

Article

Specifically Grafting Hematin on MPTS-Coated Carbon Nanotubes for Catalyzing the Oxidation of Aniline

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Abstract: Catalysts supported on nanomaterials have been widely investigated for the treatment of hazardous materials. This work has developed a novel method for grafting hematin on nanomaterials for catalyzing the oxidation of aniline in order to remove aniline from wastewater. Magnetic multi-walled carbon nanotubes (M-MWCNTs) were coated with a layer formed through the hydrolysis and condensation of 3-mercaptopropyltriethoxysilane (MPTS). Hematin was specifically grafted on the MPTS-coated M-MWCNTs through thiol-alkene reaction. Hematin-MPTS-M-MWCNTs were used to catalyze the oxidation of aniline, and a high efficiency has been obtained. Consecutive use of the conjugate of hematin-MPTS-M-MWCNTs has been investigated, and the activity has been retained to a significant extent after five reaction/cleaning cycles. The result demonstrates that hematin-MPTS-M-MWCNTs are efficient for catalyzing the oxidation of aniline. The methodology for the specific grafting of hematin is of general utility, it is an easy-to-operate method and can be extended to other supports. Potentially, hematin-MPTS-based conjugates have a widespread application in catalyzing the removal of aniline from wastewater.

Keywords: hematin; aniline; polyaniline; wastewater; carbon nanotubes

1. Introduction

In coal-conversion processes, aniline is a by-product and is soluble in process water [1]. Aniline has caused great concerns as it is very toxic to human beings and organisms [2–5]. Removing aniline from the wastewater has been paid great attention, and enormous research work on this topic has been carried out. Waste rubber tire [1], PAM/SiO₂ [6], activated carbons [7,8], modified sawdust [9,10] and PMAA/SiO₂ [11] have been studied as adsorbents for adsorption of aniline from wastewater. Chemical and biochemical processes, such as moving bed biofilm reactor [4], heterogeneous catalytic wet air oxidation of aniline [5], aniline degradation by photocatalysis and ozonation [12,13], and degradation of aniline with bacteria [13,14] have been investigated for converting aniline. Enzymatic catalysis for the conversion of aniline is also attractive. Hemoglobin was encapsulated in a silica-based matrix for catalyzing the removal of aniline from aqueous solution [15]. Horseradish peroxidase has been intensively studied for the enzymatic catalysis of the oxidative polymerization of aniline [16–18]. Polymerization of aniline catalyzed by enzymes can be carried out under milder conditions, and the formed polyaniline can be removed from the wastewater through precipitation. However, when pH conditions are more acidic or more alkaline, not around the optimal pH conditions that are most suitable for the enzymatic catalysis, the enzymatic activity may significantly decrease.

