Laccase-catalyzed poly(ethylene glycol)-templated 'zip' polymerization of caffeic acid for functionalization of wool fabrics

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Abstract

A cleaner approach for functionalization of wool fabrics via laccase-catalyzed polymerization of caffeic acid was developed. Caffeic acid was first polymerized to poly(caffeic acid) in the system where poly(ethylene glycol) was used as a template under the catalysis of laccase/O_2. Ultraviolet-Visible spectroscopic measurements verified that poly(ethylene glycol) formed a complex with caffeic acid though hydrogen bonds based on the 'zip' mechanism. During the catalysis of caffeic acid by laccase, poly(ethylene glycol) template promoted the enzymatic polymerization in term of the quantity of poly(caffeic acid) and the degree of polymerization. The data obtained from Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry and High Performance Liquid Chromatography-Electro Spray Ionization confirmed that poly(ethylene glycol) template aided the arrangement of the caffeic acid monomer, resulting in the increased degree of polymerization of caffeic acid in the laccase-catalyzed reaction. The poly(caffeic acid)/poly(ethylene glycol)-treated wool fabrics were prepared by laccase-catalyzed *in-situ* polymerization of caffeic acid under the same conditions. The treated wool fabrics showed good wettability and electroactivity. The results from fiber conductivity test and cyclic voltammetry showed that the wool fabric treated with poly(caffeic acid)/poly(ethylene glycol) complex was found to have better electrical conductivity. These data showed that the presence of poly(ethylene glycol) as a template in the laccase-catalyzed polymerization reaction greatly improved

the characteristic of poly(caffeic acid). Moreover, higher color depth of poly(caffeic acid)/poly(ethylene glycol)-treated wool fabrics further validated the important role of the poly(ethylene glycol) as a template.

Keywords: poly(ethylene glycol); enzymatic zip-polymerization; template; caffeic acid; laccase; wool functionalization

1. Introduction

There is considerable interest to develop clean processes of polymer production due to environmental concerns over the past years. Enzymatic polymerization is regarded as a green approach which can be executed under milder conditions without using toxic solvents (Dordick et al., 1987), resulting in higher efficiency and lower consumption of energy (Fukuoka et al., 2000). Premachandran et al. has discovered that it is highly feasible to synthesize fluorescent polymers using enzymatic route in the micellar systems (Premachandran et al., 1996). Polyphenol oxidases can induce polymerization of various phenol derivatives to produce phenolic polymers with high thermal stabilities (Uyama et al., 2002[1]; Pang et al., 2003[2]). For example, produced either from renewable resources or from enzyme catalyzation at room temperature, polycardol is more thermostable than polycardanol (Park et al., 2009). Lignocatechol, copolymer of lignin-based macromonomer catalyzed by laccase, has high thermal stability due to its cross-linked structures between lignin and catechol (Yoshida et al., 2009). Laccase (EC 1.10.3.2) is a type of polyphenol oxidase enzymes, which can be divided into two major groups based on its origin: either from higher plants or from fungi (Kobayashi and Makino, 2009). Laccase has been widely applied in various aspects including lignin grafting, dyes bleaching and pulp bleaching. Laccase can also catalyze the oxidation of phenolic hydroxyl groups (Ikeda et al., 1998) or aliphatic amines (Chung et al., 2003) to produce high active radicals in a redox reaction, using atmospheric oxygen as an oxidizing agent.

[1] Polymers from dihydroxydiphenylmethanes
[2] Cyclodextrins/hydrophobic monomer complex

Caffeic acid (3, 4-dihydroxycinnamic acid; CA), one of the natural phenolic compounds, is widely found in coffee, olive oil (Chen and Ho, 1997), white wine, cabbage, etc (Michaluart et al., 1999). In addition, caffeic acid is a suitable substrate of polyphenol oxidases. That is why caffeic acid can be oxidized under certain conditions in plant tissues (Kerry and Rice-Evans, 1998) or in products of plant origin (Bassil et al., 2005). Caffeic acid and its ester derivatives also possess antiradical-scavenging activity *in vitro* (Kim et al., 2003) and can be used as carcinogenic inhibitors (Sato et al., 2011).

Rompel et al. has studied the oxidation mechanism of CA and found that carboxyl group could inhibit activity of catechol oxidase (Rompel et al., 1999). Pati et al. has identified two different classes of caffeic acid dimers produced from a reaction catalyzed by tyrosinase. These dimers have caffeine-like structures formed by C-C coupling between the benzene rings (Pati et al., 2006). However, it is very difficult to control the regioselectivity of the enzymatically oxidative polymerization of phenol derivatives because such polymerization proceeds via the coupling of free radical intermediates (Sun et al., 2013).

In order to overcome regioselectvity problem, monomers were organized by natural matrices (template), which then proceed according to the ‘zip’ and ‘pick-up’ polymerization mechanisms (Połowiński, 2002). For the ‘zip’ mechanism, monomer units connect with a matrix by relatively strong attraction forces (electrostatic, hydrogen bridges) before reaction or polymerization. Alternatively, if monomer and matrix do not have sufficient attraction forces between them, the polymerization of monomer starts without the involvement of the matrix as a template until oligoradicals reach a critical length of the polymers, leading to build up the attraction forces between the polymers and the matrix, then the complexation with the template occurs. These two mechanisms are influenced by the degree of the attraction forces between the matrix and the monomer units or the resultant polymers (Shimomura et al., 2003). Kim et al. has demonstrated that the presence of poly(ethylene glycol) monododecyl ether (PEGMDE) as a template in an aqueous medium can greatly improve the regioselectivity of phenol polymerization (Kim et al., 2004).

The task of the work presented in this paper was to synthesize poly(caffeic acid) (PCA) by laccase catalyzation of caffeic acid in the presence of poly(ethylene glycol) (PEG) as a template. The role of PEG in the polymerization of CA was examined using UV-Vis spectra, which demonstrated that CA bonded to PEG through hydrogen bonds according to the 'zip' mechanism. The probable structures of PCA and PCA/PEG complex were analyzed by FT-IR, MALDI-TOF and HPLC-MS measurements. In addition, the electroactivity, wettability and dyeing properties of treated wool fabrics were also investigated. During the laccase catalyzation, the addition of PEG not only efficiently induced the polymerization of CA, but also greatly improved its degree of polymerization. Furthermore, the synthesis of PCA by this cleaner method can be simultaneously applied to wool dyeing, resulting in the coloration of wool imparting extra conductive functionality.

2. Materials and Methods

2.1 Materials and Chemicals

Wool fabrics (100% gabardine) with a density of 220 g/m^2 used in the experiment were kindly supplied by Wuxi Xiexin Wool Textile Co., Ltd.

Laccase (EC 1.10.3.2) from *Ascomycete M. Thermophila* and caffeic acid (3,4-dihydroxybenzeneacrylic acid, $(HO)_2C_6H_3CH{=}CHCO_2H$) and PEG (poly(ethylene glycol), H-(O-CH_2-CH_2)$_n$-OH, molecular weight = 6,000 Da) were both provided by Sigma-Aldrich and used without further purification. 2, 2'-azino-bis-(3-ethylthiazoline-6-sulfonate) (ABTS, ε_{420} = 36,000 $M^{-1} \times cm^{-1}$), also supplied by Sigma-Aldrich, was used for determining enzyme assay. All other chemicals used were analytical grade and supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2 Activity Assay of Laccase

The activity of laccase was measured by incubating 0.1 mL of 2 g/L laccase with 2.9 mL of 0.5 mM ABTS in 50 mM acetate buffer at pH 5. The absorbance due to enzymatic oxidation of ABTS was monitored at 420 nm using a Spectrophotometric UV-1800. The spectrometric measurements were carried out every 0.05 absorbance

increment. One unit of laccase (U) is defined as the amount of laccase required to oxidize 1 μmol of ABTS in one minute (Sun et al., 2013).

