

A novel silk fibroin-supported iron catalyst for hydroxylation of phenol

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Abstract: The aim of this study was to explore the potential use of silk fibroin (SF) as a catalyst support material for phenol hydroxylation reactions. Iron-substituted silk fibroin fibers were prepared using formic acid at room temperature and characterized using inductively coupled plasma atomic-emission spectrometry, scanning electron microscopy, Fourier transform infrared spectroscopy (FTIR) and optical microscopy. Measurement of an FTIR spectrum showed that the secondary structure was β -structure before and after iron substitution. To evaluate the catalytic properties of prepared catalyst, phenol hydroxylation reaction was carried out using aqueous hydrogen peroxide as an oxidant. An excellent transformation of phenol into dihydroxybenzenes (catechol and hydroquinone) was achieved. Phenol conversions of 3.3%, 61.2%, and 80.3% were obtained at room temperature, 40 °C and 60 °C respectively. It was found that no further phenol conversion proceeded because catalysts became separated from the reaction system during the reaction. No significant leaching of the iron was detected. Catalyst could be reused several times without a significant change in activity. Parent silk fibroin fibers without iron were inactive.

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INTRODUCTION

The hydroxylation of phenol is industrially a very important reaction, since it produces diphenols (e.g., hydroquinone and catechol), which are important starting materials in the production of chemicals used as agrochemicals, pharmaceuticals, flavors and aromatic essences. Hydroquinone is used as an intermediate to produce antioxidants for rubber, food and pharmaceuticals. Catechol is used as rubber hardening agent, electroplate additive, skin preservative as well bactericide.

H₂O₂-based catalytic hydroxylation can be environmentally as well as economically very favorable because H₂O is formed as the only by-product. H₂O₂ has high content of active oxygen and is rather inexpensive compared to various organic peroxides and peracids. Hydroxylation of phenol with hydrogen peroxide using water as the reaction solvent is mainly performed for producing diphenols in an environmentally friendly way.^{1–5}

Homogeneous catalysts such as mineral acids, metal ions and metal complexes are difficult to separate and recover from the reaction mixture, which prevents their practical utilization. Therefore, numerous heterogeneous catalysts such as metal oxides,¹ metal complex oxides,^{2,3} encapsulated metal complexes^{4,5} and hydrotalcite-like compounds⁶ have been studied. Although activated carbon and oxides (alumina,

silica, zeolites) are the most used supports, polymeric supports have recently attracted considerable attention for their low cost, ease of preparation, catalyst recycling, and versatile conditioning.^{7,8} Many researchers have focused studies on applying polymers as the matrices for dispersing metallic centers.⁹ Specific properties of polymers, such as their ability for stabilization of finely dispersed metallic nanoparticles, swelling of polymer matrix and changing reactivity of catalysts due to various hydrophobic/hydrophilic characters, make polymeric carriers very attractive as potential supporting materials. Supporting catalytic metals on specially tailored supports can add new functionalities such as reaction enantioselectivity. Biopolymers have been extensively studied during the past decade for chiral separation. For this reason, interest is now focusing on the use of biopolymers as supports for catalysis.¹⁰ Practically all classes of natural biopolymers such as silk,^{11–18} starch, cellulose and chitosan have been investigated as supports for metal complexes.⁷

Chitosan and silk fibroin have been used as catalytic supporting materials. Recently, research into supported catalysis has been carried out on chitosan.^{19–21} The main characteristics of this biopolymer are its high amine group content and its cationic behavior in acidic solutions. The presence of amine groups leads to interesting chelating properties for metal

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cations. Chitosan-supported palladium catalyst was studied for chlorophenol dehalogenation¹⁹. On the other hand, utilization of silk fibroin-supported zero-valent metal catalyst for asymmetric hydrogenation was reported by Akabori and co-workers during 1956 to 1961.^{11–14} More recently, a core-shell nanostructured gold colloid-silk fibroin bioconjugate was reported by Zhou *et al.*¹⁵ and, lately, silk fibroin-supported palladium catalyst has been studied for chemoselective hydrogenation.^{16–18,22}