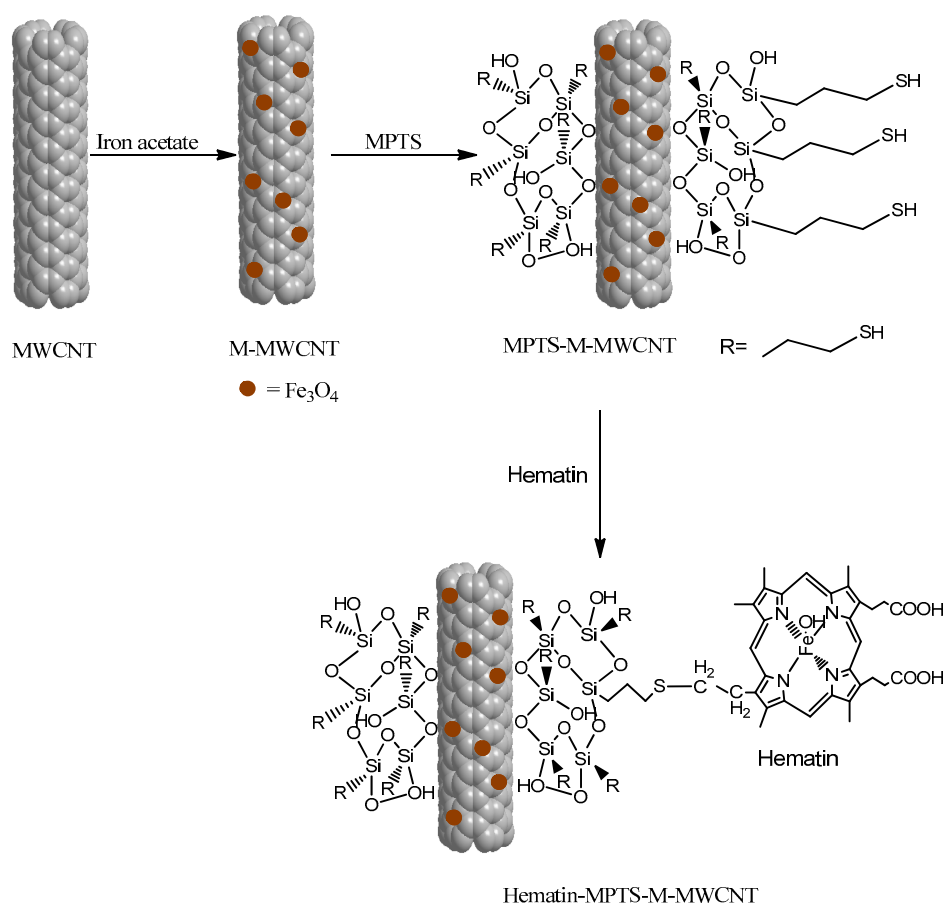
Hematin is a hydroxyl ferri protoporphyrin. It is an inexpensive alternative to horseradish peroxidase and has been used for the catalysis of polymerization of aniline [16]. Hematin is more stable in a wider range of pH conditions and more tolerant of H_2O_2 than horseradish peroxidase [17,18]. Thus, polymerization of aniline can be catalyzed by hematin under various conditions [19]. However, hematin has a low solubility in aqueous solutions and tends to aggregate at acidic conditions [16]. This is the obstacle for application of hematin [16]. Grafting hematin on supports can be a solution to the problem [20]. Catalysts supported on nanomaterials have been investigated for the treatment of hazardous materials. Magnetite-supported hematin has been used in the aniline elimination and in the removal of phenol [20]. Polymerization of aniline was catalyzed by the halloysite nanotubes-supported hematin [21]. In this work, 3-mercaptopropyltrimethoxysilane (MPTS) was used to coat carbon nanotubes (CNTs), hematin was specifically grafted on the MPTS-coated CNTs. The grafted hematin has demonstrated a high catalytic efficiency in the conversion of aniline. Potentially, hematin grafted on MPTS-coated supports can have a widespread application in catalyzing the removal of aniline from wastewater.

2. Results and Discussion

2.1. Grafting Hematin

The procedures for the formation of magnetic nanoparticles, MPTS coating, and grafting hematin on MWCNTs are illustrated in Scheme 1. Figure 1 shows the transmission electron microscope (TEM) images for the functionalized MWCNTs. Magnetic nanoparticles were clearly observed on the MWCNT (M-MWCNT in Figure 1b). By hydrolysis and condensation of MPTS, a thin layer was formed on the surface of the M-MWCNT (MPTS-M-MWCNT in Figure 1c). As MPTS has a smaller density than water, MPTS-M-MWCNT stayed on the top of the solution. After adding 5,5'-Dithiobis(2-nitrobenzoic Acid) (DTNB), the solution exhibited a yellow color. This is due to the reaction of the $-SH$ groups with DTNB [22]. Through the thiol-alkene reaction as illustrated in Scheme 1, hematin was specifically grafted on MPTS-M-MWCNTs. As hematin has a relatively large density (7.87 g/cm^3), the precipitation of hematin-MPTS-M-MWCNTs at the bottom of the solution was observed (hematin-MPTS-M-MWCNT in Figure 1d). DTNB was added to the solution of hematin-MPTS-M-MWCNTs, color change was not observed. The result indicated that hematin had reacted with the $-SH$ groups of MPTS-M-MWCNTs. The morphology of MPTS-M-MWCNTs was not changed after grafting hematin. Figure 2 shows Fourier transform infrared (FTIR) spectra for hematin-MPTS-M-MWCNTs and other CNTs. The bands at 1096 and 1030 cm^{-1} were assigned to $Si-O-C$ and $Si-O-Si$ asymmetric stretching, respectively [23]. $C=O$ stretching vibrations of the $-COOH$ groups of hematin was reflected by the band at 1720 cm^{-1} [24]. The FTIR spectra confirmed the grafting of hematin on MPTS-M-MWCNTs.

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Scheme 1. Schematic presentation of the procedures for grafting hematin. MWCNT: multiwalled carbon nanotube; M-MWCNT: magnetic MWCNT; MPTS-M-MWCNT: MPTS-coated M-MWCNT. Hematin was grafted on MPTS-M-MWCNT through the reaction between the -SH and alkene groups.