2.3 Enzymatic Oxidative Polymerization of Phenols

Based on the previous studies on laccase catalyzation of phenolic compounds (Sun et al., 2013) and the template polymerization (Kim et al., 2004), the procedures of laccase-catalyzed polymerization of phenol were further investigated. The optimal temperature and reaction time were set at 50^{o}C and 5 hours, respectively.

The role of PEG in enzymatic polymerization of caffeic acid was examined as follows. CA (0.9 g, 5.0 mmol) and PEG were mixed at their different weight ratios of CA to PEG from 1:0.1 to 1:2.0 and incubated in 0.2 M acetate buffer (pH 5) in the absence or presence of laccase (0.16 U/mL enzyme activity). The mixture was agitated at a speed of 30 rpm (revolution per minute) in a shaker water bath set at 50^{o}C for 5 hour. After that, the precipitated polymeric materials were obtained through evaporation and repeated washes with methanol, and subsequently lyophilizing at -50^{o}C to obtain the PCA/PEG complex. The same reaction without PEG was also conducted for comparison.

2.4 Preparation of Functionalized Wool

Wool samples were enzymatically treated with laccase in the presence of caffeic acid and PEG by following same procedure as described in Section 2.3. Swatch of wool fabric samples (about 5 cm^2) was immersed in 0.2 M acetate buffer (pH 5) containing caffeic acid (0.9 g, 5.0 mmol) and PEG (0.9 g) at CA-to-PEG weight ratio of 1:1 with 0.16 U/mL of laccase at a liquor-to-goods ratio of 100:1 and treated at 50^{o}C for 5 hour. The treatment was undertaken under agitation at a speed of 30 rpm (revolution per minute) in a shaker water bath. After enzymatic reaction, the wool samples were washed thoroughly with deionized water and then hydro-extracted to remove excess wetness and left to air-dry.

2.5 UV-Vis Spectra Analysis

The role of PEG in polymerization of caffeic acid catalyzed by laccase was monitored using a UV-1800 UV-Visible Spectrophotometer (Shimadzu, Japan) at a

wavelength range of 200-800 nm. The polymerization kinetics was analyzed based on the change in absorption at 500 nm (Zhang et al., 2016).

2.6 Characterization of Polyphenol and Polyphenol-PEG Complex

2.6.1 MALDI–TOF Mass Analysis

MALDI–TOF mass measurements of polymer products were conducted using a Bruker ultraflextreme MALDI–TOF Mass Spectrometer (Bremen, Germany) equipped with a nitrogen laser (λ = 337 nm). 2, 5-dihydroxybenzoic acid (DHB) was used as the matrix compound. The product samples were dissolved in water to form a concentration of 2 mg/mL followed by mixing with the matrix compound at 1:1 (vol:vol) ratio of the dissolved sample to DHB (2µl each). The sample/matrix mixture was deposited on MALDI target plate and allowed to air-dry at room temperature. The positive-ion mass spectra were acquired from the dried sample spots in reflective mode (Dong et al., 2015).

2.6.2 HPLC and ESI-MS Conditions

HPLC separation of laccase-catalyzed polymeric compounds of caffeic acid was conducted at room temperature using a Waters Acquity UPLC set at a flow rate of 0.3 mL/min through gradient elution. The product from laccase-catalyzed oxidation of caffeic acid was prepared by dissolving in deionized water. The injection volume was 0.5 µL. Acetonitrile and formic acid were selected as mobile phases A and B respectively to perform on the column, BEH C18, 1.7 µm (2.1 mm internal diameter × 50 mm length). The column was flushed with the mixture of 2:98 (v/v) acetonitrile:formic acid for the first 0.1 min, followed by 60:40 acetonitrile:formic acid for 8 min, then with 100% acetonitrile for 4 min, and finally with 2:98 acetonitrile:formic acid for 0.1 min to complete. During the HPLC-PDA (photo-diode array) analysis, the PCA and PCA/PEG complex were detected at a wavelength of 280 nm (Rompel et al., 1999; Pedrosa et al., 2000).

HPLC-ESI-MS data were acquired in negative ion mode using Waters Maldi Synapt Q-Tof mass software. The optimized electrospray parameters were shown in Table 1.

Table 1 The optimized electrospray parameters

The optimized electrospray parameters	
Capillary voltage	3.5 kV
Cone voltage	20 kV
Source block temperature	100°C
Desolvation temperature	400°C
Desolvation gas flow	500 L/h
Cone gas flow	50 L/h
Collision energy	6 V
Detector voltage	1800V
m/z range	0-1000

2.6.3 FTIR Spectroscopy

The spectra of polymeric compounds from catalysis of caffeic acid by laccase were obtained using a Nicolet IS10 FT-IR Spectrometer (Thermo Fisher Scientific, USA). Prior to data collection, background signal was scanned using potassium bromide (KBr) powder. The polymer products, which were lyophilized as described in Section 2.3, were mixed with small amount of the matrix KBr. At least 32 scans were carried out in order to achieve adequate signal-to-noise ratio. The spectra were obtained at room temperature from 450 to 4000 cm^{-1}, with a resolution of 8 cm^{-1}.

2.6.4 Thermogravimetric Analysis (TGA)

The laccase-catalyzed polymeric products were analyzed for TGA using a TGA/SDTA 851e Thermogravimetric Analyzer (Mettler Toledo, Switzerland), in which approximately 5 mg of each sample was used. The samples were heated from 50 to 600°C at a heating rate of 10°C / min under nitrogen atmosphere.

2.7 Characterization of Wool Fabric

2.7.1 Cyclic Voltammetry (CV) Test

Cyclic voltammetry measurements of the treated wool samples were performed on a CHI 660D Electrochemical Workstation (CH Instruments Inc., Austin, USA). A glassy-carbon electrode was used as the working electrode, whereas platinum and Ag/AgCl electrodes were used as the counter and the reference electrodes, respectively. Prior to the test, a small piece of wool sample was fixed onto the glassy-carbon

electrode using dried Nafion emulsion (1.5 wt%) (Karamyshev et al., 2003).

2.7.2 Conductivity Measurement

The resistance of wool fibers was determined using a 4200-SCS semiconductor parameter analyzer (Keithley Instruments Inc., USA). Two ends of a wool yarn were tied to the two wires that were drawn from a fixture box (Zhang et al., 2014). The resistance of the treated wool yarns before and after standard washing was measured to assess durability of conductivity to wash. Washing of the treated wool fabrics (dimension: 5 cm × 5 cm) were carried out according to ISO 105-C06-B1S:2010 standard. After 5, 10, or 25 washing cycles, the treated wool fabrics were rinsed with deionized water and dried at room temperature for conductivity measurement (Liu et al., 2015).

2.7.3 Color Measurement of Treated Wool Fabrics

During the laccase-catalyzed functionalization process, wool fabrics were also colored into brown color shade. After enzymatic process, wool samples were thoroughly washed several times with 1:3 (v/v) acetone/deionized water solution to remove residual laccase or unfixed color, and then dried overnight at room temperature.

