

## Research Article

# Environmentally Friendly and Recyclable Natural-Mediator-Modified Magnetic Nanoparticles for Laccase-Catalyzed Decolorization

Kun Zhang,<sup>1</sup> Yeying Wu,<sup>2</sup> Juan Huang<sup>1,3</sup> ,<sup>3</sup> and Youxun Liu<sup>1</sup> 

<sup>1</sup>School of Basic Medical Sciences, Xinxiang Medical University, Jinsui Avenue 601, Xinxiang, Henan 453003, China

<sup>2</sup>School of Life Science, Wuchang University of Technology, Jiangxia Avenue 16, Wuhan, Hubei 430223, China

<sup>3</sup>School of Life Sciences and Technology, Xinxiang Medical University, Jinsui Avenue 601, Xinxiang, Henan 453003, China

Correspondence should be addressed to Juan Huang; [huangjuan@xxmu.edu.cn](mailto:huangjuan@xxmu.edu.cn) and Youxun Liu; [liuyouxun@xxmu.edu.cn](mailto:liuyouxun@xxmu.edu.cn)

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The high cost, potential toxicity, and possible enzyme inhibition ability of artificial mediators have limited the large-scale application of laccase (Lac)/mediator systems. Here, sinapic acid (SA), a natural mediator, was covalently attached to amino-functionalized magnetic nanoparticles (MNPs) via amide bond formation. The as-prepared SA@MNPs were characterized by Fourier-transform infrared spectroscopy, scanning electron microscopy, cyclic voltammetry, and thermogravimetric analysis. The SA@MNPs were then applied to evaluate the activity of the immobilized mediator for Lac-catalyzed dye decolorization using indigo carmine (IC) as a model dye. When SA and SA@MNPs were used as Lac mediators, IC decolorization yields of ~93% and 96%, respectively, were obtained after 60 min. Moreover, SA@MNPs exhibited an IC decolorization yield of ~90% after being reused for 8 cycles. The Lac/SA@MNP system was shown to degrade IC by breaking down the chromophoric group. The easy recyclability, good reusability, nontoxicity, and relatively low cost of SA@MNPs make this immobilized natural mediator a promising tool for dye treatment.

## 1. Introduction

Laccases (Lacs; *p*-diphenol:oxygen oxidoreductase EC 1.10.3.2) are multicopper oxidases that can use molecular oxygen to oxidize a great variety of aromatic substrates, typically phenolic compounds [1]. However, the direct oxidation of nonphenolic compounds by Lacs is limited because the redox potentials of these enzymes (typically 0.5–0.8 V) are lower than those of such substances [2]. However, Lacs are able to oxidize nonphenolic substrates with the help of redox mediators, which are small molecules that facilitate electron transfer between enzymes and substrates [3]. Because Lacs exhibit substrate universality and produce water as the sole reaction byproduct, they are considered green biocatalysts. Thus, there has been increasing interest in the application of Lacs in various fields

such as wood pulping, electrochemical analysis, organic synthesis, and, especially, detoxification of recalcitrant environmental pollutants or decolorization of synthetic dyes [4].

Lac mediators are divided into two types: artificial and natural. Artificial mediators are synthetic small molecules such as 2,2-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), 1-hydroxybenzotriazole (HBT), and 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO). Such mediators have been applied in Lac-mediator-based catalytic reactions, and the oxidation mechanisms have been elucidated [3, 5–7]. However, the large-scale application of artificial mediators has been limited by their high cost, potential toxicity and possible enzyme inhibition activity. Therefore, great efforts have been made to find alternative mediators of natural origin. In the past decade, many natural mediators, typically

lignin-related phenols including sinapic acid (SA), syringaldehyde, ferulic acid, acetosyringone, vanillin, and acetovanillone, have been investigated [8, 9]. Some natural mediators have been found to be as efficient as artificial mediators [10]. In particular, dimethoxyphenol compounds show even higher efficiencies than artificial mediators for the oxidation of recalcitrant pollutants by Lac [11]. Furthermore, compared with artificial mediators, natural mediators are environmentally friendly, relatively inexpensive, and nontoxic.

Typically, mediators are dissolved in solution during use, which makes recovery from the reaction medium and reuse difficult, leading to a significant cost increase for large-scale industrial applications [3, 12, 13]. Thus, a few researchers have made great efforts towards recovering and reusing mediators by immobilizing them on support materials. For instance, ABTS immobilized on silica nanoparticles or encapsulated in a metal-organic framework (MIL-100(Fe)) has been successfully applied as a mediator for Lac-catalyzed decolorization [14, 15]. However, to the best of our knowledge, there has been little research on the immobilization of natural Lac mediators.

SA, a precursor of lignin, has been confirmed to act as a highly efficient mediator for Lac oxidation of various recalcitrant compounds, including industrial dyes [11]. The oxidation mechanism of the Lac/SA system has been elucidated and is similar to a hydrogen atom transfer oxidation mechanism. SA, a substituted phenol, is a good Lac substrate because its electrochemical potential is well matched to that of Lac. Oxidation of SA by Lac results in the fast production of highly reactive phenoxyl radicals (PhO), which play a similar role to nitroxyl radicals ( $>N-O\cdot$ ) generated by the oxidation of an artificial mediator (HBT) through a hydrogen atom transfer oxidation mechanism. Subsequently, these phenoxyl radicals can oxidize target substrates. It has been confirmed that fast oxidation of SA by Lac provides a stable, high concentration of phenoxyl radicals that efficiently promote the Lac-catalyzed decolorization of various dyes [4, 11, 16]. For practical applications of the Lac/SA system to dye decolorization, the immobilization of SA would improve the reusability of the mediator, thus reducing the running cost. The molecular structure of SA consists of a phenol moiety and a carboxyl moiety (Figure 1(a)). As the phenol group of SA is responsible for the mediator activity of this compound, the carboxyl group could potentially be exploited to attach SA to an immobilization matrix such as magnetic nanoparticles (MNPs). However, it is necessary to determine whether the mediator activity of SA is maintained following immobilization.

In the present work, our main aim was to immobilize a natural mediator, SA, on MNPs to facilitate its recovery and reuse. First, SA was covalently attached to amino-modified MNPs via amide bond formation. Second, the feasibility of using SA-modified MNPs (SA@MNPs) as a Lac mediator was evaluated for the Lac-catalyzed decolorization of the dye indigo carmine (IC). Finally, it was confirmed that the SA@MNPs could be easily recovered by magnetic separation and effectively reused as a Lac mediator.

## 2. Materials and Methods

**2.1. Materials.** Lac (EC 1.10.3.2, enzyme activity  $\geq 0.6$  U/mg) was provided by Sunson Industry Group (Beijing, China). ABTS was supplied by Sigma-Aldrich (St. Louis, MO, USA). SA and 3-aminopropyltriethoxysilane (APTES) were purchased from Energy Chemical (Shanghai, China). All other chemicals and reagents were of analytical grade.