Silk fibroin is an attractive natural fibrous polymer produced by the species *Bombyx mori*.^{23,24} It is known that besides material for clothing, fibroin has also been investigated as one of the promising resources of biotechnology and biomedical materials.²⁵ Another important reason for interest in this material is its versatility.²⁶ It can be conditioned in forms as different as beads, powder, films, sponges, gels, fibers, membranes and hollow fibers, or as a coating material on mineral membranes that may be helpful in the design of new catalytic systems. The total content of glycine, alanine, serine and tyrosine comprised more than 90 mol% of the whole volume of the silk fibroin, and very few residues of sulfur amino acids, which can be a strong catalyst poison of metals, are included.¹⁷ The fibroin macromolecules are closely packed owing to higher content of hydrophobic residues as compared to that of hydrophilic polar groups. Fibroin sparingly swells in water and is soluble in concentrated solutions of LiCNS, LiBr, CuCl₂, Ca(CNS)₂, and ZnCl₂, copper ammonia solution (CuSO₄ + NH₄ OH), phosphoric acid, and some other inorganic solvents.²⁷ Although several organic solvents have been tried to obtain stable protein solution, formic acid is known as a good solvent at present because this solvent allows producing a stable solution and has an excellent film and fiber formation properties for silk protein polymer.²⁸

The powdery form of supported catalysts often becomes a great problem for practical uses in fine chemicals synthesis. Hence, much research has been directed at immobilizing catalyst powders on a carrier material for convenient handling. We have studied the preparation and catalytic application of iron loaded silk fibers. Catalytic silk fibers were successfully prepared by a simple technique. To the best of our knowledge, no report concerning the use of silk fibroin supported iron catalyst for the hydroxylation of phenol has been published in the literature. The aim of the present paper is to explore the application of silk fibroin as catalyst support material for phenol hydroxylation reactions.

EXPERIMENTAL

Materials

The native silk of *Bombyx mori* (domestic silkworm) was obtained in reeled form from the Silk Industry Research Center, Bursa, Turkey. Nitrate of Fe(III), phenol, hydroquinone, catechol, hydrogen peroxide

(30%) and formic acid were purchased from Sigma-Aldrich. Other reagents and solvents used were HPLC grade and obtained from Sigma-Aldrich.

Catalyst preparation

The raw silk fibers of *Bombyx mori* were processed three times (40 min each) in 0.5 wt% Na₂CO₃ solution at 98–100 °C to remove sericin (degumming), rinsed with deionized water, and dried at room temperature. The degummed fibroin (1 g) was dissolved in 50 mL of formic acid (98%), by stirring at 30 °C for 2 h. The ratio of degummed silk to formic acid was always kept at 1:50 (weight/volume). Iron nitrate (0.5 g) was added to the prepared solution and mixed for another 2 h. The resulting solution was poured into Petri dishes with a diameter of 10 cm and left under the fume hood to dry. After drying the prepared iron containing fibroin fibers was washed several times with deionized water in order to remove excess iron.

Catalyst characterization

Morphology of the catalyst samples was observed using an optical microscope (Olympus C-4040). Scanning electron micrographs were recorded on a Philips XL 30S FG. Infrared spectra in the region 400–4000 cm⁻¹ were recorded in KBr pellets using a Shimadzu 8101 FT-IR spectrophotometer. The iron content of the catalyst was determined by an inductively coupled plasma spectrometer (ICP, Varian 8410). Standard solutions for ICP calibrations were prepared by using ICP multi-element standard solution. A digestion procedure (contact of the catalyst with 65% nitric acid) was used to disrupt the support and dissolve the iron.

Phenol hydroxylation and analytical methods

Phenol hydroxylation was carried out in a three-necked flask (250 mL) equipped with a magnetic stirrer, reflux condenser and temperature controller. First, 0.2 g of phenol was dissolved in 100 cm³ deionized water (initial phenol concentration = 0.021 mol dm⁻³), then a certain amount (0.1 g) of catalyst was added to the flask. After heating the mixture containing phenol solution and catalyst to the desired reaction temperature, an appropriate amount of 30 wt% aqueous H₂O₂ was added for the required phenol-to-hydrogen peroxide molar ratio of 1 and the reaction was started. The reaction was monitored by taking aliquots at different time intervals. Immediately after sample collection, vials containing reaction mixture were covered with aluminum foil to protect them from light. Samples from the reaction mixtures were analyzed by high-performance liquid chromatography (HPLC). An Agilent 1100 HPLC system equipped with a reversed-phase C18 column (250 mm × 4.6 mm i.d., 5 μm particle sizes, Lichrospher[®]) was used for HPLC analysis. Aliquots of 20 μL were injected and separation was carried out with an isocratic mobile phase consisting of methanol-water (60:40, v/v) with a flow rate of 1 cm³ min⁻¹ and at 30 °C column

temperature. The detection wavelengths were 280 nm for detecting phenol, catechol, and hydroquinone. Prior to injection of a sample, the column was equilibrated with the mobile phase at a flow rate of $1 \text{ cm}^3 \text{ min}^{-1}$ for at least 20 min or until a steady baseline was obtained. The percentage of each compound in the reaction mixtures was estimated directly from the corresponding peak areas.

