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Enzymatic polymerization of aniline in the presence of different inorganic substrates

E. Flores-Loyola [a,b,*], R. Cruz-Silva [c], J. Romero-García [a], J.L. Angulo-Sánchez [a,✠], F.F. Castillon [d], M.H. Farías [d]

[a] *Centro de Investigación en Química Aplicada, Blvd. Enrique Reyna No. 140, CP 25100 Saltillo, Coah, Mexico*
[b] *Escuela de Ciencias Biológicas, UA de C. Carr. Torreón-Matamoros Km 7.5, Ciudad Universitaria, CP 27400 Torreón, Coah., Mexico*
[c] *Centro de Investigación en Ingeniería y Ciencias Aplicadas, UAEM. Av. Universidad # 1001, Col. Chamilpa, CP 62210, Cuernavaca Mor., Mexico*
[d] *Centro de Ciencias de la Materia Condensada de la UNAM, Apdo. Postal 2681, CP 22800 Ensenada, B.C., Mexico*

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Abstract

The effect of different inorganic substrates in the structure of polyaniline synthesized by enzymatic oxidation was studied. The polymer characterization was done by electronic absorption and X-ray photoelectron spectroscopy. The substrates studied were: controlled pore glass, mordenite, zeolite Y, zeolite MCM-41, Wollastonite, silica gel, fuming silica and short glass fibers type E. Polyaniline was synthesized in the presence of the substrates under acidic aqueous conditions, using hydrogen peroxide as oxidizer and HRP or SBP enzymes as catalyst. The composition of the substrates strongly affected the degree of electronic conjugation of the synthesized polyaniline, whereas the pore size and the enzyme type apparently had no effect. The chemical structure of polyaniline enzymatically synthesized was more sensitive to the substrate composition than that chemically synthesized. Apparently substrates containing alkaline ions, such as sodium and calcium, promoted the formation of the branched, non-conductive polyaniline form. The effect of the substrates on the polyaniline structure can be explained considering the local pH effect of the templates surface on the coupling reaction of aniline radicals.

Keywords: Polyaniline; Enzymatic synthesis; Porous materials; Peroxidases

1. Introduction

Polyaniline (PANI) has attracted considerable attention due to its interesting electrochemical behavior [1], the reversible nature of its electrical conductivity as well as its environmental stability. Furthermore, its conductivity is not only controlled by the degree of chain oxidation but also by the protonation level [2,3]. All these properties make PANI attractive for applications as an electrically conductive material in the preparation of composites with new and specific properties. Dunsch [4] studied the aniline polymerization mechanism, and found that a linear structure is obtained only under acidic conditions due to 1,4 coupling of aniline radicals. Under near-neutral or alkaline pH, the aniline radicals may undergo substitution at the *ortho* and *para* positions, or even undergo multisubstitution, yielding a branched structure [5]. The polymerization mechanism for the enzymatic reaction in aqueous conditions at neutral pH [6,7] proceeds preferentially by 1,2 substitutions rather than by the 1,4 substitution observed under acid conditions. There is also the possibility to have multi-substitution in the rings yielding branched chains, which reduce mostly the optical and electric properties of PANI, restraining their potential applications.

A different approach to control the structure during the synthesis of a polymer is by using a porous inorganic material as template. Mesoporous and nanoporous materials often provide environments within their pores where the polymerization reaction is carried out under different conditions to that of the reaction media. These materials may act as a template by promoting certain coupling reactions of the radicals. For these reasons, confined monomers usually polymerize in a more ordered and efficient way, and undesirable reactions, such as

* Corresponding author at: Centro de Investigación en Química Aplicada, Blvd. Enrique Reyna No. 140, CP 25100 Saltillo, Coah, Mexico. Tel.: +52 871 757 1785; fax: +52 871 757 1795.
E-mail address: erika-flores@mail.uadec.mx (E. Flores-Loyola).
✠ Deceased on 28 October 2006.

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branching or crosslinking, are avoided due to steric hindrances, resulting in a polymer with better properties compared to those synthesized in solution media [8]. Indeed, PANI has been synthesized by *in situ* chemical oxidation of aniline in the presence of different substrates and, in this case, the polymer is adsorbed both on the surface and within the pores of the substrate. These hybrid materials have potential applications in new technologies such as electrorheological fluids, sensors and catalysts [9,10]. PANI has been prepared using porous materials as templates, and some of these works are those reported by Wu and Bein [8], Bein and Enzel [11] and Enzel and Bein [12], who synthesized PANI by chemical oxidation within the channels of zeolite molecular sieves. They found that aniline in acidic zeolites can form intrazeolite polyaniline by oxidative polymerization reaction, in analogy to the oxidative coupling of aniline in acidic solution and that while the level of intrazeolite acidity has a strong influence on the polymerization reaction, the nature of the channel system controls the degree of polymer oxidation that affects its electronic properties.

Besides, porous glass/polyaniline composites have been prepared by *in situ* oxidative polymerization of aniline [13,14]. These composites represent a new class of materials, where synthetic conductors are encapsulated in an insulating inorganic mesoporous host. This process has been used to modify a wide range of polymeric [15,16] and inorganic materials [12,17,18]. Mica [19], glass [13,14], silicon dioxide [20], and metallic oxides [21,22] have been successfully modified with polyaniline by *in situ* chemical polymerization. One of the most important potential applications of these materials is their use as electrically conductive fillers in advanced polymer composites.