**2.2. Preparation of SA-Immobilized MNPs.** Amino-modified  $Fe_3O_4$  nanoparticles were synthesized according to the method described by Mohapatra et al. [17] with slight modifications. Briefly, 0.324 g of  $FeCl_3$  and 0.278 g of  $FeSO_4 \cdot 7H_2O$  were added to 40 mL of deoxygenated water in a 100 mL three-necked flask. The mixture was stirred vigorously at room temperature for 20 min under nitrogen protection. Then, 3 mmol of APTES was added to the stirred reaction mixture over a period of 10 min. Subsequently, 3 mL 25%  $NH_3$  solution was quickly added to the flask. After 30 min of vigorous stirring, the black precipitate was separated and washed ten times with deionized water via magnetic decantation. The resulting nanoparticles were dried at  $60^\circ C$  under vacuum for 8 h. The obtained material is referred to as APTES@MNPs. The control MNPs were prepared without using APTES.

The MNPs coated with APTES can provide active amino groups for amide bond formation with SA. Thus, the SA-modified MNPs were fabricated as follows. First, 0.25 g of SA was dissolved in 10 mL of *N,N*-dimethylformamide in a 50 mL three-necked flask at  $0^\circ C$ . After 10 min, 0.28 g of *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride was added, and the reaction mixture was stirred for another 10 min at  $0^\circ C$ . Subsequently, 0.4 g of APTES@MNPs, 0.2 g of 4-dimethylaminopyridine, and 0.3 mL of triethylamine were added, and the reaction mixture was stirred for 24 h under nitrogen protection at room temperature. The black precipitate was recovered and then washed five times with *N,N*-dimethylformamide and five times with deionized water, respectively. The resulting nanoparticles were dried at  $60^\circ C$  under vacuum for 12 h. The obtained material is referred to as SA@MNPs.

**2.3. Dye Decolorization and Measurement Activity of Lac.** IC (Figure 1(b)) was used as the model pollutant for enzymatic decolorization. As IC is not a phenolic substrate, Lac needs the help of a mediator to decolorize IC completely. To determine the optimal conditions for SA oxidation by Lac, three buffers at pH 4.5 were used: 0.2 M sodium acetate buffer, 0.2 M citrate buffer, and 0.2 M sodium citrate buffer. The effects of pH on Lac-catalyzed decolorization using both SA@MNPs and SA were examined between pH 3.0 and 7.0 in 0.2 M sodium acetate buffer. The optimum pH was used for further experiments. The decolorization conditions were as follows: 20 mg/L dye, 3000 U/L Lac, and either the immobilized mediator (0.02 g/L SA@MNPs) or the free mediator (15.4  $\mu M$  SA) at pH 5.0. Unless otherwise specified, the decolorization experiments were carried out in 5 mL centrifuge tubes and the mixtures were shaken at 150 rpm at

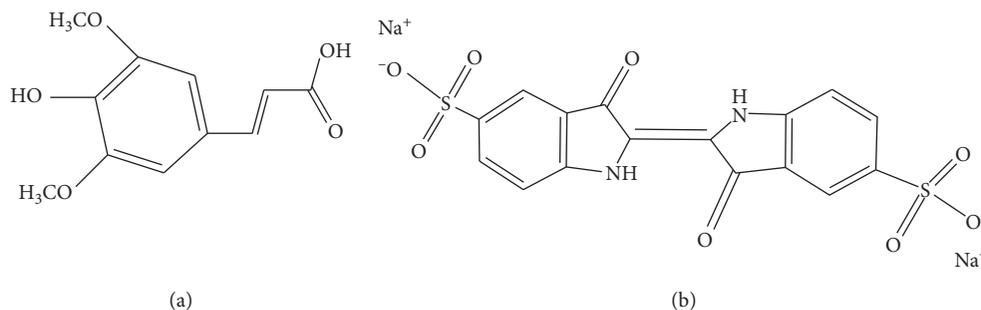


FIGURE 1: Molecular structures of (a) SA and (b) IC.

room temperature. Control reactions were also performed to study the decolorization efficiency using Lac or SA@MNPs alone. Dye degradation was monitored using UV-Vis spectroscopy by recording the absorbance spectrum of the dye between 250 and 800 nm. The extent of IC decolorization was determined based on the decrease in absorbance at  $\lambda_{\max} = 615$  nm using the following formula: decolorization (%) =  $(A_0 - A_t)/A_0$ , where  $A_0$  is the initial absorbance of the dye at  $\lambda_{\max} = 615$  nm and  $A_t$  is the absorbance after the specified reaction time. In addition, the band at 515 nm was used as a characteristic absorption peak to monitor SA oxidation in solution. All experiments were performed at least in triplicate. The data presented herein correspond to mean values with standard errors.

Assays of Lac activities were conducted spectrophotometrically using ABTS as a substrate [13]. The reaction was initiated by adding 0.1 mL of the Lac solution into 2.7 mL of sodium acetate buffer solution (50 mM, pH 4.0) and 0.2 mL of ABTS (4 mmol/L), and then the mixtures are incubated at 25°C for 3 min. The increase in absorbance of the solution was recorded at the wavelength of 420 nm.

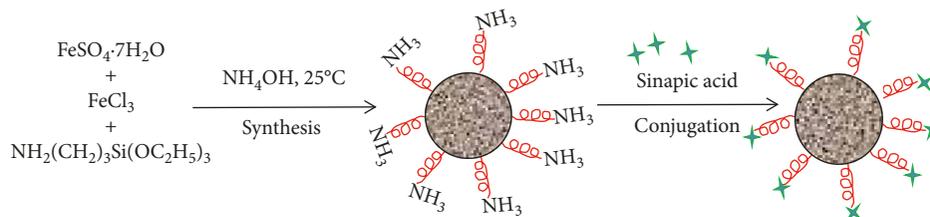
**2.4. Reusability and Stability of SA@MNPs.** The reusability of the SA@MNPs was assessed over 8 decolorization cycles. At the end of each cycle, the SA@MNPs was recovered with a magnet, washed three times with distilled water, and then used for a new cycle. The data presented herein are average values of triplicate measurements. The storage stability of the SA@MNPs was evaluated by storing the SA@MNPs at room temperature in water and in pH 5.0 and 9.0 disodium hydrogen phosphate-citric acid buffers. The amount of SA in the supernatant was determined periodically over 10 days by monitoring changes in the UV-Vis absorbance spectrum. In addition, the characteristic Fourier-transform infrared (FT-IR) vibration bands of the as-prepared SA@MNPs were compared with those of the SA@MNPs after 8 catalytic cycles. The statistical significance of differences was compared and analyzed using Student's *t* tests or one-way analysis of variance by GraphPad Prism7 Software. Differences with *p* values of less than 0.05 were considered statistically significant.