The colorimetric properties of the treated wool fabrics were studied at 400 nm using a Color-Eye 7000 A Spectrophotometer (Gretag Macbeth, USA), in terms of CIELab values (L*, a*, and b*), with the standard illuminant D65 and a 10° observer. The color depth (K/S value) was calculated based on Kubelka-Munk equation, as shown in equation (2) below:

$$K/S = (1-R)^2/2R \quad (2)$$

Where K is the absorbance coefficient, S is the scattering coefficient, and R is the reflectance.

2.7.4 Water Contact Angles (WCA) Test

Wettability of treated wool samples was determined by measuring the contact angles of a water drop on the fabric surface using a SL200B Static Contact Angle/Interfacial Tension Meter (Kino Industry Co. Ltd., USA). Water contact angle at five different spots on each sample was measured and the data presented for each sample is an average value from the five spots (Dong et al., 2014). As a control,

wettability of untreated wool sample was determined, in which its contact angles were measured before treatment (Shang et al., 2010).

3. Results and Discussion

3.1 Formation of Caffeic Acid and PEG Complex and Their Catalysis by Laccase

Caffeic acid (CA) can be catalyzed to dimeric, oligomeric, and polymeric products by oxidoreductase. The representative structures of caffeic acid dimers have been investigated previously (Sun et al., 2013). In the present work, PEG was first used as a template to arrange caffeic acid on the polymer chain of PEG through H-bonding. Then caffeic acid aligned on the template of PEG was polymerized by laccase-catalyzed oxidation. The scheme of formation of CA/PEG complex followed by catalysis of laccase was shown in Figure 1. Without PEG, caffeic acid can be catalyzed by laccase to form different formations of its dimeric as shown in Figure 1 (a, b and c) and further polymerized to the polymeric colorants.

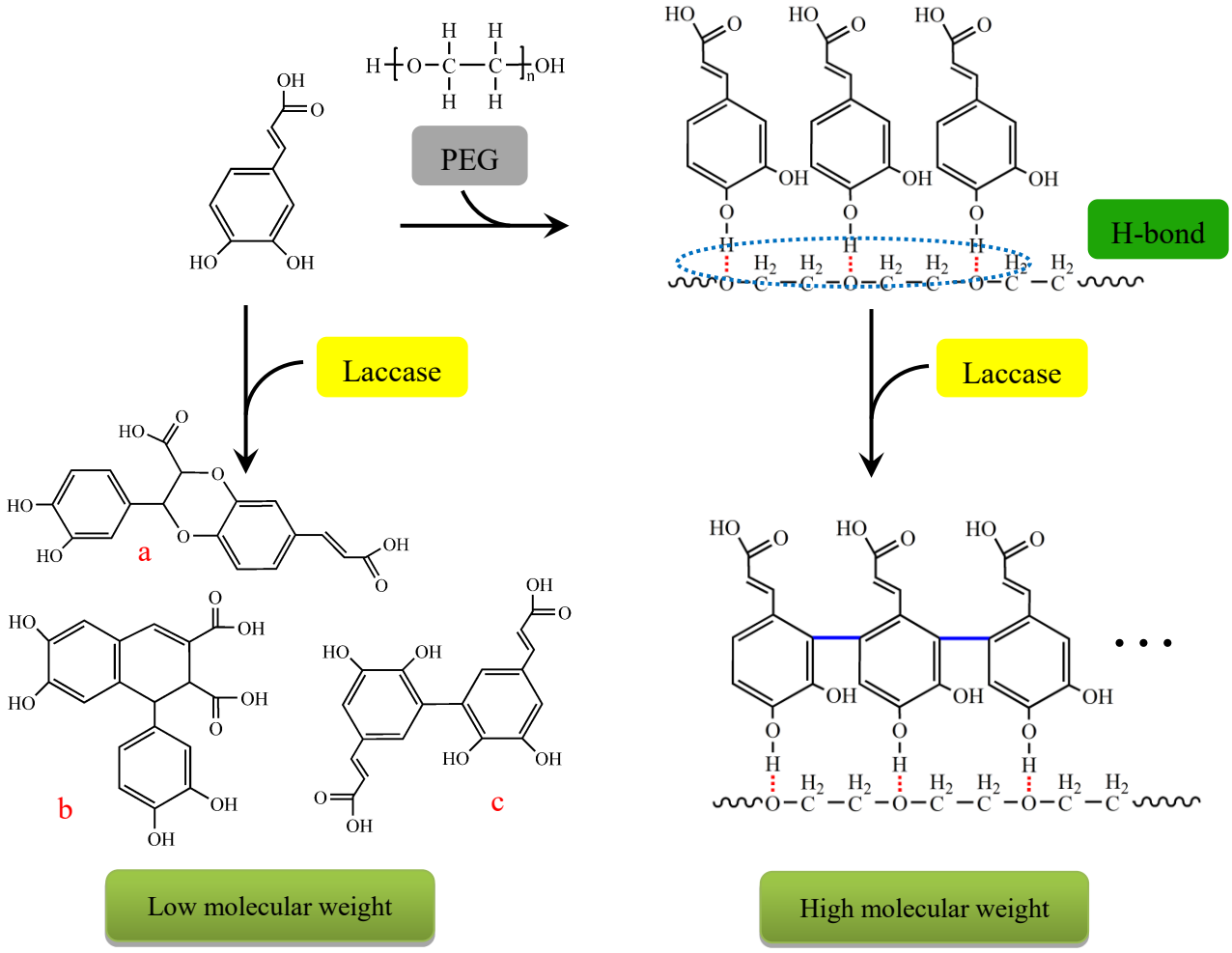


Figure 1 Schematic representation of PEG templated polymerization of Caffeic acid

The role of PEG in polymerization of CA was examined. UV absorption spectra of CA and PEG mixture at the different ratios in the acetate buffer was monitored and shown in Figure 2. Each bar at different wavelength was obtained by subtracting the spectrum of CA in the absence of PEG from that in the presence of PEG. When PEG was added to a solution containing caffeic acid, the absorption intensities of specific peaks at the wavelengths of 216, 288, and 313 nm increased due to the formation of CA/PEG complex through hydrogen bonding interactions (Oguchi et al., 2002). The absorbance of the mixture at three different wavelengths all reached the maximum value for the mixture ratio of CA to PEG at 1:1. It is suggested that the hydroxyl groups of CA and PEG are bound through hydrogen bonding interactions according to the 'zip' mechanism (Połowiński, 2002).

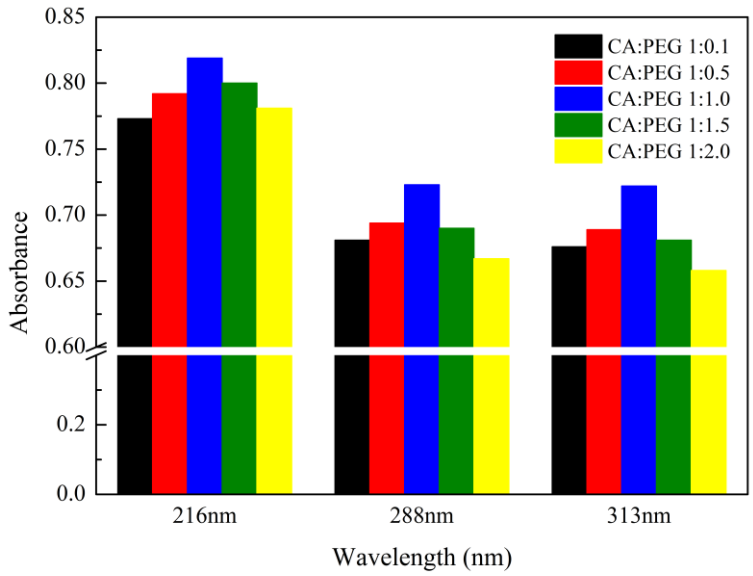


Figure 2 UV-Vis absorption data of CA/PEG complex with different weight ratios of CA to PEG in acetate buffer (pH 5.0).