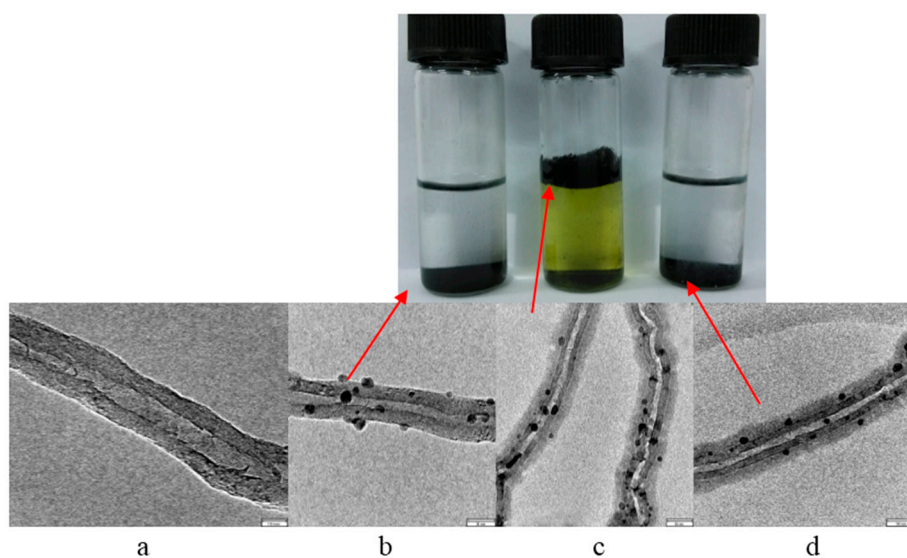


Figure 1. Transmission electron microscope images. MWCNTs: (a) purified; (b) magnetic; (c) MPTS-coated (MPTS M-MWCNTs); (d) hematin functionalized (hematin-MPTS-M-MWCNT). Scale bars: a—10 nm; b—20 nm; c—30 nm; d—30 nm. The arrows indicate the corresponding MWCNTs in the solutions (photographs in the upper section).

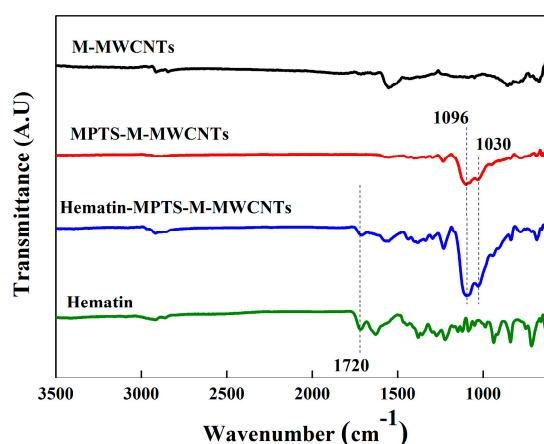


Figure 2. Fourier transform infrared spectra for functionalized carbon nanotubes. MWCNTs: black; magnetic (M-MWCNT); red: MPTS-coated (MPTS M-MWCNT); blue: hematin functionalized (hematin-MPTS-M-MWCNT).

2.2. Oxidation of Aniline Catalyzed by Hematin-MPTS-M-MWCNTs

Prior to catalyzing the oxidation of aniline, hematin-MPTS-M-MWCNTs was used to catalyze the oxidation of 3,5,3',5'-tetramethylbenzidine (TMB) in the presence of H_2O_2 . Thus the catalysis capability of hematin-MPTS-M-MWCNTs can be directly observed. In the control tube only containing hydrogen peroxide and TMB, there was no color change after incubation for 5 min (Figure 3a), indicating that TMB was almost not oxidized within the incubation period of time. While in the tube containing hematin-MPTS-M-MWCNTs, TMB and hydrogen peroxide, the solution exhibited a blue color (Figure 3a) [25], indicating that TMB was oxidized under the catalysis of hematin-MPTS-M-MWCNTs. The absorbance peaks at 367 nm and 650 nm (Figure 3b) were ascribed to the product from the oxidation of TMB.

