching of the remaining acetate groups of PVA.

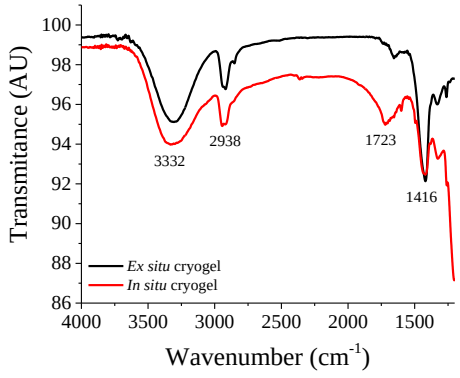

Figure 6: FTIR spectra of PVA PANI cryogels.

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The swelling degree was calculated during a time lapse of 24 hours on a controlled temperature of 37 °C, samples were lyophilized, weighted and immersed on phosphate buffer pH 4 and 7. Swollen samples were taken out, the superficial solution was wiped off using absorbent paper, and weighted. The swelling degree at equilibrium was obtained using the following equation:

$$Wt = \frac{Ws - Wd}{Wd} * 100$$

Where Ws is the weight at different times and Wd is the weight after the samples were immersed for 24 h and lyophilized. Figure 7 shows the comparison between cryogels with PANI synthesized via *in situ* and *ex situ*, all samples presented a higher swelling on pH 4 medium. The best result was obtained by *in situ* cryogel fabrication.

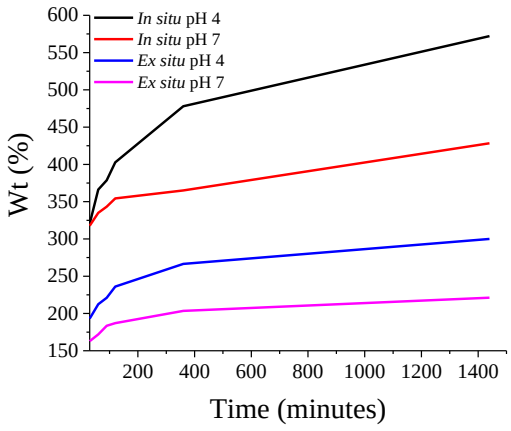

Figure 7: Swelling behavior of cryogels on phosphate buffer pH 4 and pH 7.

4 CONCLUSIONS

PANI was synthesized effectively with HemHNT as biomimetic catalyst and the emeraldine salt form of this polymer was confirmed with the UV vis spectra. PANI functionalization was corroborated by XPS because it was possible to observe that the metallic oxide gradually decreases from the original nanotubes to the final product. The cryogel that showed the higher swelling degree (572%) was the one obtained via *in situ* and in a lower pH. Conductivity results also confirmed the emeraldine form of PANI, being highly conductive ($1.23x10^{-1}$ S/cm) and the best conductive properties of PVA cryogels with PANI were observed with the *in situ* fabrication. It is expected that cryogels obtained by this reported biomimetic synthesis are free of possible contaminants commonly present in any chemical synthesis and/or chemical crosslinking.

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