CA and PEG were mixed at a ratio of 1:1 and incubated with laccase in the acetate buffer at pH 5 for 5 hour. Laccase-catalyzed polymerization of CA on the template of PEG produced brown color in the solution due to the formation of polymeric colorant, poly(caffeic acid) (PCA) as shown in Figure 3. A control was also conducted, in which caffeic acid was catalyzed by laccase without PEG at the same initial pH (using acetate buffer) and temperature. It was found that polymerization of CA on the template (PEG) showed darker brown color than that without PEG. It is apparent that the amount of

PCA formed in the presence of PEG was higher according to the higher extent of color achieved. Reaction rate of the oxidative polymerization was measured to study whether PEG promotes the polymerization of CA. Absorbance of the reaction solution at 500 nm as a function of reaction time shown in Figure 3b demonstrated that the absorbance of CA/PEG mixture rapidly increased with increasing time due to the formation of larger conjugated structures of phenolic rings. Moreover, because the increase in the absorbance was found to be in a linear relationship with the reaction time, the reaction rate catalyzed by laccase could thus be determined from the slope of a plot of absorbance versus time. It was noted that in the presence of PEG, the reaction rate was three times higher than that in the absence of PEG, which is incoherent with those observed in Figure 3a.

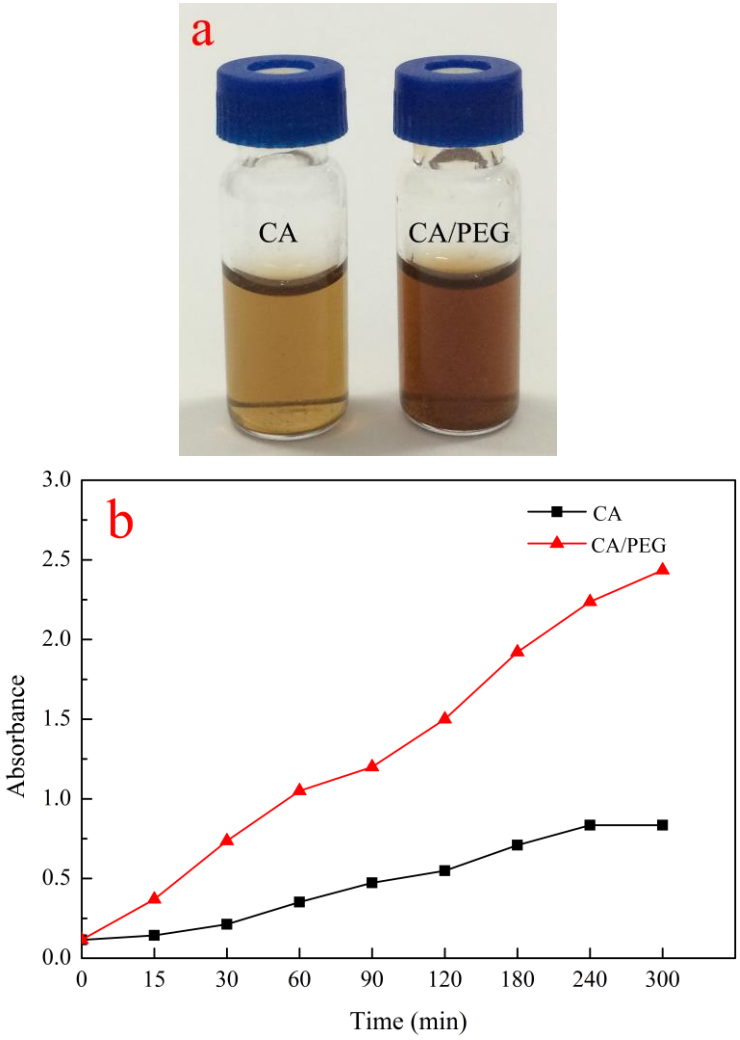


Figure 3 UV-vis absorption data of CA and CA/PEG mixture under the catalysis of laccase at 500 nm as a function of reaction time at 50°C

These results indicate that PEG significantly induced the oxidative polymerization of CA. Prior to the addition of laccase, caffeic acid monomers bond to PEG via hydrogen-bond interactions according to the 'zip' mechanism, which can lead to a regularly arranged structure. Upon the addition of laccase, caffeic acid was then catalyzed to form phenoxy radicals, and then further polymerized, resulting in higher degree of PCA polymerization as shown in Figure 1.

3.2 Characterization of PCA and PCA/PEG Complex

3.2.1 HPLC-ESI-MS Analysis of PCA

HPLC-ESI-MS profiles obtained at 280 nm from poly(caffeic acid) solutions catalyzed by laccase (dissolved in deionized water) were analyzed. As shown in Figure 4, the presented peaks are related to unreacted CA monomers, intermediates species, and stable oxidation products. Due to different polarities of the product compounds, they were separated by HPLC into eight major peaks. The corresponding information of MS fragments (the major ions observed in the ESI-MS spectra averaged under each HPLC peak in Figure 4) observed during HPLC-ESI-MS analysis was shown in Figure 5 and Table 2. Comparing these MS data (for all compounds), it was shown that intermediate and stable oxidation products were observed in most peaks. The simple MS pattern of caffeic acid (peak A, disappeared in MS spectra) arose from decarboxylation (*m/z* = 135, corresponding to 44 Da loss) was observed. Because this typical decarboxylation was observed in almost all species, the fragmentation dominated with decarboxylation of the parent ion ($[M–H]^-$; M: *m/z* = 180) was thus used as the base peak in ESI-MS spectra (Pati et al., 2006). The deprotonated dimer and tetramer of caffeic acid with *m/z* ratios of 359 and 715, respectively, could explain the formation of these oligomers with different molecular weights. The dimer of caffeic acid (*m/z* = 358) could lose its two CO_2 molecules and become neutral fragment (*m/z* = 715 in Table 2) which could be the $[2M–H]^-$ adduct of the dimer. The dominant peak at *m/z* = 311 was originated from ions at *m/z* = 401, due to the loss of CO_2 and HCOOH. Both ions at *m/z* ratios of 715 and 401 generated a minor fragment of *m/z* = 311 (peaks C and E in Figure 4). Each main peak was different by about 44 Da, indicating that CO_2 can be easily lost in the polymerization process. Moreover, other fragments, such as those at *m/z* ratios of 669, 313, and 401 (Table 2), were due to the loss of $[CO_2+2H]$

ion (46 Da). These results suggested that the polymerization of caffeic acid was irregular, and structure of the polymer compounds was extremely complex. In addition, caffeic acid trimer and its derivatives were the compounds of the highest molecular weights among those detected by MS.