Leaching tests

To test if metal ions were leaching out from the catalyst, the fiber catalyst was removed from the hot reaction mixture. Then the reaction mixture was monitored by HPLC analysis to check the progress of the reaction by leached metal ions, if any. The leaching test of the prepared catalyst was also evaluated by digesting the reaction mixture after each reaction completion. The reaction mixture was digested in a Teflon beaker by the addition of HNO_3 after all the organic solvent was completely evaporated. Metal contents of digested residue for all catalysts were determined using inductively coupled plasma (ICP) technique.

RESULTS AND DISCUSSION

Catalyst characterization

Photographs of fibroin fibers without iron loading and with iron loading are given in Fig. 1(a) and (b),

respectively. With iron loading the color of fibers changed to red-brown, as expected. As seen from Fig. 1(b), color changes throughout the fiber showed that iron content of fibers was not evenly distributed. Sufficient mixing during catalyst preparation and drying stages could overcome this problem. Using optical microscopy, we studied the changes in color and texture of these fiber catalysts. Figure 1(c) shows one example of the polarized light optical microscopy pictures of iron-loaded fibers. The data showed that a sample with no added iron has a dull texture that did not reflect light very well. Fibers with iron appeared to acquire a 'glassy' cover which, under optical microscopy, gave fibers a sharper definition and glossy appearance, making them more reflective to light. Increasing levels of iron on fibers further enhanced the observed glassy layer and, as expected, changed the particle colour to red-brown, similar to the familiar color of iron oxide. Only the color changed, from white, to a light ferric oxide red/brown and finally to a darker red-brown.

Scanning electron microscopy (SEM) images evidenced the fibrous structure and the smooth surface of the fibroin fiber catalyst (Fig. 2a, b). The iron dispersion was attributed to an interaction between the iron and amino acids of the silk fibroin, increasing the resistance to growth of the iron cluster.

Figure 3 shows the FTIR spectra of fibroin fiber before (Fig. 3a) and after iron loading (Fig. 3b). It