**2.5. Characterization Methods.** Transmission electron microscopy (TEM) was carried out using a model 9000

transmission electron microscope (Hitachi, Japan). Field-emission scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) were performed with a JSM 6700F scanning electron microscope (JEOL, Japan). FT-IR spectra were recorded with a Tensor 27 spectrometer (Bruker, Germany) using the KBr pellet technique. Cyclic voltammetry (CV) measurements were performed with a CHI600E electrochemical workstation (CH Instruments, USA). Thermogravimetric analysis (TGA) of the as-synthesized MNPs was carried out using a LENSES STAPT-1000 calorimeter (Germany) by scanning up to 700°C at a heating rate of 10°C/min. UV-Vis spectroscopy was performed with a CARY 50 spectrophotometer (Varian, USA).

### 3. Results and Discussion

**3.1. Synthesis and Characterization of SA-Immobilized MNPs.** Owing to their attractive features, such as easy surface modification, convenient separation, and nontoxicity [18–20], Fe<sub>3</sub>O<sub>4</sub> MNPs were selected as a support material for the immobilization of SA. The preparation of SA@MNPs is illustrated in Scheme 1. The FT-IR spectra of MNPs, SA, APTES@MNPs, and SA@MNPs are given in Figure 2. Compared with the FT-IR spectrum of the MNPs, the FT-IR spectrum of APTES@MNPs exhibited new peaks at 1052 cm<sup>-1</sup>, corresponding to the Si–O–Si stretching vibration, ~1125 cm<sup>-1</sup>, corresponding to the C–N stretching vibration, and ~3420 and ~1620 cm<sup>-1</sup>, corresponding to the –NH<sub>2</sub> groups of the APTES [21]. In addition, a strong peak was observed at ~575 cm<sup>-1</sup> corresponding to the Fe–O–Fe vibration. These results indicated that the MNPs were successfully coated with APTES to give amino-functionalized MNPs. In the FT-IR spectrum of SA@MNPs, peaks were observed at ~3352 and 1680 cm<sup>-1</sup> corresponding to the –NH stretching vibration and C=O stretching vibration, respectively, of an amide bond [21]. Amide bond formation occurred via the reaction between the carboxyl group of SA and an amino group on the APTES@MNPs. Furthermore, a comparison of the FT-IR spectra of SA, APTES@MNPs, and SA@MNPs shows that the characteristic bands of both of SA and APTES@MNPs are clearly observable in the FT-IR spectrum of SA@MNPs, indicating that the amino-functionalized MNPs were successfully modified with SA. Owing to magnetic dipole interactions, both MNPs and APTES@MNPs tended to agglomerate, resulting in clusters, as shown



SCHEME 1: Schematic illustration of the preparation strategy for SA@MNPs.

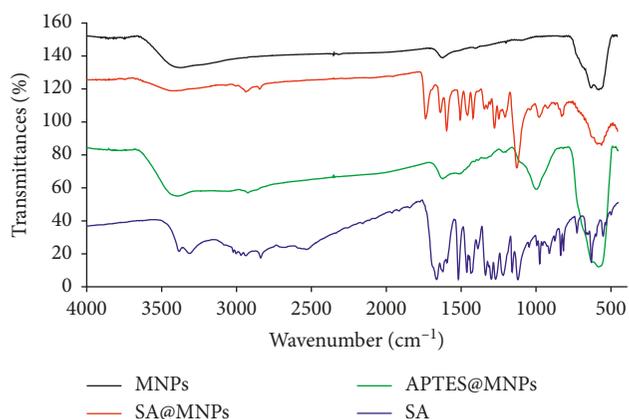


FIGURE 2: FT-IR spectra of MNPs, SA, APTES@MNPs, and SA@MNPs.

in the TEM images (Figures 3(a) and 3(b)). As observed in the TEM images, the mean size of the APTES@MNPs was slightly larger than that of the MNPs, and this difference was attributed to the APTES coating.

TGA showed that MNPs, APTES@MNPs, and SA@MNPs had different decomposition temperatures (Figure 4). The total weight loss observed for the MNPs was  $\sim 4\%$ , whereas that for the APTES@MNPs was  $\sim 10\%$ . Unlike the MNPs, the APTES@MNPs showed a two-step decomposition process. The first weight loss ( $115\text{--}200^\circ\text{C}$ ) corresponded to the loss of water from the sample. The second significant weight loss of  $\sim 6\%$  ( $250\text{--}420^\circ\text{C}$ ) corresponded to the thermal degradation of APTES. Notably, the total weight loss of SA@MNPs was much greater ( $\sim 28\%$ ) and a three-step decomposition process was observed. The first two weight loss steps were similar to those of the APTES@MNPs, whereas the third weight loss step of approximately  $15\%$  was assigned to the thermal decomposition of SA. These results indicated that the MNPs were coated with APTES and that SA was covalently attached to the APTES@MNPs.

As shown in Figure 5(a), the SEM-mapping images confirm the presence of C, Si, and Fe in the SA@MNPs. In addition, the dispersion of Si and C on the surfaces of the SA@MNPs suggests that the MNPs were homogeneously coated with APTES and SA. Moreover, the EDS spectrum (Figure 5(b)) further confirmed that C, O, N, Si, and Fe were present in the SA@MNPs, providing clear evidence for the formation of SA@MNP nanocomposites. The amount of amino groups on the surface of MNPs was determined using a spectrophotometric assay according to the method described by Mohapatra et al. [17]. The change in the amount

of amino groups before and after SA grafting indicated that the concentration of SA on the surface of MNPs was  $\sim 770 \mu\text{mol/g}$ .

The electrochemical behavior was examined by CV using glassy carbon electrode (GCEs) coated with films of the as-synthesized MNPs at pH 5.0 in sodium acetate buffer (Figure 6). Although the electrochemical behavior of APTES@MNPs/GCE and SA@MNPs/GCE was similar, different behavior was observed in the presence of Lac. Lac/APTES@MNPs/GCE exhibited no obvious redox peaks at applied potentials between 0 and  $+1.0 \text{ V}$ , whereas Lac/SA@MNPs/GCE showed a well-defined reversible signal at  $0.4 \text{ V}$ , which was ascribed to the reversible oxidation of immobilized SA by Lac. This result indicated that SA immobilized on MNPs plays a role in electron transfer between the electrode and Lac and can still act as a Lac mediator, similar to free SA.