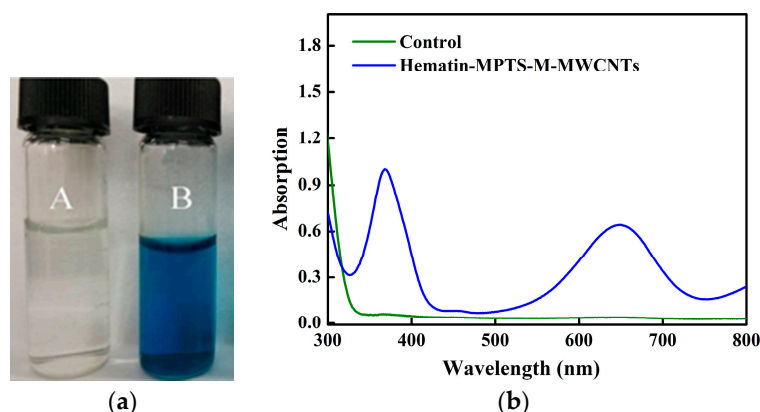


Figure 3. Oxidation of 3,5,3',5'-tetramethylbenzidine under the catalysis of hematin-MPTS-M-MWCNTs. (a) Samples. A: H_2O_2 (1 μ L/mL), TMB (0.02 mg/mL); B: H_2O_2 (1 μ L/mL), TMB (0.02 mg/mL), hematin-MPTS-M-MWCNTs (1 mg/mL). To observe to the color more clearly, hematin-MPTS-M-MWCNTs in sample B was removed by centrifugation; (b) UV-vis spectra.

The conjugate of hematin-MPTS-M-MWCHTs was further used as a biomimetic catalyst for the oxidation of aniline. Hematin-MPTS-M-MWCHTs could be well dispersed in the reaction media (Figure S1) and easily separated from the solution by using a magnet. The reaction liquid exhibited a purple-black color as shown in Figure 4a. The black color is due to hematin-MPTS-M-MWCHTs, and the purple color is ascribed to the oxidation state of polyaniline, indicating the availability of

hematin-MPTS-M-MWCHTs for catalyzing the oxidation of aniline forming polyaniline. With the polymerization proceeding, polyaniline associated to form insoluble products and precipitated to the bottom of the reaction media. Pernigraniline is an intermediate state of polyaniline [26], and has a relative strong absorbance at 528 nm. The evolved pernigraniline in the reaction media was monitored through measuring a series of UV-vis spectra at a regular time interval. In Figure 4b, the absorbance peak at 528 nm was assigned to pernigraniline [26], and the absorption of pernigraniline decreased with the reaction time, consistent with the polymerization and precipitation procedures that occurred in the solutions.

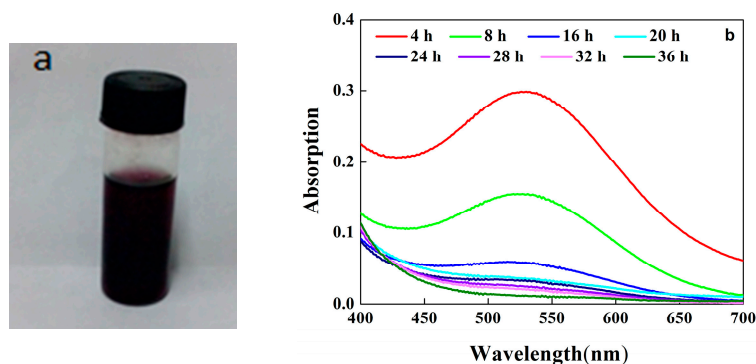


Figure 4. UV-vis spectra monitoring the oxidation process under the catalysis of hematin-MPTS-M-MWCNTs. (a) The liquid exhibited a purple-black color after reaction; (b) UV-vis spectra.

Figure 5a shows the effect of pH conditions on the conversion of aniline. Acidic conditions are favorable for the oxidation of aniline, with an optimal pH 5. However, in the range of pH 2 to pH 7, the effect of pH is not significant. In comparison, the concentration of hydrogen peroxide has a relatively larger effect on the oxidation of aniline (Figure 5b). The conversion of aniline increased with the concentration of hydrogen peroxide until 1 M.