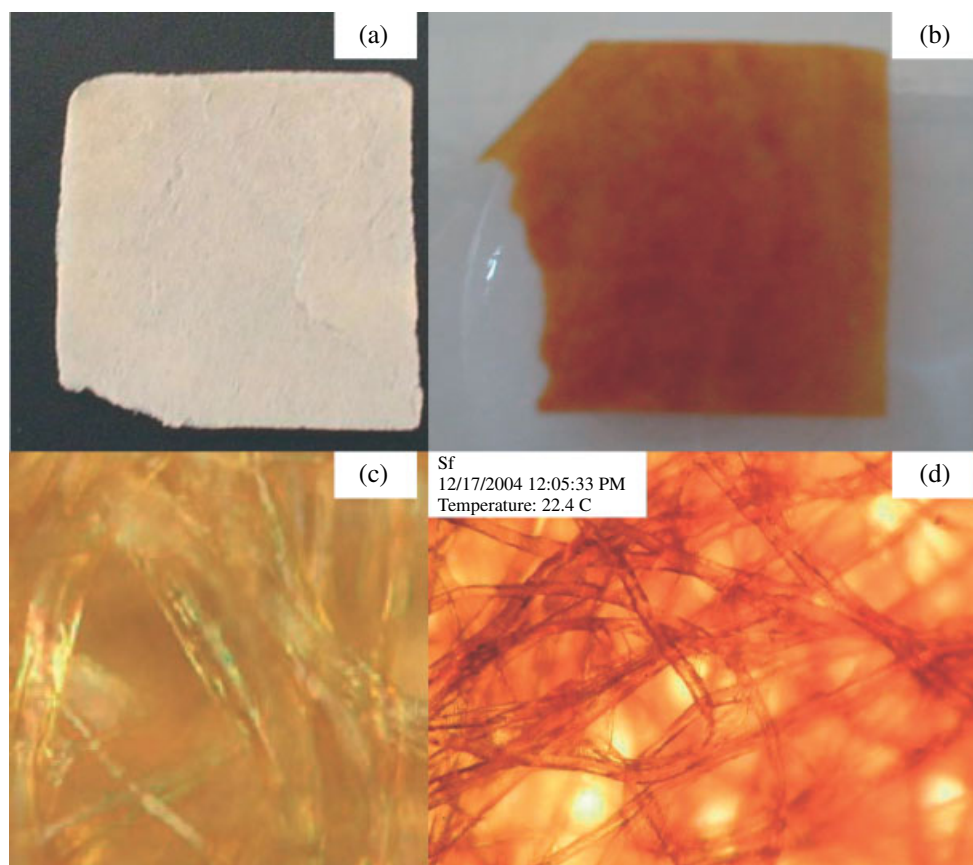


Figure 1. Photographs of silk fibroin fiber without iron (a), and with iron (b), polarized light microscopy image (c) and optical microscopy image of fibroin fiber with iron (d).

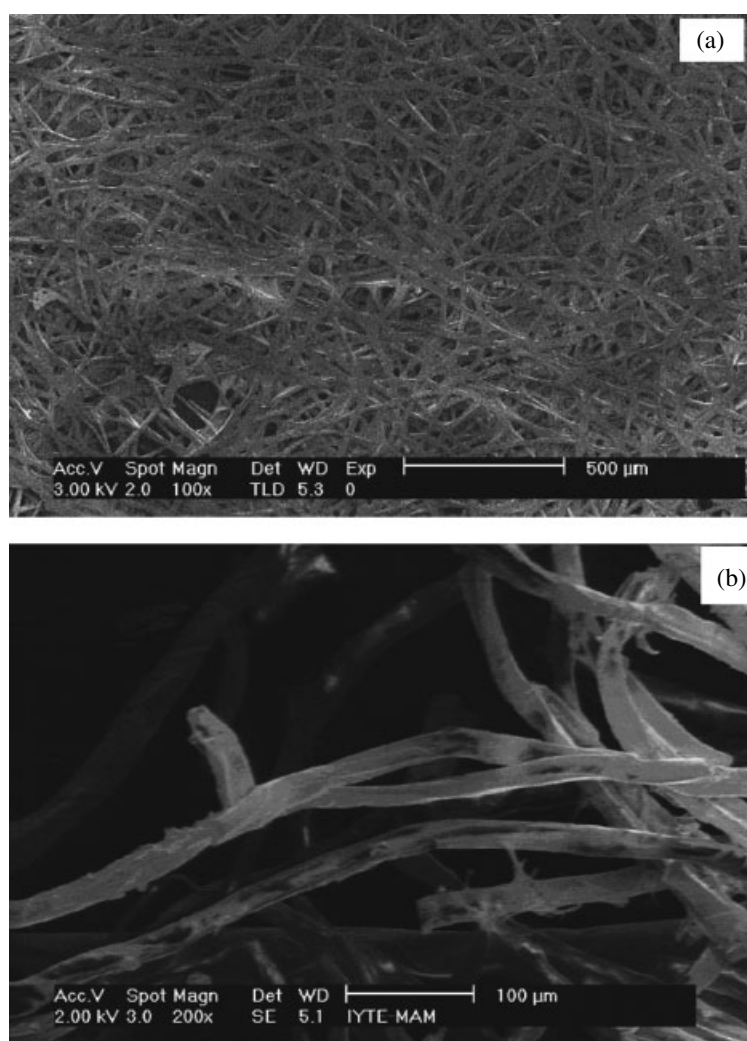


Figure 2. SEM micrographs of fibroin fiber catalyst at $\times 100$ (a) and $\times 200$ (b) magnifications.