**3.2. Effects of pH on Dye Decolorization.** To explore the ability of SA to act as a Lac mediator, the effects of the buffer type and pH on SA oxidation by Lac were evaluated. To monitor SA oxidation, the absorbance spectra of the SA were recorded between 200 and  $750 \text{ nm}$  before and after Lac oxidation. As shown in Figure 7(a), SA in solution showed two characteristic absorption bands at 232 and  $300 \text{ nm}$ . Upon incubation of SA with Lac at pH 5 in sodium acetate buffer for approximately 30 min, an intense brown-red color appeared. Simultaneously, the band at  $300 \text{ nm}$  nearly disappeared and a new band appeared at  $515 \text{ nm}$  suggesting the formation of the new product resulting from SA oxidation by Lac. Therefore, the band at  $515 \text{ nm}$  was used as a characteristic absorption peak to monitor SA oxidation in solution. The catalytic activity of Lac for SA oxidation was compared in various buffers. As shown in Figure 7(b), the oxidation rate in sodium acetate buffer was highest in three kinds of buffer ( $F(2, 6) = 64.24$ ,  $p < 0.05$ ). Thus, sodium acetate buffer was used for further experiments. In addition, the optimum pH for maximizing SA oxidation by Lac was found to be 5.0, as shown in Figure 7(c).

The effects of pH on dye decolorization by Lac, Lac with the free mediator (SA), and Lac with the immobilized mediator (SA@MNPs) were then evaluated in sodium acetate buffer between pH 3.0 and 7.0. Figure 7(d) shows that the maximum decolorization by Lac was achieved at pH 4.5, likely because the catalytic activity Lac for decolorization of IC was maximized at this pH value [14]. However, both the Lac/SA system and the Lac/SA@MNP system exhibited maximum decolorization at pH 5.0. This slight shift in the

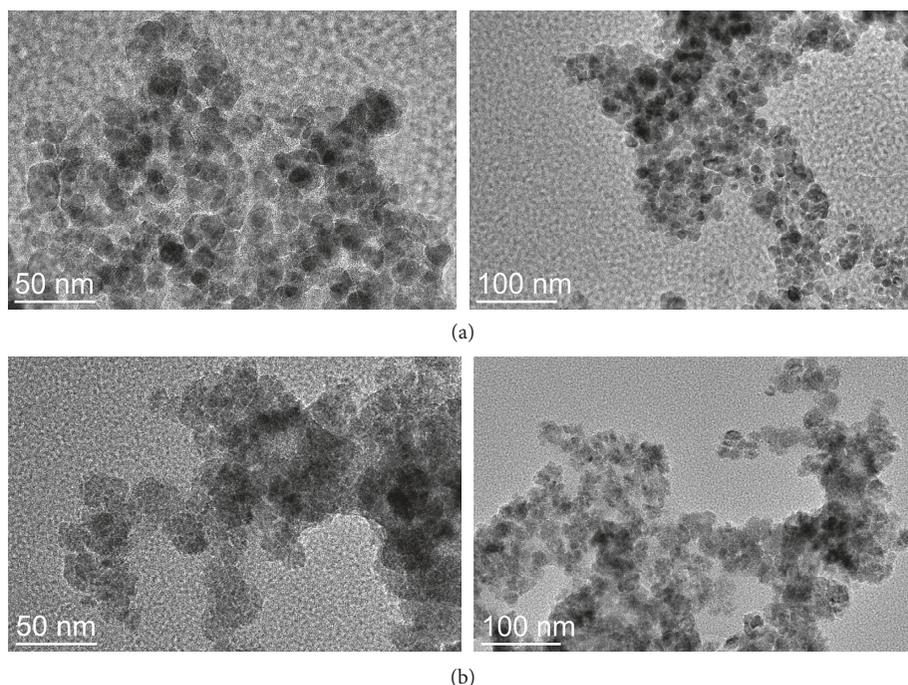


FIGURE 3: TEM images of (a) MNPs and (b) APTES@MNPs.

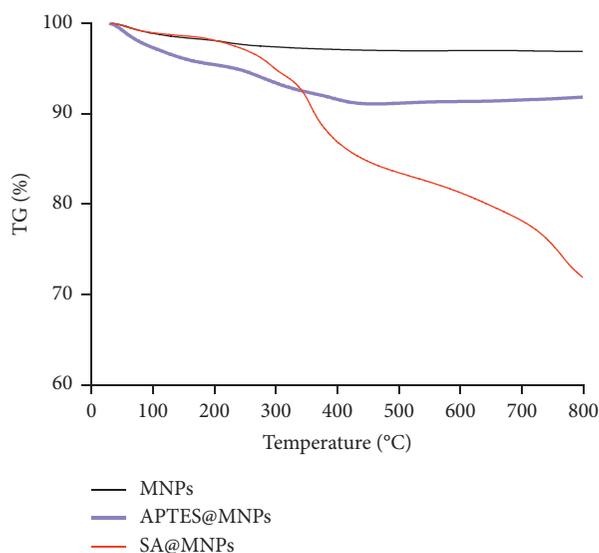


FIGURE 4: TGA thermograms of MNPs, APTES@MNPs, and SA@MNPs.

optimum pH relative to that for Lac alone may be attributable to the fact that the optimum pH for oxidation of SA by Lac was 5.0, as shown in Figure 7(c). It is also possible that electron transfer between the dye molecules, the SA mediator, the MNPs, and Lac is more favorable at pH 5.0. Many studies have found that acidic pH values enhance the activities of most Lacs [22–24]. Increasing the pH further resulted in an evident decrease in the decolorization activities of both the Lac/SA and Lac/SA@MNP systems. However, the latter displayed higher decolorization activities

than the former at pH values above 5.0, suggesting that the environmental pH value had little influence on the activity of SA as mediator following immobilization.

**3.3. Dye Decolorization Efficiencies.** To evaluate the effect of the SA@MNPs on Lac-catalyzed decolorization, the decolorization efficiency of the Lac/SA@MNP system was compared with those of the Lac/SA system, SA@MNPs, and Lac alone (Figure 8). For Lac alone, only ~10% dye decolorization was obtained within 30 min. Previous reports have shown that Lac is not able to degrade IC completely without a mediator because IC is a nonphenolic compound, which is not a typical substrate for Lac [14]. In the case of SA@MNPs, negligible decolorization (~6%) was obtained after 30 min, and this value remained almost constant throughout the reaction time. Thus, the observed decolorization is mainly due to the adsorption of the dye molecules on the SA@MNPs. These results implied that Lac degradation alone and nanoparticle adsorption have negligible effects on the decolorization of IC. In comparison, Lac with the free mediator (Lac/SA) achieved almost complete decolorization of IC (~93%) within 60 min, and Lac with the immobilized mediator (SA@MNPs) realized a similar decolorization efficiency (~96%) within 60 min. These results indicated that SA immobilized on the MNPs maintained the same activity as free SA for mediating Lac-catalyzed dye decolorization. Notably, these high decolorization efficiencies were due to the effect of the mediator instead of Lac alone or the MNPs. In comparison, our previous reports have shown that ABTS, an artificial mediator, immobilized on silica nanoparticles or encapsulated in a metal-organic framework exhibited a higher