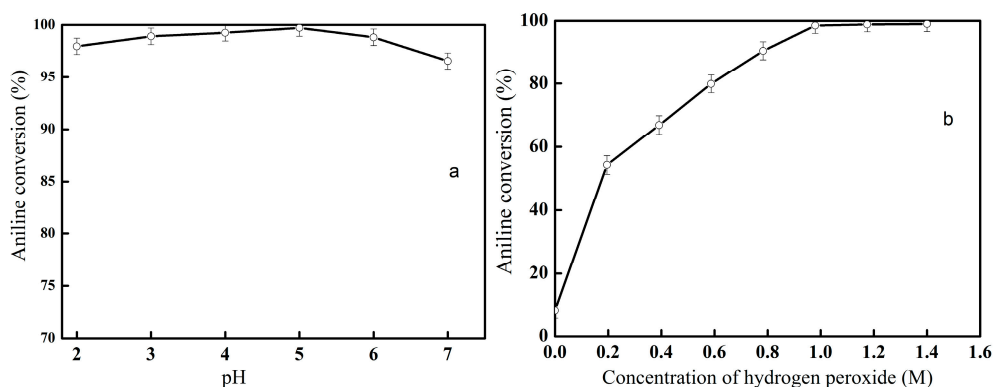


Figure 5. Effect of pH and concentration of hydrogen peroxide on the conversion of aniline. (a) Effect of pH conditions on the conversion of aniline; (b) Effect of hydrogen peroxide on the oxidation of aniline.

Figure 6 shows the aniline conversion under the catalysis of hematin-MPTS-M-MWCNTs as a function of reaction time. In Figure 6, one curve is for the result with polyaspartic acid, another one without using polyaspartic acid. The control reaction catalyzed by free hematin is also shown. The aniline conversion catalyzed by free hematin is higher than that of hematin-MPTS-M-MWCNTs, but the difference is not great. In contrast, the reaction catalyzed by the immobilized hematin using polyaspartic acid achieved the highest aniline conversion. Polyaspartic acid is a biodegradable polymer and has been produced at a relatively low cost. Herein, we used polyaspartic acid to promote the

aggregation and precipitation of the synthesized polyaniline. The aniline conversion increased with the reaction time. The aniline conversion reached 97.7% after 5 days of reaction time for the case without using polyaspartic acid. In comparison, for the oxidation reaction with addition of polyaspartic acid, the aniline conversion reached 98.9% after 3 days of reaction time, indicating that the conversion of aniline was improved by using polyaspartic acid to promote the aggregation and precipitation of the synthesized polyaniline. Using polyaspartic acid in aniline conversion has several advantages. Polyaspartic acid has a very good solubility in aqueous solutions. It can be easily dissolved in the solution of aniline. Polyaspartic acid is biodegradable, addition of polyaspartic acid does not cause extra pollution. Polyaspartic acid has many carboxyl groups, and polyaniline has amine and amino groups. The carboxyl groups of polyaspartic acid had electrostatic interactions with the amino groups and hydrogen bonding interactions with amine groups. As a result, only a small amount of polyaspartic acid was enough to promote the aggregation and precipitation of the synthesized polyaniline. The polyaniline clusters obtained by using polyaspartic acid were larger than that obtained without using polyaspartic acid (Figure 7), confirming the promotion of polyaniline aggregation by polyaspartic acid. This facilitated the conversion of aniline.

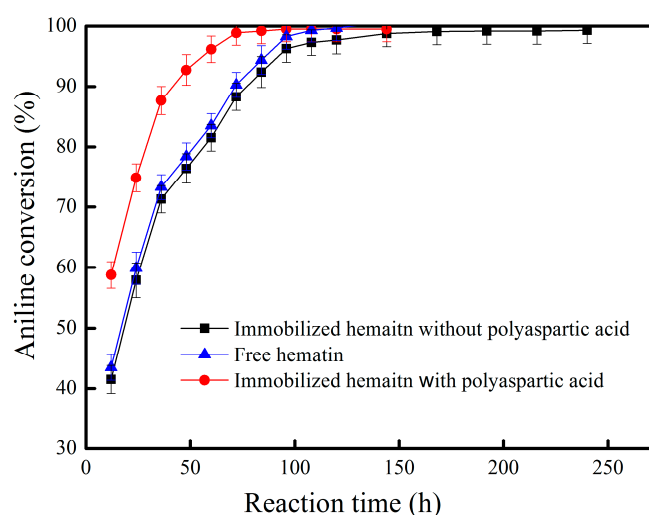
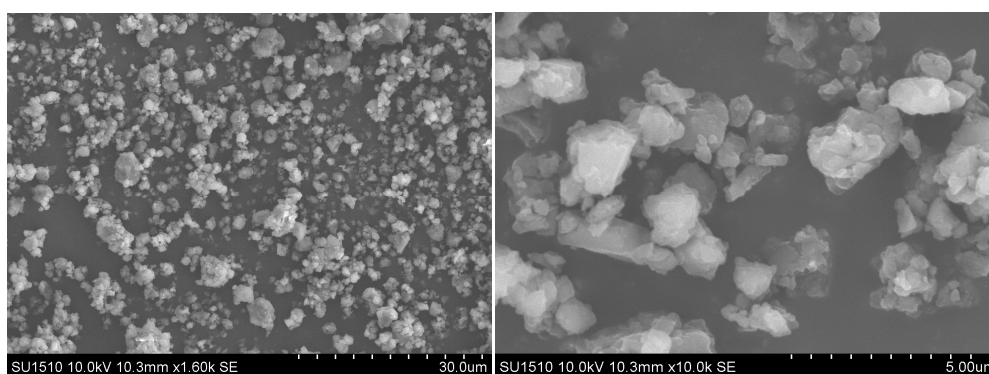