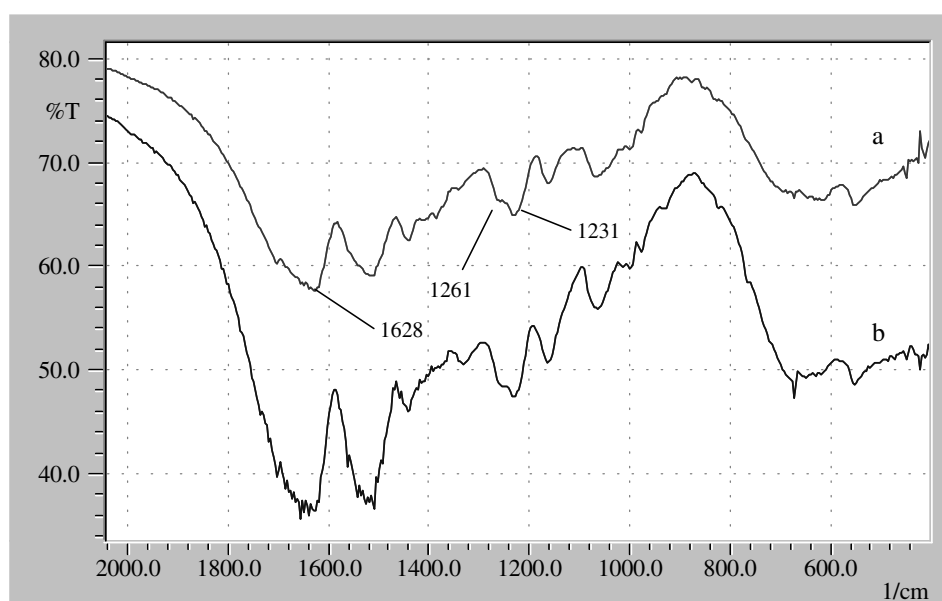


Figure 3. FTIR spectra of fibroin fiber (a) without iron (b) with iron loading.

was well established for fibroin and other proteins that the peak position of the absorption frequency was quite sensitive to the secondary structure. Absorption bands

at 1628 (amide I), 1533 (amide II), and 1261 cm^{-1} (amide III) were assigned to the β -sheet conformation. Both spectra before and after iron loading were

apparently ascribable to the β -sheet structure. There was no structural changes caused by iron loading by analyzing the amide I, II, III bands in IR spectroscopy. This result suggested that no conformational change of fibroin fiber occurred from the β -sheet to random coil structure due to iron loading. The insolubility property and crystal structure findings were consistent with the literature.²⁸

Phenol hydroxylation

The catalytic hydroxylation of phenol usually gives two products: catechol and hydroquinone. These products are the only expected ones, as the –OH group of phenol is *ortho* and *para* directing. The reaction simply adds a new –OH group to the molecule. In this study, catechol and hydroquinone were the major products of phenol hydroxylation reaction using H₂O₂ as oxidant and using the iron loaded fibroin fiber catalysts. Minor product, if any, was not detectable in HPLC under the conditions used here. Table 1 summarizes the results of phenol hydroxylation with H₂O₂ over silk fibroin-supported iron catalysts in powder and fiber form. There was only a small difference in the activities of the powder form and fiber form, as both catalysts contained 4.4 wt% Fe. For both types of catalysts, catechol and hydroquinone were produced as major products. A high selectivity toward catechol was detected for the Fe–SF catalysts. A plausible explanation for the above experimental results of phenol hydroxylation was that iron sites in all the iron-containing solid samples were mainly responsible for the heterogeneous phase transformation of phenol into catechol and hydroquinone under the selected reaction conditions. The phenol conversion increased from 61.2% to 80.3% with increasing temperature from 40 to 60 °C, while a low activity (3.3%) of phenol hydroxylation was observed at 25 °C.

When using a supported metal catalyst a crucial issue is the reusability of the catalyst. The Fe–SF

fiber catalyst was therefore tested for recoverability and reusability over successive runs. After each run, the original Fe–SF catalyst was removed, washed several times with water, and reused under the same reaction conditions as for the initial run without any regeneration. Results from Table 1 showed that the Fe–SF catalyst could be recovered and reused in further reaction without a significant decrease in activity. Although the catalytic activity was gradually diminished, a conversion of 57% was still achieved in the second run, and 51% conversion was achieved in the third run. It was found that, with recycling of the catalysts, catechol selectivity increased along with a slight decrease in hydroquinone selectivity. This might be due to higher iron content in the catalyst, which was probably lost after each catalyst recycling. Another point of great concern for most anchored catalysts is the possibility that some active metal migrates from the solid support to the liquid phase and that this leached metal would become responsible for a significant part of the catalytic activity. After the first and second runs, reaction samples were analyzed with the inductively coupled plasma atomic-emission spectrometry (ICP-AES) technique. No significant amount of iron was determined within the detection limit of ICP-AES. Therefore, metal leaching could not be the direct reason for the decrease in yield. The decrease in catalytic activity could be attributed to catalyst deactivation since the color of fibers became darker compared to its initial color. In order to determine whether leaching was a problem, another experiment was performed to estimate the contribution of leached iron to the catalytic activity by performing a filtration during the course of the reaction to remove the solid phase. If the catalytic reaction continued, this would indicate that the active species was leached metal rather than the supported catalyst. Thus an experiment using fiber catalyst (Fig. 4) under the same reaction conditions as given in Table 1 was interrupted after 5 min. The hot aqueous phase without the catalyst