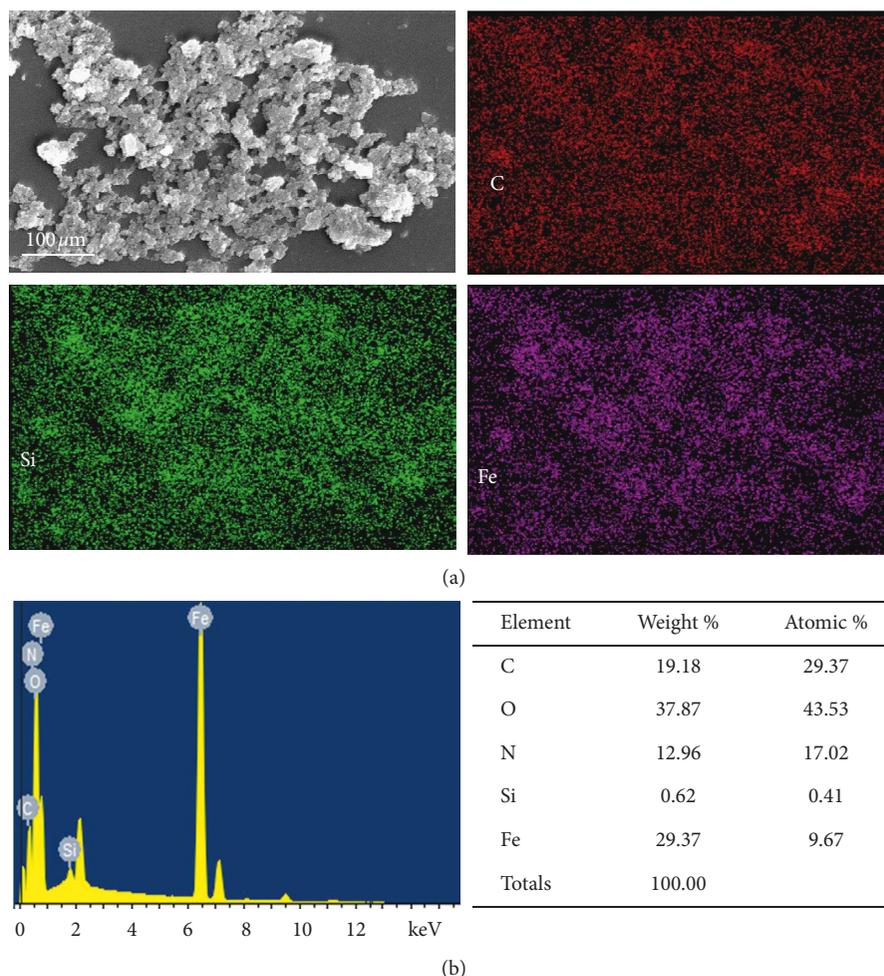


FIGURE 5: (a) SEM-mapping images of the distribution of various elements on the surfaces of SA@MNPs. (b) EDS spectrum and corresponding relative elemental contents.

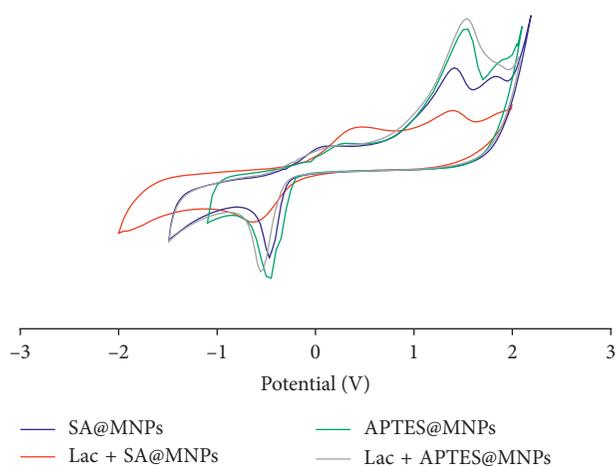


FIGURE 6: Cyclic voltammograms of APTES@MNPs and SA@MNPs coated on GCEs at a sweep rate of 20 mV/s in 0.05 M sodium acetate buffer at pH 5.0 in the absence and presence of Lac.

decolorization efficiency than SA@MNPs, which may attribute to the oxidation mechanism of SA and ABTS as mediators in the Lac-catalyzed reaction [14, 15]. It had been

reported that ABTS-mediated oxidations belong to an electron transfer mechanism, and SA-mediated oxidations are a hydrogen atom transfer mechanism [11].

**3.4. Reusability of SA@MNPs.** To evaluate the reusability of the immobilized mediator, the SA@MNPs were recovered and reused for dye decolorization for up to 8 cycles, as shown in Figure 9(a). Using a reaction time of 60 min, very high decolorization rates were obtained by employing the same SA@MNPs over 8 runs. However, the decolorization efficiency decreased significantly ( $F(7, 16) = 2.786$ ,  $p < 0.05$ ) after 8 cycles, and approximately 90% decolorization was achieved. On the one hand, this decrease may be due to partial destruction of the SA@MNPs during the reaction and/or the loss of some SA@MNPs during magnetic recovery. Similar observations have been reported previously for immobilized-mediator-catalyzed dye decolorization reactions [15, 25, 26]. On the other hand, the accumulation of dye degradation products on the surface of the SA@MNPs may depress the activity of the enzyme, resulting in a reduction in the decolorization efficiency during the subsequent cycle [27].