Figure 6. Aniline conversion with reaction time.



(a)

Figure 7. Cont.

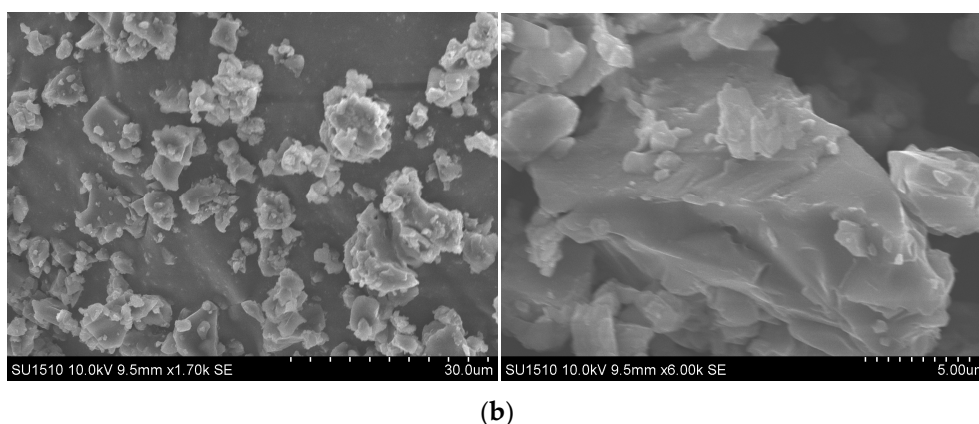


Figure 7. Scanning electron microscope image of the synthesized polyaniline. Under catalysis: (a) hematin-MPTS-M-MWCNTs; (b) hematin-MPTS-M-MWCNTs with addition of polyaspartic acid.

Fourier transform infrared spectra for the synthesized polyaniline are shown in Figure 8. The bands at $1235/1240/1241\text{ cm}^{-1}$ and $1292/1304/1313\text{ cm}^{-1}$ are for C–N asymmetric stretching. The bands at $1235/1240/1241\text{ cm}^{-1}$ result from the structure of benzenoid, and the bands at $1292/1304/1313\text{ cm}^{-1}$ are due to the structures of quinonoid, benzenoid, and phenazine ring [27,28]. The bands at $1443/1444\text{ cm}^{-1}$ were assigned to C=C stretching. The bands at $1499/1508\text{ cm}^{-1}$ and $1569/1575/1585\text{ cm}^{-1}$ are ascribed to benzene ring stretching and C–C stretching, respectively [27,28]. The FTIR spectra confirmed that the main structures of the synthesized polyaniline are similar for the three cases.