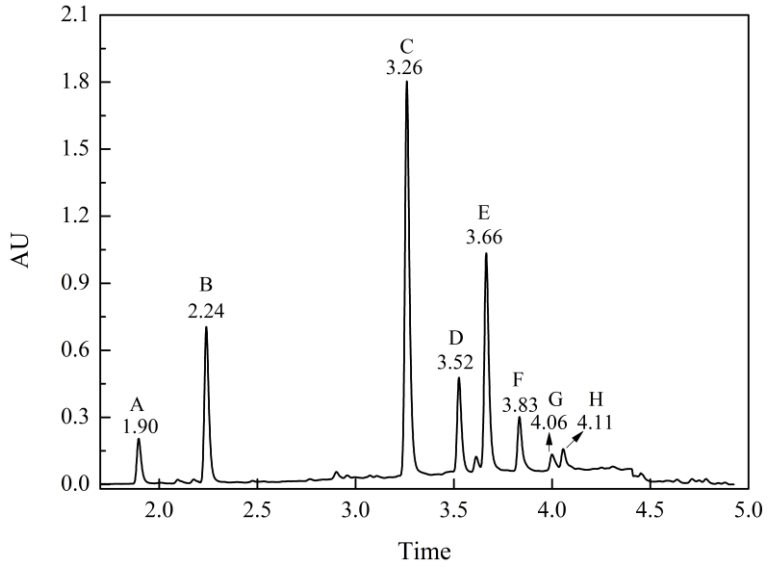


Figure 4 HPLC-ESI-MS UV spectra (absorbance at 280 nm) of PCA solution

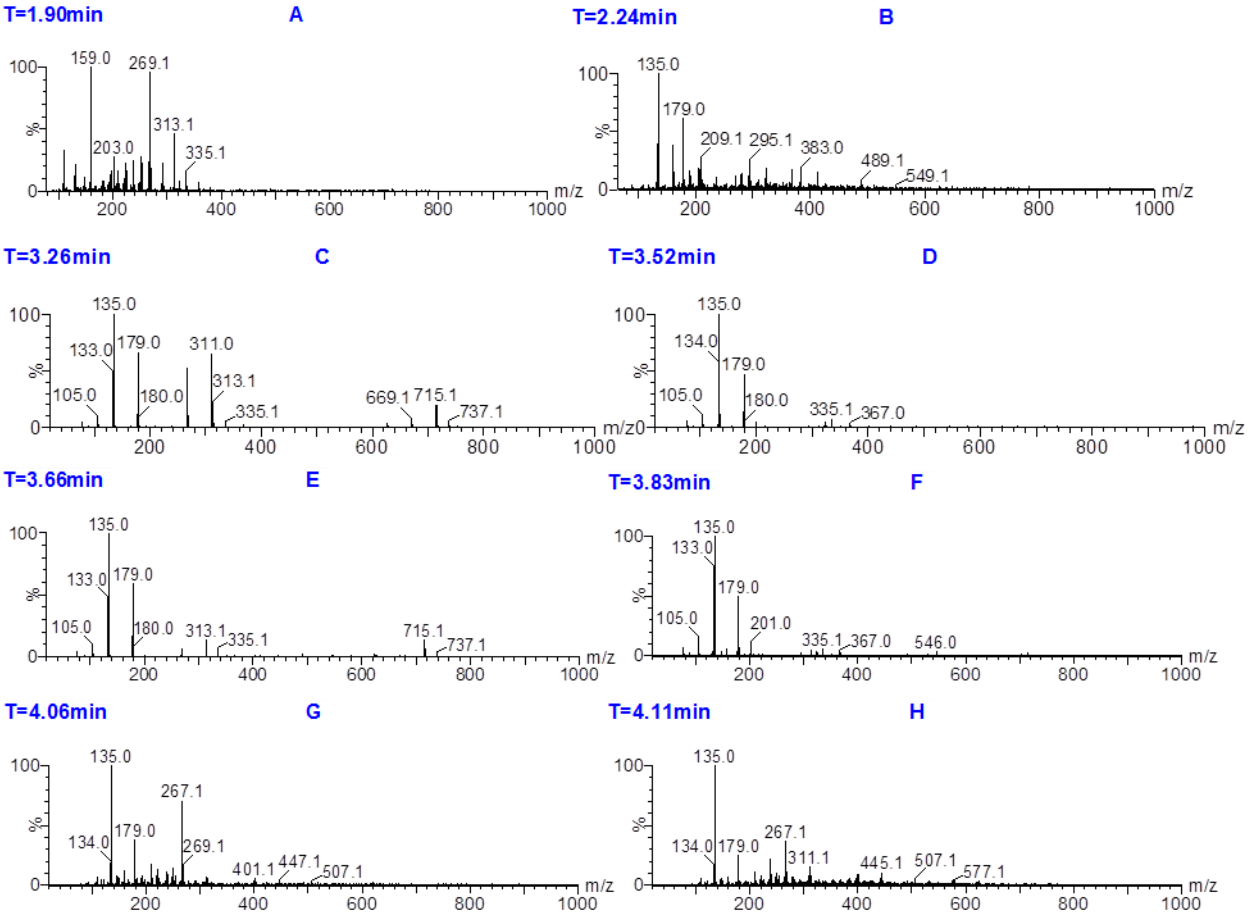


Figure 5 ESI-MS full-scan spectra relevant to all products from enzymatic oxidation of caffeic acid

Table 2 Summary of peaks and MS information obtained by HPLC-ESI-MS separations of PCA

Peak Label	Retention time (min)	m/z ratios for the *base peak*	m/z ratios for the MS/MS *main fragments*
A	1.90	313	269; 159
B	2.24	179; 180; 489	295; 135
C	3.26	179; 311; 715	669; 267; 135; 105
D	3.59	179; 180	135
E	3.66	179; 180; 313; 715	269; 135; 105
F	3.83	179	135; 105
G	4.00	179; 447	401; 269; 267; 135
H	4.06	179; 311	267; 135

3.2.2 FT-IR Analysis

The FT-IR analyses of CA, PEG, PCA and PCA/PEG complex were carried out

and described in Figure 6. The structure of PCA products from tyrosinase-catalyzed oxidation of caffeic acid was previously investigated by Sun et al. (Sun et al., 2013). CA spectrum showed the characteristic phenolic O-H stretching and in-plane bending at 3434.3 and 1294.7 cm^{-1}, respectively. The O-H stretching vibration bands of carboxyl group were observed at 3233.3, 3026.5, and 2568.1 cm^{-1}. Peaks at 1645.1 and 1619.2 cm^{-1} were attributed to the C-O and C-C stretching vibrations, respectively. Peaks between 1449.8 and 1620 cm^{-1} belonged to the aromatic skeletal vibration. Adsorption bands at 1280.4 and 1218 cm^{-1} were due to the phenolic C−O stretching and CH=CH in plane bending, respectively (Kalinowska et al., 2010; Xing et al., 2012). Broad peaks of PCA and PCA/PEG over the range of 3600 - 2500 cm^{-1} suggest strong hydrogen bond interactions. Strong absorption band of two spectra at 1624.9 cm^{-1} （PCA/PEG）could be attributed to both the C=C stretching and increased intensity of aromatic skeletal vibration. In addition, a new strong adsorption band of PCA in the presence of PEG appeared at 1100 cm^{-1}. This could be attributed to the C-O-C stretching of aromatic−aliphatic ether and PEG, which were the results of benzodioxane moiety formation (Sanchez-Cortes and Garcia-Ramos, 1999) and the PEG and phenolic acid linkage.