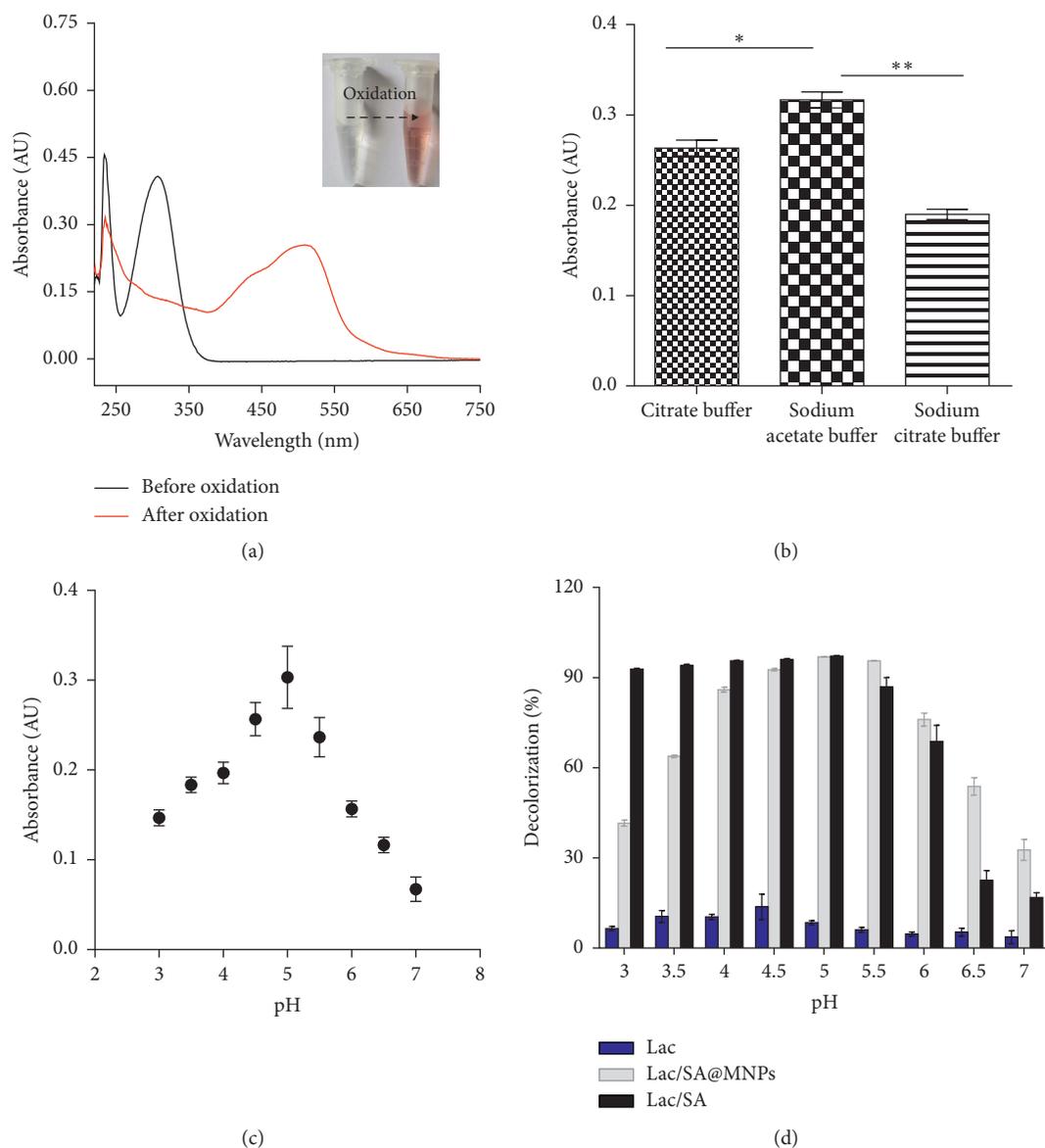


FIGURE 7: (a) UV-Vis spectra of SA before and after oxidation by Lac. (b) Effects of buffer type on SA oxidation by Lac. (c) Effects of pH on SA oxidation by Lac. (d) Effects of pH on dye decolorization by Lac, Lac/SA@MNPs, and Lac/SA.

As shown in Figure 9(b), the UV-Vis absorption spectrum of IC exhibits two characteristic absorption bands at 288 and 615 nm. The blue color of the solution decreased in intensity following treatment with the Lac/SA@MNP system, with the reaction mixture turning pale yellow after complete decolorization. Simultaneously, the absorbance peaks at 288 and 615 nm disappeared. These results indicated that destruction of the molecular structure of the dye was catalyzed by Lac in the presence of the SA@MNPs, resulting in complete decolorization of IC. According to the previous reports, the decomposition of chromophores and benzene rings removes the toxicity of dyes by preventing the formation of aromatic amines [28].

Although Lac mediators have been used in various applications, their high cost, especially for artificial mediators, has limited large-scale applications [29]. Moreover,

most mediators used in various applications are dissolved in the reaction medium. Thus, after use and abandonment, the potential toxicity of the mediators can cause environmental problems [30]. Considering the potential economic and environmental benefits, it is very important to recover and reuse mediators for large-scale applications of Lac/mediator systems in various fields [8, 10, 30, 31]. It has been reported that mediator immobilization is an effective way to achieve this goal, and the successful immobilization of several artificial on various materials has been reported. For example, a Lac redox mediator (ABTS) was immobilized on a macroporous cryogel by electron irradiation to assist the Lac-catalyzed degradation of bisphenol A [32]. In addition, ABTS had been successfully attached to silica nanoparticles and a mesoporous metal-organic framework, and the immobilized ABTS played a similar role to free ABTS in