Recently, enzymatic polymerization of aniline has become very attractive because it is a clean and environmentally friendly process [23] that is carried out under milder conditions with high reaction yield. Although a wide range of peroxidases has been used to synthesize PANI, commercially available horseradish peroxidase (HRP) and soybean peroxidase (SBP) [24] have been the most studied. This biocatalytic approach has been applied mainly to synthesize PANI in the form of water–soluble complexes using different polyacids as templates [25–27]. These polyelectrolyte-templates provide a local acid pH and promote the head-to-tail coupling of aniline radicals. However, under acidic conditions, the template-free approach also yields electrically conductive PANI with a structure quite similar to that chemically synthesized [28]. Immobilization of peroxidase on polymeric substrates has been used to synthesize thin films of polyaniline [29], and recently, nanowires of PANI have been obtained by the enzymatic approach [30]. However, the usefulness of the enzymatic polymerization approach to synthesize PANI by *in situ* polymerization on inorganic substrates has not been examined. The aim of the present work was to study the PANI enzymatically synthesized in the surface and within the pores of several inorganic substrates. The electronic conjugation degree was studied by UV–vis and X-ray photoelectron spectroscopic techniques, whereas the thermal stability was studied by thermogravimetry. The effects of substrate composition and morphology, enzyme type, and monomer confining on PANI properties are also discussed.

2. Experimental part

2.1. Materials

Hydrogen peroxide (29.9 wt%), Horseradish peroxidase (HRP) (238 U/mg), Soybean peroxidase (SBP) (53.4 U/mg), and *p*-toluensulfonic acid (99%) (TSA), were purchased from Sigma. Aniline and ammonium hydroxide were acquired from Química Dinámica, Mex. *N*-Methyl-2-pyrrolidinone (NMP) was acquired from Aldrich. Aniline was distilled at reduced pressure and stored at −28 °C prior to use, and all other reagents were used as received. The porous inorganic substrates studied were: controlled pore glass (CPG-LP), 72.9 nm average pore diameter (Electro-nucleonics), controlled pore glass (CPG-SP), 7.5 nm average pore diameter (Sigma), sodium zeolite Y (NaY) and silica gel (SIL) (mesh size 70–230, 500 m^2/g BET surface) were both acquired from Aldrich. Molecular sieve MCM-41 was synthesized following the procedure reported by Beck et al. [31] and characterized in a previous work [32]. Natural zeolite, mordenite-type from the San Luis Potosi State in Mexico, whose characterization has been reported [33], was used in two forms: as received (MOR) and acid treated (MOR-AT). Solid substrates were: Wollastonite NYAD grade F-1 was a gift from Nyco Minerals (WOL-F1), Glass Fiber Type E (SGF-TE) that was acquired from Grupo Vitro, Mex. and fuming silica (Cab-O-Sil, M5) was acquired from Cabot, Co (FSIL).

2.2. Polyaniline synthesis

PANI synthesized by the chemical method reported by Wei and Hsueh [34] was used as reference for polymer structure. Acid-treated mordenite was prepared by stirring overnight the as-received mineral in HCl aqueous solution (1.0 N). The PANI was synthesized by enzymatic polymerization in the inorganic porous hosts as follows: aniline was absorbed into the different hosts from the liquid phase, at room temperature, for 7 days. The aniline saturated hosts were immersed in 60 ml of distilled water containing an equimolar amount of TSA with regard to aniline. Afterwards, HRP or SBP peroxidase was placed into the mixture reaction and the hydrogen peroxide added during the total reaction time. The rate of addition was adjusted to a maximum value of 3.6 μl/min in order to prevent denaturing of the enzyme. This mixture was kept at 0 °C under continuous stirring for a time period depending on the aniline concentration, typically between 5 and 8 h. The product thus obtained was recovered from the polymerization vessel, filtered and washed thoroughly with water followed by methanol, to remove residual monomer and oligomers, and then lyophilized.

2.3. Characterization

Pore size measurements in mordenite were performed by thermoporometry, using a modulated DSC TA Instrument Model 2920. The experimental details can be found elsewhere [35]. Alternatively, the pore size distribution was determined by nitrogen physisorption using the BJH model [36] on the desorption isotherm. The electronic absorption spectra of the PANI were acquired in NMP solutions, using a diode array spectrophotometer (Hewlett Packard HP 8452A). For these analyses, the solid samples were dedoped with NH_4OH 0.2 M, lyophilized and extracted with NMP under stirring. Monomer content and PANI thermal stability in the inorganic substrates was determined by thermogravimetric analysis (TA Instruments TGAQ500), using a 20 °C/min heating rate under nitrogen atmosphere from room temperature to 600 °C and under air from 600 to 800 °C. X-ray scattering microanalysis was carried out using an energy-dispersive X-ray spectrometer (EDAX) coupled to a scanning electron microscope (TOPCON SM-510). The samples were coated with an Au-Pd alloy before analysis. The X-ray photoelectron spectroscopy (XPS) analysis was carried out on a modified laser ablation system, Riber LDM-32, using a Cameca Mac3 analyzer. The base pressure in the analysis chamber was in the low 10^{-10} Torr range, and about 10^{-9} Torr in the sample loading chamber. The X-ray Al Kα line at 1486.6 eV was used for excitation. The binding energies were calibrated with reference to Cu $2p_{3/2}$ at 932.67 eV and Ag $3d_{5/2}$ at 368.26 eV, respectively. The resolution attained with this set-up is 1.1 eV measured on the C 1s signal of a graphite target. Spectra were collected by acquiring data every 0.2 eV and the energy resolution was 0.5 eV. The core-level spectra for C 1s, and N 1s, were obtained. Background subtraction was done using the Tougaard