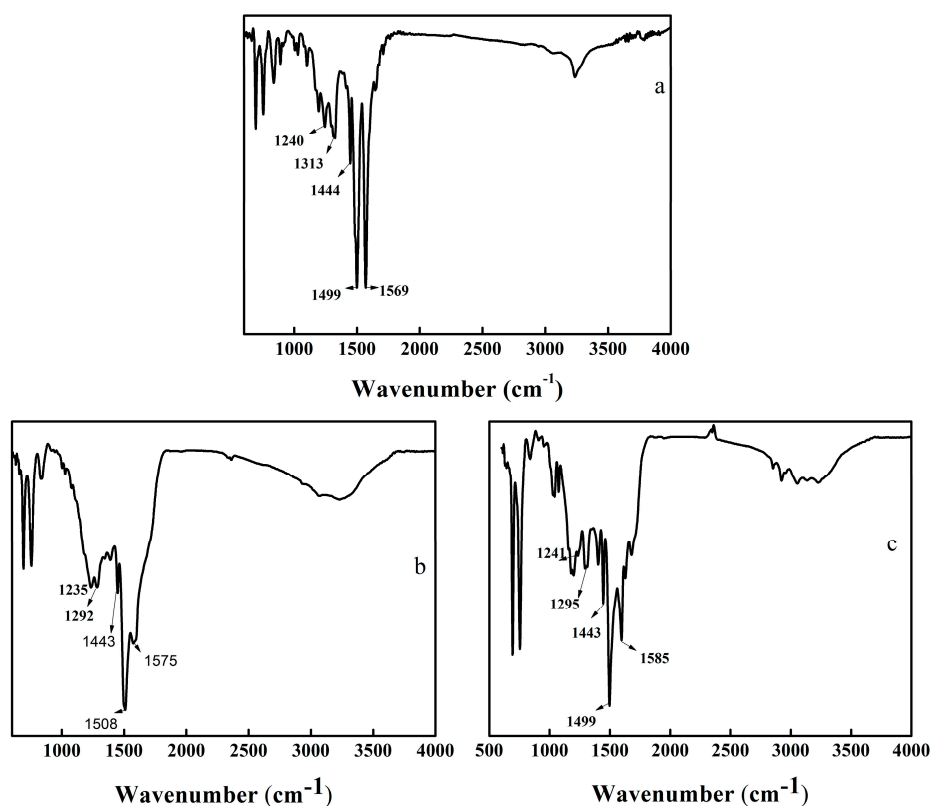


Figure 8. Fourier transform infrared spectra for the synthesized polyaniline. Under catalysis: (a) free hematin; (b) hematin-MPTS-M-MWCNTs; (c) hematin-MPTS-M-MWCNTs with addition of polyaspartic acid.

Consecutive use of hematin-MPTS-M-MWCNTs was carried out. The catalyst was separated from the reaction solution by using a magnet, and then washed with ethanol to remove the possibly adsorbed polyaniline. Afterwards the ethanol was recovered through evaporation under vacuum condition. Therefore, the reuse of hematin-MPTS-M-MWCNTs has been carried out in a simple way, and no extra materials were taken into the reaction media. Consecutive use of the catalyst showed that there was some decrease in the aniline conversion after five reaction/cleaning cycles, but this was not significant (Figure 9).

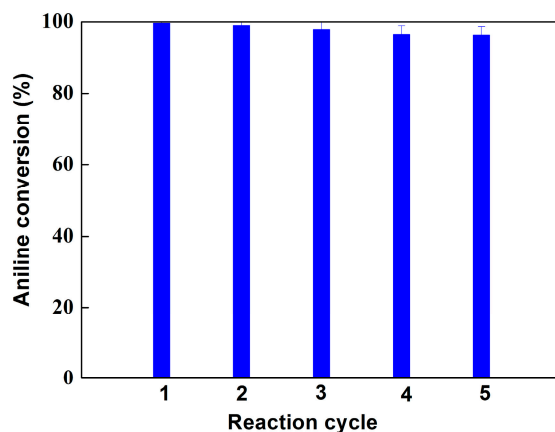


Figure 9. Consecutive use of hematin-MPTS-M-MWCNTs.

3. Experimental Section

3.1. Materials

Multi-walled carbon nanotubes (MWCNTs) were purchased from Nanotech Port Co., Ltd (Shenzhen, China). Iron(II) acetate (99.995%), 3-mercaptopropyltriethoxysilane (MPTS), and azobisisobutyronitrile (AIBN) were purchased from Sigma-Aldrich (Shanghai, China). All other reagents are analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China) or Sigma-Aldrich (Shanghai, China).

3.2. Grafting Hematin

MWCNTs were purified by refluxing in HNO_3 (2.6 M) at 70 °C [29]. After 30 h, the nanotube suspension was rinsed with distilled water by filtering through a 0.2 μm polycarbonate membrane. The samples were dried at 60 °C under vacuum.

Magnetic MWCNTs (M-MWCNTs) were prepared according to the methods reported by Tan et al. [30]. 130 mg Iron(II) acetate was grinded and then mixed with 300 mg purified MWCNTs. The sample was then put into a quartz tube which had been purged by introducing nitrogen for 20 min. The sample was thermally treated under nitrogen at 300 °C for 3 h.

A quantity of 200 mg M-MWCNTs were dispersed in 40 mL ethanol and sonicated for 10 min. 200 mg MPTS and 6 mL ammonia solution were then added to the suspension, sonication was carried out for 4 h at 50 °C. M-MWCNTs coated with MPTS (MPTS-M-MWCNTs) were rinsed sequentially with ethanol and water, each for 10 min, by filtering through a membrane (0.2 μm).