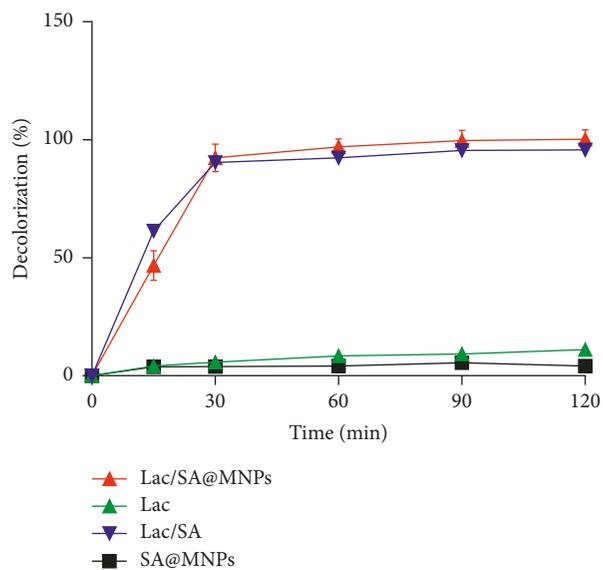
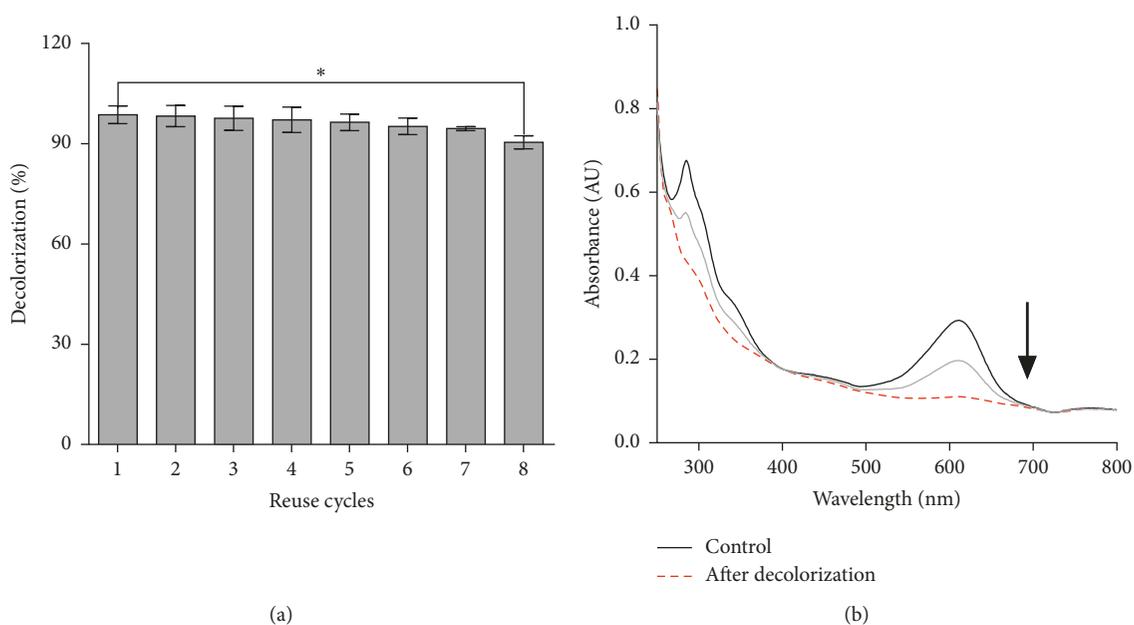
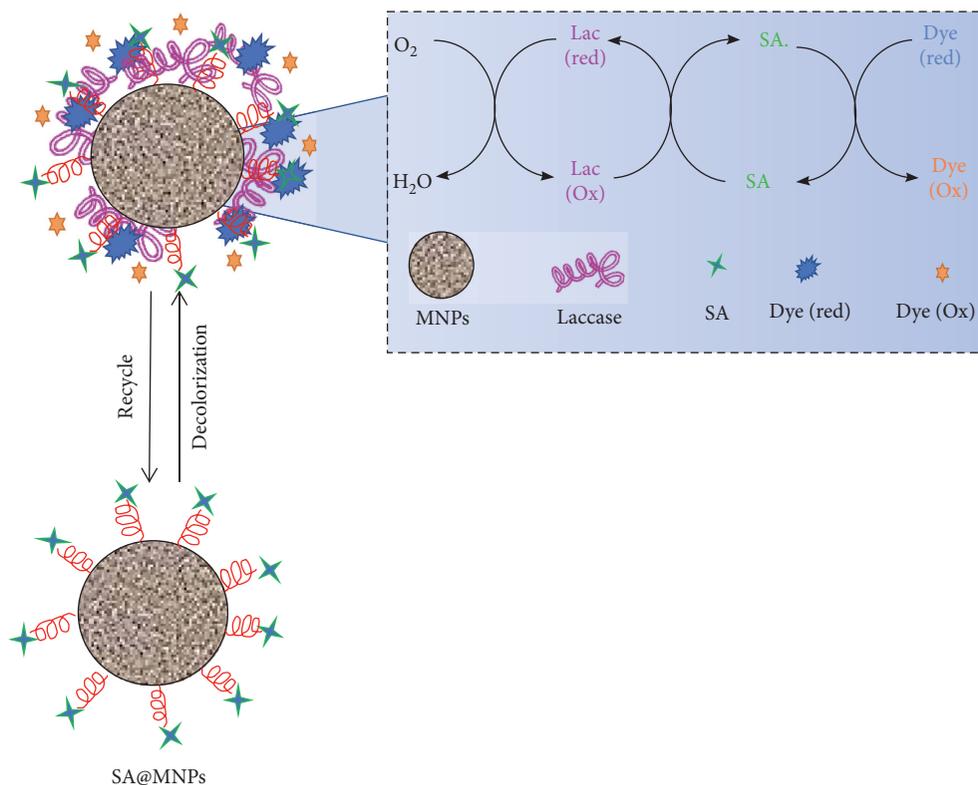


FIGURE 8: Dye decolorization by Lac/SA@MNPs, Lac/SA, SA@MNPs, and Lac alone as a function of time.



(c)

FIGURE 9: (a) Reusability of SA@MNPs over 8 successive cycles. (b) UV-Vis spectra of IC at various times during the enzymatic degradation process. (c) Photograph of the dye solutions before and after decolorization and the magnetic recovery of SA@MNPs.



SCHEME 2: Schematic illustration of the catalytic cycle of SA@MNPs.

mediating Lac-catalyzed dye decolorization [14, 15]. Moreover, polyethylene glycol was modified with TEMPO, and the resulting immobilized redox mediator exhibited reusability for Lac-catalyzed dye decolorization [26]. Furthermore, efficient catalytic performance and easy recoverability were achieved by co-immobilization of Lac and a mediator in the same matrix [33, 34]. In this work, the natural mediator SA was immobilized on magnetically separable MNPs to facilitate its recovery and reuse. As shown in Figure 9(c), the SA@MNPs could be efficiently separated from the reaction solution using a magnet after being applied as a decolorization mediator. This strategy provides two advantages. First, the easy recoverability of the SA@MNPs contributes to this system having lower running costs than systems with nonmagnetic immobilization matrices. Second, and more importantly, as SA is a natural compound that is environmentally friendly and nontoxic, this system avoids the environmental problems that may be associated with the use of artificial mediators.

However, there is an important limitation that should also be pointed out. In fact, a powdered form of SA@MNPs is too small to leak out easily from wastewater treatment systems. One solution to the problem is to entrap SA@MNPs and laccase together into polysaccharides like chitosan, pectin, and alginate, to form beads. Another solution to the problem is to co-immobilize SA@MNPs and laccase onto the membrane to form enzymatic membrane bioreactor. It has been reported that the degradation of phenolic and non-phenolic trace organic contaminants was significantly enhanced in enzymatic membrane bioreactor fabricated by

laccase and redox-mediator [35]. Thus, these methods will further contribute to the reusability of SA@MNPs. In addition, as a new system, there are some problems remain unresolved, one of which is the environmental safety of the usage of the nanoparticles.