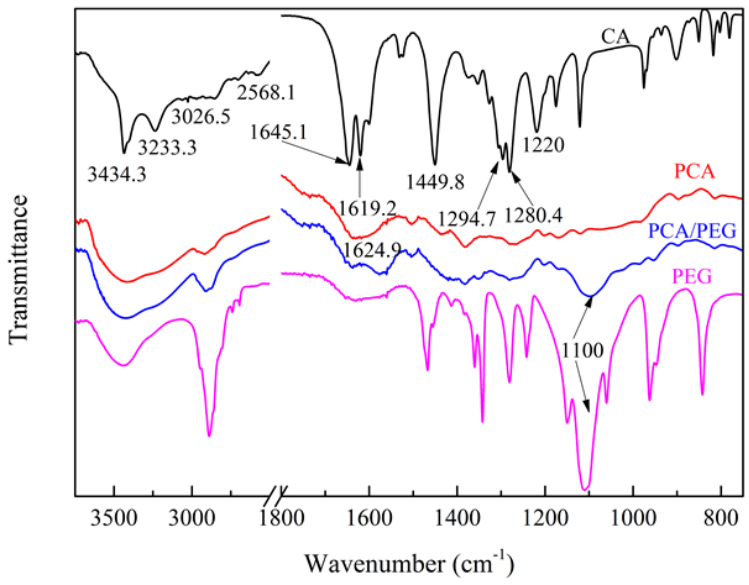


Figure 6 FT-IR spectra of CA, PCA, PEG and PCA/PEG complex

3.2.3 MALDI-TOF Analysis

The formations as well as the properties of the polymers obtained from laccase-catalyzed reactions were first investigated by MALDI-TOF MS. It was found that 2, 5-dihydroxybenzoic acid (DHB) was the best matrix to be used for generation of the ionization. Figure 7 shows MALDI-TOF mass spectra of the products generated from laccase-catalyzed polymerization of CA and CA/PEG complex respectively. Their polymerization reactions were carried out in which similar amounts of laccase and CA monomer were used. The molecular weight distribution was found to be from 300 to 1300 Da. Fine oligomeric structures observed in the spectra could be attributed to different degrees of oligomerization (i.e. from dimers to heptamers). These oligomers were further characterized by MALDI spectra and found to be caffeic acid dimers, trimers, tetramers, pentamers, hexamers, and heptamers with molecular weights of about 373, 550-573, 726-749, 903-926, 1078-1101, and 1253-1276 Da, respectively. Meanwhile, PCA/PEG complexes with higher molecular weights of about 1454-1652 Da were observed in the MS spectra. These PCA/PEG complexes had higher degree of polymerization than those of PCA (without PEG), indicating that PEG, as a template, could promote the polymerization of caffeic acid. The successive peaks were separated by 176 Da which were corresponding to the mass of one caffeic acid unit $[M-2H]^+$, and most of them match those protonated species, $[nM-H]^+$ (where n is the number of monomeric units containing phenolic and benzoic acid end groups). Because some peaks appeared regularly in all spectra, this may suggest the diversity of oligomers formed by the polymerization reaction. In addition, in each peak group, smaller peaks were observed in the regions of higher m/z than those of protonated species. These peaks may arise due to additives, such as sodium or potassium. The glycol unit of PEG was not detected in the MS spectra, and it is possible that H-bonds between PCA and PEG were deteriorated by the laser.

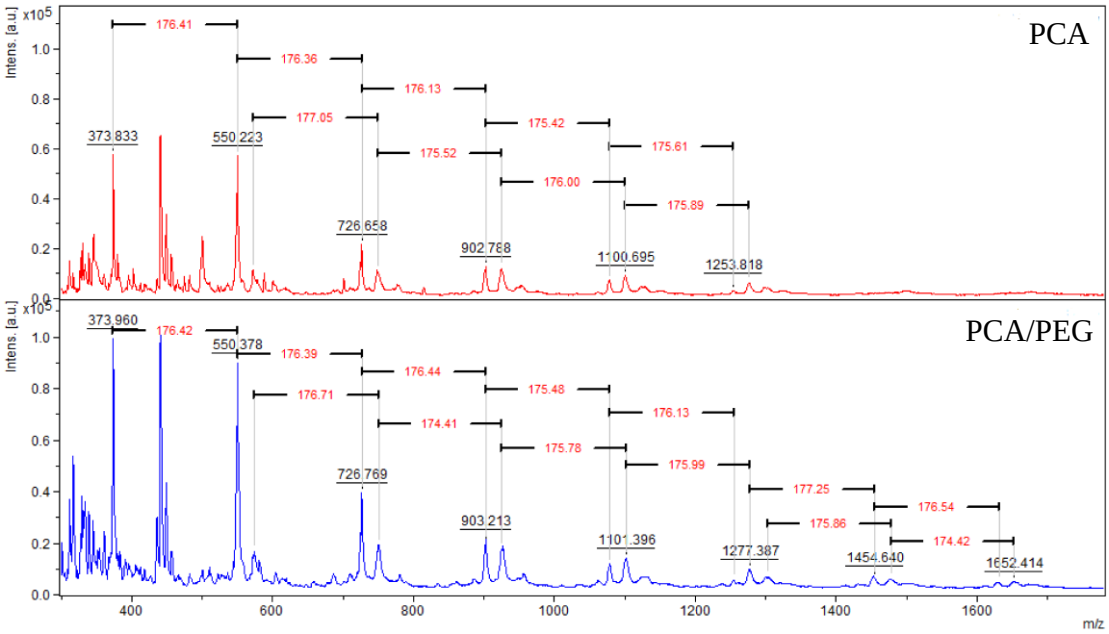


Figure 7 MALDI-TOF mass spectra of PCA (upper) and PCA/PEG composite (lower)

3.3 Properties of Polymerized Caffeic Acid and Functionalized Wool Fabrics

3.3.1 Thermal Property of PCA and PCA/PEG Complex

The TG and DTG plots for the PCA and PCA/PEG complex were shown in Figure 8. Initial weight loss corresponding to loss of water from the materials was observed at around 100^{o}C. The TG-DTG curves for PCA and PCA/PEG also showed that the two-step weight losses were observed at the temperature range of 100-600^{o}C. The TG-DTG curves of PCA showed that in the first step, the weight loss of PCA was 8.2% at the temperature range of 300-320^{o}C with a minimum DTG (0.045 wt%/^{o}C) at 305^{o}C; and in the second step, 26% weight loss was observed at the temperature range of 400-500^{o}C with a maximum value of DTG (0.58 wt%/^{o}C) at 475^{o}C. On the other hand, the curves for PCA/PEG showed that in the first step, the weight loss was 14.4% at the temperature range of 300-400^{o}C with a minimum DTG (0.148 wt%/^{o}C) at 378^{o}C; and in the second step, 28.1% weight loss was detected at the temperature range of 400-500^{o}C with a maximum DTG (0.62wt%/^{o}C) at 480^{o}C. Sharp decomposition peaks over the temperature range of 100-600^{o}C were observed in both the curves for PCA and for PCA/PEG complex suggest that structures of the two polymers are very similar. Moreover, decomposition peak for PCA/PEG that was discovered at higher temperature demonstrates the thermal stability of PCA/PEG was slightly higher than that of PCA.