Table 1. Conversion and selectivity in the liquid-phase hydroxylation of phenol with H₂O₂ in the presence of iron-loaded silk fibroin^a

Catalyst type	Temperature (°C)	Phenol conversion (%)	Selectivity (product) ^b (%)	
			Catechol	Hydroquinone
Powder	25	3.3	100.0 (3.3)	–
Powder	40	61.2	64.2 (39.3)	35.8 (21.9)
Powder	60	80.3	57.5 (46.2)	42.5 (34.1)
Fiber (1st run)	40	60.0	55.0 (33.0)	45.0 (27.0)
Fiber (2nd run ^c)	40	57.0	63.8 (36.4)	36.2 (20.6)
Fiber (3rd run ^d)	40	51.0	70.6 (36.0)	29.4 (15.0)
Fiber (hot filtration ^e)	40	36.0	70.7 (25.5)	29.3 (10.5)
Unloaded fiber	40	no activity ^f	–	–
No catalyst	40	no activity ^g	–	–

^a Reaction conditions: 0.2 g phenol in 100 cm³ water as solvent, 0.1 g catalyst, time = 2 h, phenol/H₂O₂ molar ratio = 1.

^b Conversion and selectivity were determined by HPLC. Yield for a specific product can be calculated as conversion × selectivity.

^{c,d} Catalyst thoroughly washed with water and used in a second and third reaction cycles respectively.

^e The fiber catalyst was removed from the reaction mixture by hot filtration, after 5 min.

^f No catalytic activity was detected without catalyst towards hydroxylation of phenol.

^g No catalytic activity was detected with fibroin fiber containing no iron as support material.

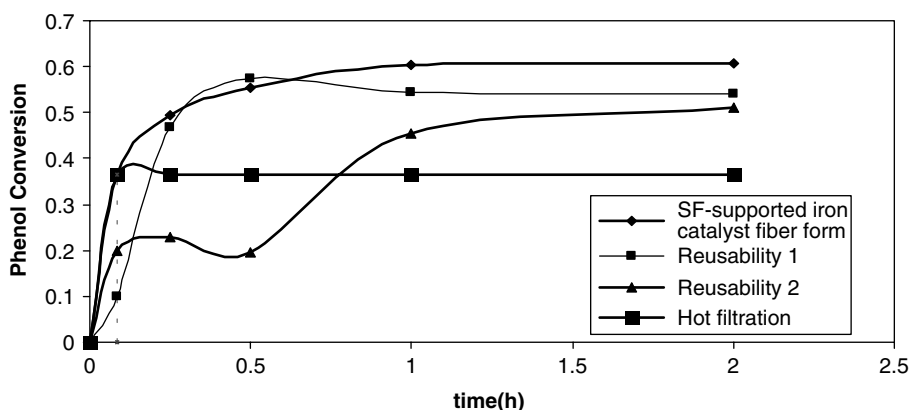


Figure 4. Phenol conversion using fiber form of catalyst (reaction conditions: phenol/H₂O₂ molar ratio = 1, 0.1 g catalyst, 40 °C).

obtained by removing fiber catalyst was kept in the reactor at 40 °C for another 2 h reaction. The composition of the reaction mixture was determined using HPLC at regular intervals. The data from HPLC determinations gave quantitative information about residual, catalytically active iron in solution after supported catalyst separation. Within experimental error, no increase in the amount of product was detected, proving there to be no contribution from leached species (homogeneous catalysis). In order to determine the absolute amount of the iron species dissolved in solution caused by leaching, the crude reaction mixtures were evaporated to dryness and analyzed using ICP-AES. It was shown that a trace amount of the original iron species was lost into solution during the course of a reaction. This almost negligible leaching level, which is at the limit of detection of the ICP-AES, accounts for the recoverability and reusability of the Fe-SF catalyst.

CONCLUSIONS

A new biopolymer metal has been prepared by a simple method using fibroin and iron as raw materials, and has been found to catalyze hydroxylation of phenol to catechol and hydroquinone. The iron-loaded silk fibroin catalyst can be used by cutting up the fibers with scissors and is removed easily from the reaction mixture using a pair of tweezers or by simple filtration. We believe that the present method should find broad catalytic application since the flexible and easy-handling catalyst fibers would be expected as a promising material for catalytic applications, clean energy production and material conversion process based on green chemistry. The techniques described here for preparing significantly active silk fibers are effective and economic ways of providing new catalytic materials for industrial application.

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