**3.5. Catalytic Cycle of SA@MNPs.** Several authors have described the role of mediators in Lac/mediator catalytic systems [35, 36]. Previous reports have suggested that a hydrogen atom transfer oxidation mechanism can be used to understand the role of SA as a mediator in the Lac-catalyzed reaction [11]. The catalytic cycle of the SA@MNPs in Lac-catalyzed dye decolorization is shown in Scheme 2. First, SA covalently attached to the MNPs is oxidized by Lac to form highly reactive phenoxyl radicals (SA·). Subsequently, the phenoxyl radicals participate in nonenzymatic oxidation reactions with dye molecules cannot be efficiently oxidized by Lac alone. The phenoxyl radicals can be reduced to their original form by dye molecules, resulting in the simultaneous oxidation of the dye molecules to their intermediates, thus completing one catalytic cycle. After the dye is completely decolorized by Lac/SA@MNPs-catalyzed oxidation, the SA@MNPs are recovered and recycled. The redox mediators act as electron shuttles and provide an indirect oxidation step. The oxidized radical form of the SA is able to oxidize a wide range of substrates, including compounds with a high redox and/or nonphenolic compound. Previous research has reported that phenolic compounds related to lignin have shown their capability and efficiency to act as

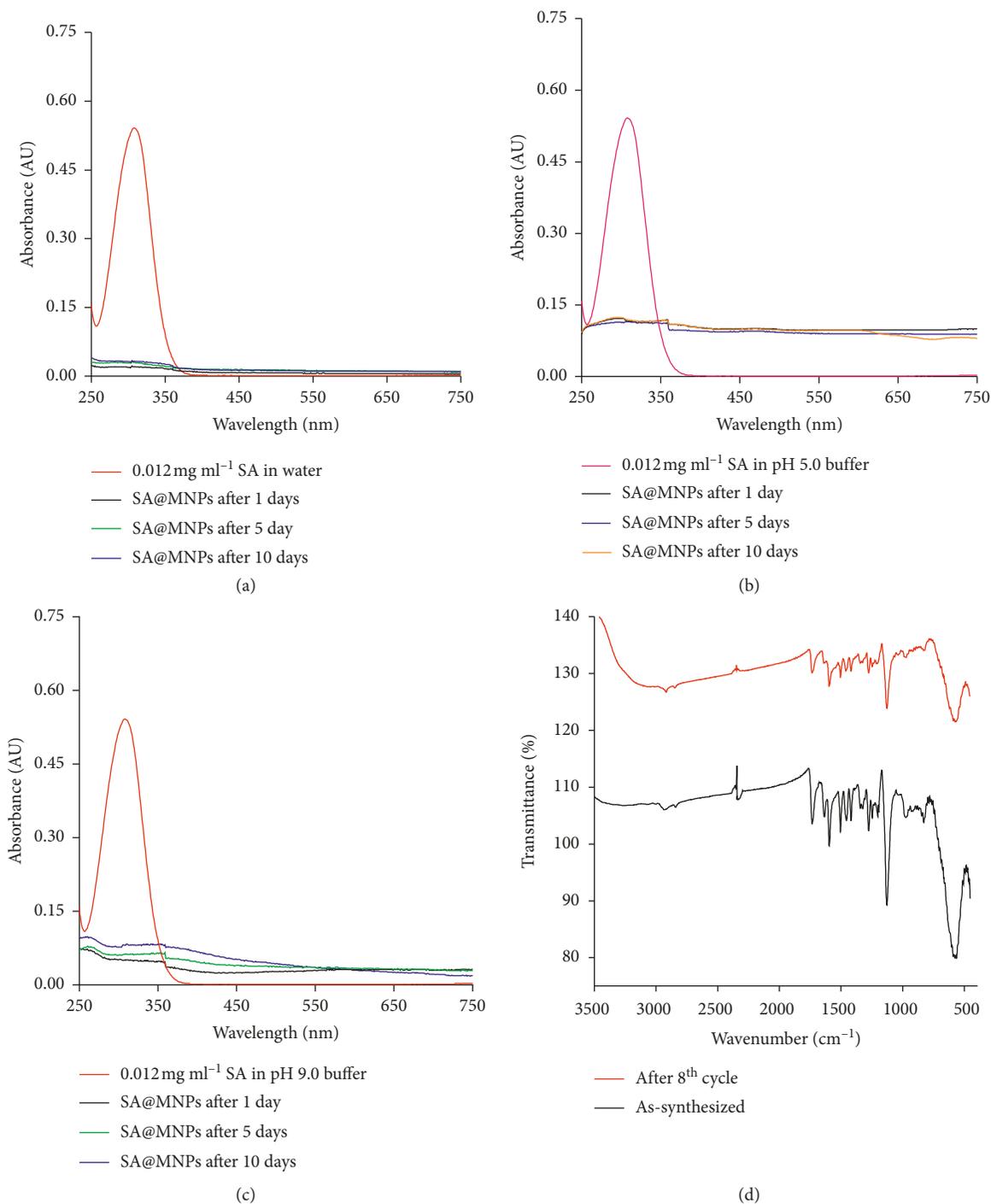


FIGURE 10: UV-Vis spectra of SA@MNPs dispersed in (a) water, (b) pH 5.0 buffer, and (c) pH 9.0 buffer for 1, 10, and 5 d (d) FT-IR spectra of the as-synthesized and recycled SA@MNPs samples.

natural Lac mediators [37]. Although ABTS and HBT are well-known artificial redox mediators for Lac, these mediators are not economically feasible. Furthermore, Lac stability and activity was decreased when incubated with artificial these redox mediators in the long run. Thus, natural mediators have been used due to their environmental friendliness, low-cost, and good biocompatibility to laccase [38].

**3.6. Stability Analysis.** Recovery and recycling of the mediator will only be efficient if the SA@MNPs are stable in various solutions. Thus, the storage stability of the SA@MNPs and the potential detachment of SA from the SA@MNPs should be assessed. The characteristic absorption band of unoxidized SA at 300 nm (Figure 7(a)) was used to estimate the concentration of SA in solution. As shown in Figures 10(a)–10(c), following the dispersion of the SA@

MNPs in water, pH 5.0 buffer, and pH 9.0 buffer, respectively, little SA was observed in the supernatant solution after 1, 5, and 10 d, implying that SA was not detached from the SA@MNPs via hydrolysis of the amide bond. These results confirmed that the SA@MNPs exhibited good stability in solution under different pH conditions. In addition, the characteristic FT-IR bands of the SA@MNPs after 8 catalytic cycles were compared with those of the as-prepared SA@MNPs, as shown in Figure 10(d). There were no obvious changes in the characteristic bands, which further confirmed that the structure of the SA@MNPs was maintained with negligible loss of SA. This stability may be attributed to the fact that SA was covalently attached to the MNPs rather than adsorbed on the surface.

#### 4. Conclusion

In view of the high costs of synthetic mediators and their potential toxicities, the practical application of Lac/mediator systems on a large scale is severely limited. In this work, a natural mediator (SA) was covalently attached to amino-modified MNPs via amide bond formation. Similar to free SA, SA immobilized on the MNPs was shown to be an efficient mediator for Lac-catalyzed dye decolorization. Moreover, the structure of the SA@MNPs was maintained over 8 cycles and essentially no SA was released into the supernatant after storage in solution for 10 days, thus confirming the stability of the SA@MNPs. Furthermore, the SA@MNPs were magnetically separable, facilitating easy recovery and recycling. These results confirmed that SA@MNPs can improve the efficiency of mediator utilization and reduce both the costs and the secondary pollution associated with the use of synthetic mediators. Thus, this immobilized natural mediator shows potential for practical application in the treatment of dye wastewater.

#### Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

#### Conflicts of Interest

The authors declare that they have no competing financial interest.

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