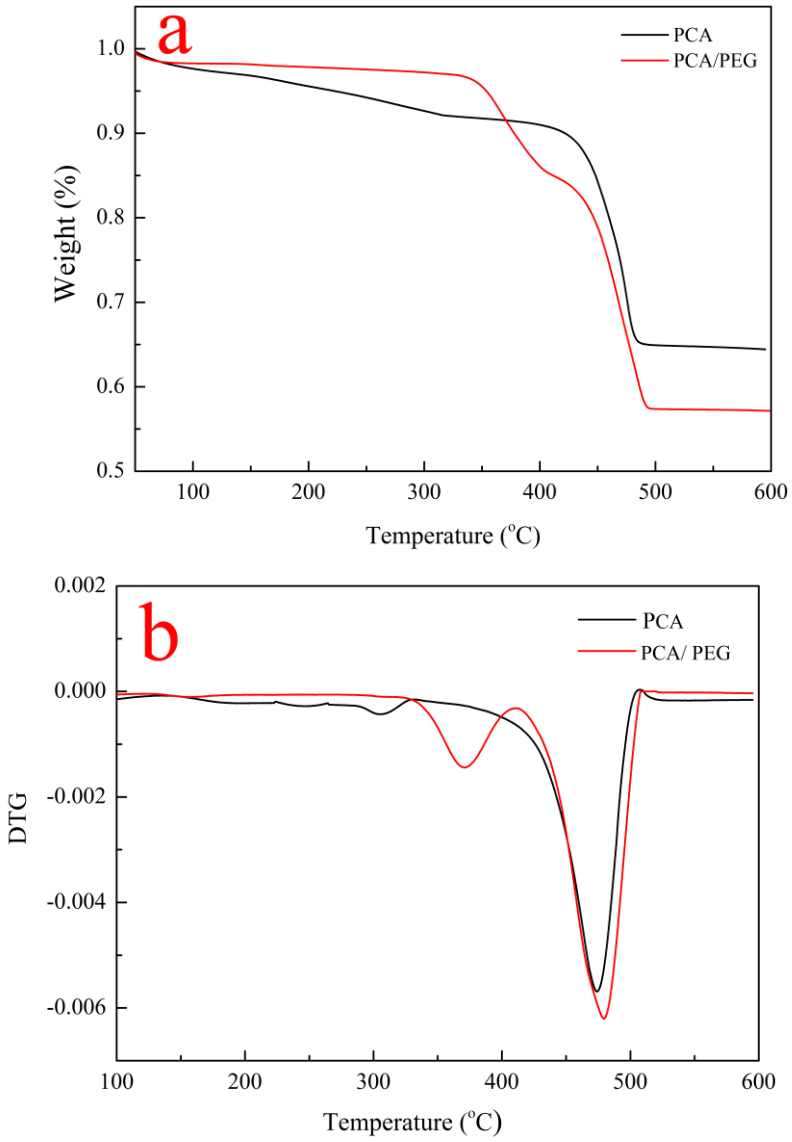
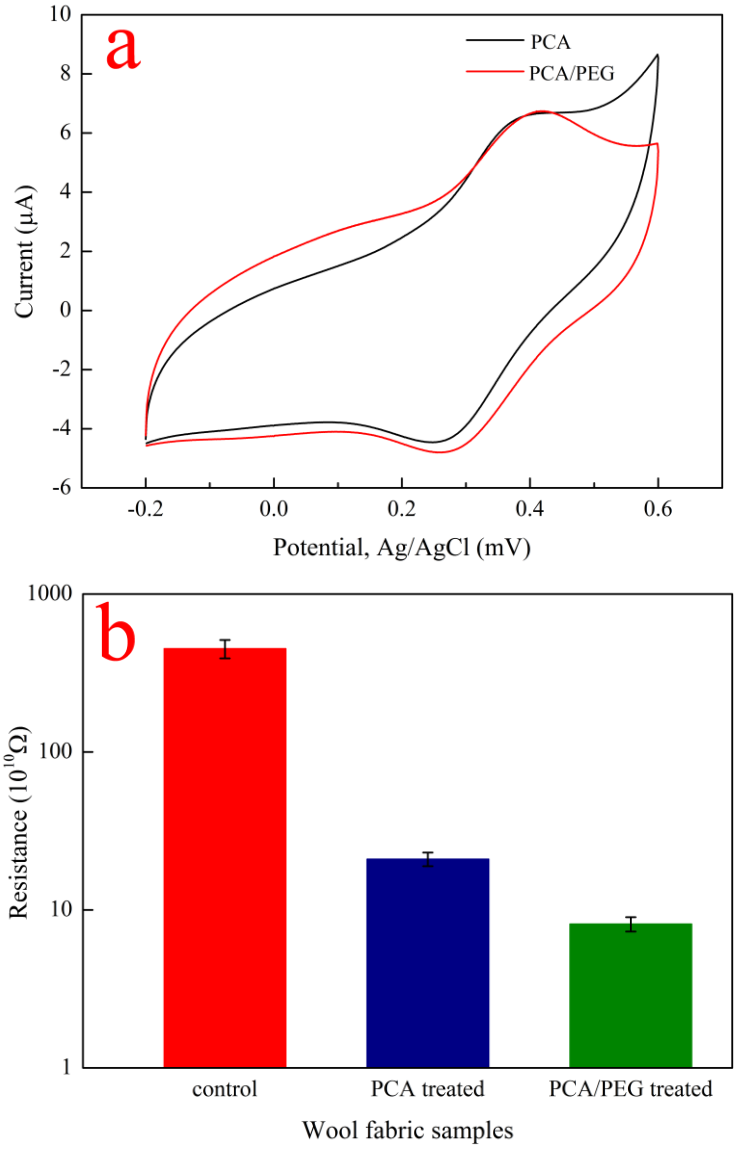


Figure 8 TG (a) and DTG (b) plots for PCA and PCA/PEG complex

3.3.2 Electroactivity of PCA- and PCA/PEG-treated Wool Fabrics

Cyclic voltammetry (CV) was used to determine the redox property of wool fabrics treated with PCA and PCA/PEG complex. The CV values of wool fabrics treated with PCA or PCA/PEG complex were compared and shown in Figure 9a. The broader oxidation and reduction peaks in PCA/PEG curve suggested that PEG existed in the complex. Peaks at +0.42 V for oxidation and at +0.29 V for reduction against Ag/AgCl, which were observed for PCA/PEG complex had similar characteristics to those for PCA. Figure 9b presents the resistance of wool fabric treated with PCA and PCA/PEG complex. The resistance of wool fabric treated with PCA was higher than that treated with PCA/PEG complex. It was indicated that the PCA/PEG complex has higher

conductivity. The electrical durability of wool fabrics treated with PCA or PCA/PEG complex was presented in Figure 9c. Comparison of all resistance data in this chart demonstrated that the resistance of both types of the treated wool fabrics remained almost the same during the first 10 washing cycles. After 25 washing cycles, the resistance of wool fabric treated with PCA increased more than that treated with PCA/PEG complex. These data suggested that PCA/PEG-treated wool fabric had good electrical durability after repeated water wash and confirmed that PCA/PEG-treated wool fabric had higher degree of electrochemical activity.