A quantity of 200 mg MPTS-M-MWCNTs were dispersed in 100 mL DMSO. Homogeneous dispersion was achieved through sonication for 20 min. Then 40 mg AIBN and 40 mg hematin were added. Incubation was carried out for 12 h at 60 °C under shaking (200 rpm). The suspension was then filtered through a 0.2 μm membrane, rinsed sequentially with DMSO and water, each for 10 min. Hematin-MPTS-M-MWCNTs were dried with nitrogen-blowing.

The amount of SH groups on MPTS-M-MWCNTs was determined using the Ellman method [25]. 5,5'-Dithiobis(2-nitrobenzoic Acid) (DTNB) was used as the Ellman agent. DTNB reacted with

3-mercaptopropionic acid, and the adsorption at 412 nm was measured. For SH groups, the calibration curve was obtained as illustrated in Figure S2.

A quantity of 200 mg MPTS-M-MWCNTs were dispersed in 60 mL water by sonication for 10 min. Then 2 mL DTNB solution (4.0 mg/mL) was added. The reaction was carried out for 5 min, then MPTS-M-MWCNTs were removed by filtering the suspension. For the filtrate, the adsorption at 412 nm was measured. The calibration curve for hematin is shown in Figure S3. By subtracting the amount of hematin in the original solutions with that in the filtrate, the amount of hematin that was grafted on MPTS-M-MWCNTs was determined. It was 0.11 mg hematin/mg MPTS-M-MWCNTs.

3.3. Oxidation of Aniline

A quantity of 100 mg of functionalized carbon nanotubes (hematin-MPTS-M-MWCNTs) were added to 10 mL aniline solution (0.055 M), the mixture was sonicated for 30 min, then 1 mL hydrogen peroxide (30%, *v/v*) was added dropwise. The mixture was then incubated under shaking (160 rpm) at 25 °C. After 2 h, 1 mL HCl (37%) was added. Samples were taken at regular time intervals to monitor the progress of aniline oxidation.

The concentrations of initial and remaining aniline after reaction were analyzed through HPLC (Shimadzu LC-10A) using C18 column (Dikma Technologies, Beijing, China). The solution of CH₃OH/H₂O (70/30 by vol) was used as the mobile phase with a flow rate 1 mL/min. The retention time and calibration curve for aniline are shown in Figure S4 and Figure S5, respectively.

3.4. Measurement of Optical Spectrum

Infrared spectra were measured using a FTIR spectrometer (Bruker TENSOR 27, Beijing, China), which is equipped with a temperature-controlled ATR with ZnSe Crystal (Pike Technology, New Jersey, NJ, USA) and a specular reflectance accessory. A liquid-nitrogen-cooled mercury-cadmium-telluride detector was used. It collected 128 scans per spectrum at a resolution of 2 cm⁻¹. The ATR element spectrum was used as background. Ultrapure nitrogen gas was introduced for purging water vapor.

Ultraviolet-visible absorption spectroscopy measurements were performed in a Shimadzu UV2550-PC spectrophotometer (Beijing, China) using 1-cm-path length quartz cuvettes. Spectra were collected within a range of 190–800 nm.

4. Conclusions

Mediated by 3-mercaptopropyltriethoxysilane, hematin has been specifically grafted on magnetic carbon nanotubes. This is a novel and easy-to-operate method for grafting hematin on micro and nanomaterials. Hematin-MPTS-M-MWCNTs have been used to catalyze the oxidation of aniline, and high conversion of aniline has been achieved. Biodegradable polymer polyaspartic acid has been found to efficiently promote the conversion of aniline. Recovering the catalyst from the reaction media has been carried out in a simple way, and the activity has been retained to a significant extent after five reaction/cleaning cycles, demonstrating the potential of consecutive use of the catalyst.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4344/6/8/123/s1, Figure S1: (a) Hematin-MPTS-M-MWCNTs were well dispersed in the solution; (b) Hematin-MPTS-M-MWCNTs were separated from the solution with a magnet. The yellow colour was due to the initial reaction after the dropwise addition of H₂O₂. Figure S2: Standard curve for SH group. Figure S3: Standard curve for hematin. Figure S4: Retention time of aniline. Figure S5: Calibration curve for aniline.

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Author Contributions: Peijun Ji provided the idea and design for the study. Kunkun Zheng, Peng Li, Hao Wu performed the experiments. Mengmeng Du analyzed the data.

Conflicts of Interest: The authors declare no conflict of interest.

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