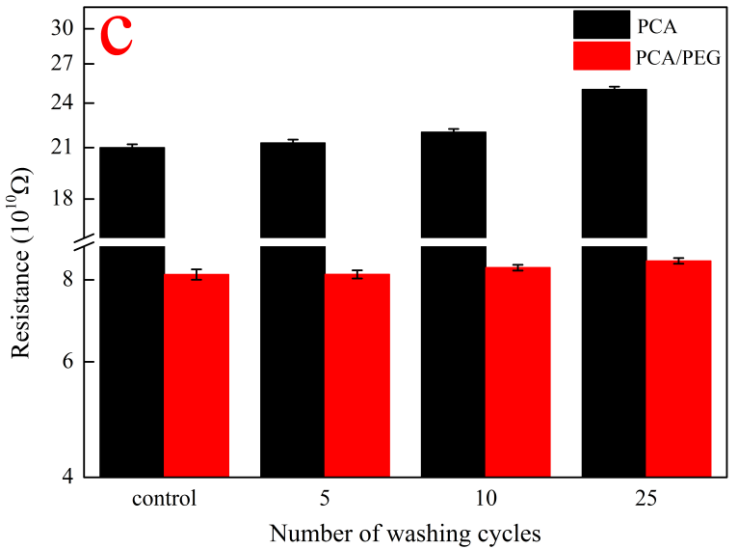


Figure 9 Cyclic voltammograms of the wool fabric treated with PCA and PCA/PEG complex (a), resistance of wool samples (b) and resistance of treated wool samples after different washing times (c)

3.3.3 Wettability of Treated Wool Fabrics

The water contact angles of treated wool fabrics were presented in Figure 10. The water contact angle of the control wool fabric was found to be 121.51° at 5 s, which remained constant until 1 min, whereas that of wool fabrics treated with PCA and PCA/PEG complex were 75.02° and 45.39°, respectively. Wool fabric treated with PCA took about 6 s to soak up the water droplet, but the fabric treated with PCA/PEG complex took only less than 5.5 s. The increase in hydrophilicity of PCA/PEG-treated wool fabrics could be due to that the poly(caffeic acid), which was connected with wool fibers, brought together a number of phenolic hydroxyl groups as well as carboxyl groups onto the surface of wool fabrics. PCA/PEG-treated wool fabrics had higher hydrophilicity compared with that treated with PCA, indicating that PCA/PEG complex has higher degree of polymerization. The hydrophilicity of the surface was further confirmed by photographs of water droplet on the surface of the treated wool fabrics in comparison with that of the control (Figure 10, insert) at 5 s.

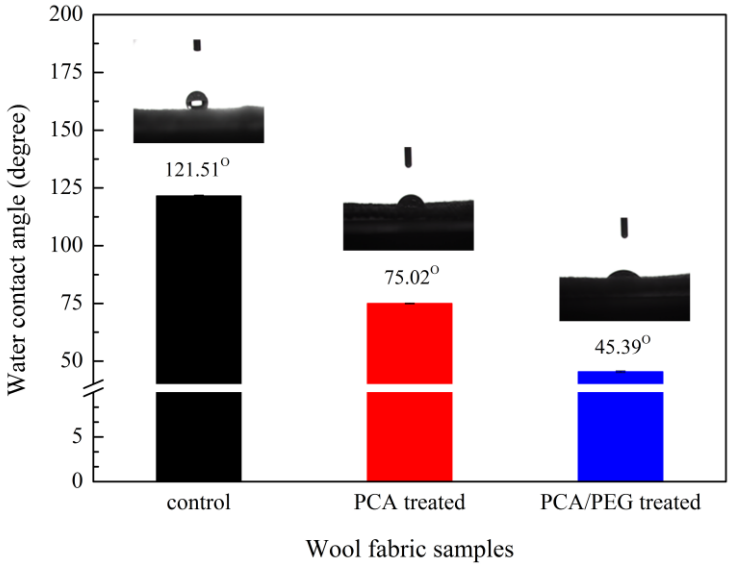


Figure 10 Water contact angles of treated wool. Insets are images of water droplet on the treated wool surfaces.

3.3.4 Dyeing Depth of Treated Wool Fabrics

PCA, as a colored polymer, can be used as a dye for dyeing of wool fabric. In this work, K/S values of wool fabrics treated with PCA or PCA/PEG complex were tested, and the data were shown in Figure 11. Photographs of the treated wool fabrics were also presented. The K/S values of both curves (associated with both types of wool fabrics) decreased with increasing wavenumber, indicating that the maximum adsorption peak of poly(caffeic acid) was not in the visible region. According to the photographs of dyed wool fabrics, the dyeing depth of wool fabric treated with PCA/PEG complex was higher than that of wool fabric treated with PCA. This could be due to the following reasons: (i) conjugated structure of PCA polymerized with PEG may lead to darker coloration of the catalyzed products; (ii) PEG may be adsorbed onto the surface of the wool fabrics prior to coloration, providing a template for more CA monomers to be fixed onto the wool fabrics through hydrogen-bonds.

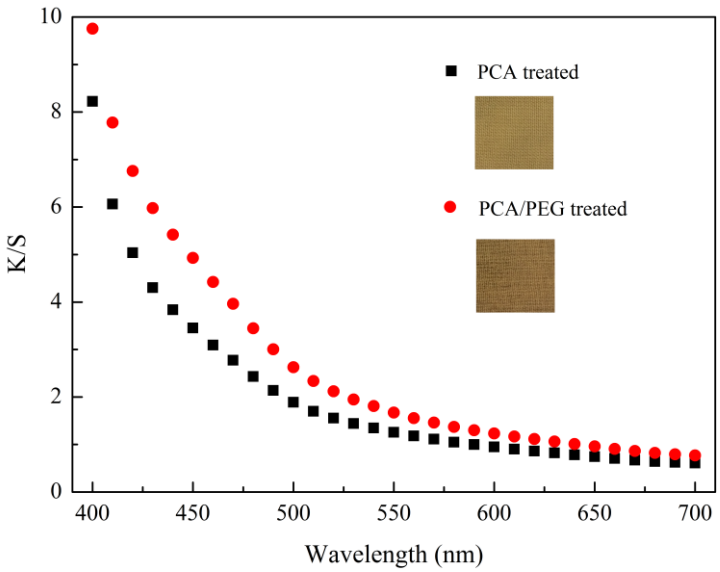


Figure 11 K/S values of wool fabrics dyed by laccase-catalyzed polymerization of CA with or without PEG

4. Conclusions

Poly(caffeic acid) (PCA) was synthesized in a novel approach to form polymeric colorant which was structurally arranged on the PEG template by the laccase-catalyzed oxidative polymerization. Compared to chemical polymerizations, this enzyme-based synthesis is more environmentally friendly method due to milder reaction conditions and use of atmospheric oxygen as electron provider for oxidation. During the polymerization of caffeic acid, PEG acted as a template to arrange CA align with the polymer chain of PEG through hydrogen bonding interactions between the hydroxyl groups of PCA and ether groups of template according to the 'zip' mechanism. Less amount of oligomers generated after adding PEG demonstrated the improved efficiency of polymerization of caffeic acid and reducing the yield of wastes. It was found that high degree of polymerization was achieved by laccase-catalyzed polymerization of caffeic acid in the form of PCA/PEG complex. Possible structures and bonding modes of PCA and PCA/PEG complex were analyzed by UV-Vis, FTIR, TG, MALDI-TOF and HPLC-MS measurements. This study presents a novel promising approach in preparation of functional wool textiles. Wool fabrics were successfully dyed with PCA/PEG complex through in-situ laccase-catalyzation of caffeic acid on the template of PEG. The higher depth of brown color shade was achieved for wool fabrics treated with PCA/PEG complex than PCA during laccase-catalyzed polymerization. Only enzyme laccase as biocatalyst and low dosage of CA monomer was required to achieve the coloration of wool fabrics, resulting in the reduction of chemicals used. The

PCA/PEG-treated wool had better electroactivity and wettability than that treated with PCA. Further investigations involving the effect of different molecular weights of the template PEG on the polymerization of phenols are being undertaken. This may expand the scope of application of PCA for fibre coloration and functionalization in the field of the smart textiles and provide a novel approach for cleaner production of functional fabrics.

Acknowledgments

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