Bio-based Polymer from Ferulic Acid by Electropolymerization

Yasuyuki Matsushita,* Akihiko Nakamura, Dan Aoki, Sachie Yagami, and Kazuhiko Fukushima

Electropolymerization was carried out in order to obtain a bio-based polymer by a simple procedure. Ferulic acid, a component of gramineous plants, was selected for this purpose. A thin polymer film was produced in an organic solvent medium (i.e., CH₂Cl₂/methanol (4:1 v/v) in the presence of 0.2 M LiClO₄), whereas it was not obtained in a totally aqueous medium (0.2 M NaOH). Scanning electron microscopic analysis showed that the polymer film had a porous lamellar structure of ~10 μm thickness. Most of the carboxyl groups remained, and IR spectroscopy and time-of-flight secondary ion mass spectrometry (TOF-SIMS) analyses revealed that o-quinones existed in the polymer film. After electropolymerization, caffeic acid, sinapic acid, 3,6-bis(4-hydroxy-3-methoxyphenyl)tetrahydrofuro[3,4-c]furan-1,4-dione (1), and 4,4'-dihydroxy-3,3'-dimethoxy-β,β'-bicinnamic acid (2) were detected in the organic reaction medium; this suggested that demethylation, methoxyl group addition, and radical coupling reactions occurred at the electrode surface.

Keywords: Bio-based polymer; Ferulic acid; Electropolymerization; Crop waste; Thin film; TOF-SIMS

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INTRODUCTION

Biomass is carbon-neutral by nature, which means that it does not increase the levels of carbon dioxide in the atmosphere. This is because biomass grows by consuming the same carbon dioxide that it releases. Recently, much attention has been focused on the utilization of lignocellulosic biomass as an alternative source of fossil fuels because of its abundance.

Crop wastes, such as corn stover and wheat bran, are used as animal feed, yet their conversion to high-value materials is a subject of agro-industrial research. Ferulic acid is one of the major cinnamic acids, and it is found in plant cell walls incorporated with polysaccharides and lignin through ester and ether bonds (El-Basyounia et al. 1964; Higuch et al. 1967; Hartley and Haverkamp 1984; Mueller-Hartley et al. 1986; Saulnier and Thibault 1999; Saha 2003). Large amounts of ferulic acid are contained in crop wastes. Faulds et al. (1997) produced 5.7 g of ferulic acid on a laboratory scale from 1 kg of wheat bran by using a preparation of Trichoderma xylanase mixed with Aspergillus niger ferulic acid esterase.

Bio-based polymers using ferulic acid have been reported, including polyester (Mialon et al. 2010; Kreye et al. 2013; Pion et al. 2013, 2014; Oulame et al. 2015; Barbara et al. 2015; Nguyen et al. 2015; Maiorana et al. 2016; Reano et al. 2016), copolymer with methacrylic acid (Iemma et al. 2010), and liquid-crystalline forms (Wang et al. 2012). Ferulic acid has many physiological functions including antimicrobial, anti-oxidant, anti-
thrombotic, and carcinostatic activities; it has been studied for its applications in the food, cosmetic, and pharmaceutical fields (Ou and Kwok 2004; Pei et al. 2015; Takahashi et al. 2015). Bioactive polymers, which are based on ferulic acid, can also be prepared by incorporating ferulic acid into the polymer backbone, which can be regenerated again via biodegradation (Nagata and Hizakae 2003; Ouimet et al. 2015, 2013). However, these conversion processes involve several steps.

In this study, in order to prepare the bio-based polymers from ferulic acid by a simple method, electropolymerization was attempted. The operation is easy and is carried out by dipping the two electrodes (anode and cathode) into a solution containing the monomer; then a voltage is applied between the two electrodes. The oxidation area is limited, which yields a thin polymer film on the electrode surface. The electropolymerization can be carried out at atmospheric pressure and room temperature. In addition, except for the ferulic acid itself, the reagent is only in the form of electrons.

Recently, the electropolymerization of coniferyl alcohol, which is a structural analog of ferulic acid, yielded a thin polymer film (Matsushita et al. 2009). Polymer films that are obtained from ferulic acid possess significant levels of carboxyl groups; such films have applications as ion exchange films and as electroactive polymers in biomimetic sensors, actuators, and artificial muscles (Shahinpoor et al. 1998; Shahinpoor and Kim 2001; Chen et al. 2010).

**EXPERIMENTAL**

**Materials**

Ferulic acid was synthesized using vanillin and malonic acid. Vanillin (70 mmol) and malonic acid (84 mmol) were dissolved in pyridine (45.5 mL) with the addition of piperidine (7 mL) and heated at 70 °C for 4 h. The reaction mixture was acidified by adding dilute hydrochloric acid, and the precipitate was filtered and dried under reduced pressure to obtain ferulic acid (yield of 77%). The NMR spectra of the synthesized ferulic acid was as follows: $^1$H NMR (DMSO-$d_6$) δ: 3.48 (3H, s), 6.39 (1H, d, $J = 15.9$ Hz), 6.83 (1H, d, $J = 8.2$ Hz), 7.10 (1H, dd, $J = 8.2, 1.9$ Hz), 7.29 (1H, d, $J = 1.9$ Hz), and 7.53 (1H, d, 15.9 Hz); and $^{13}$C NMR (DMSO-$d_6$) δ: 55.6, 111.1, 115.5, 115.6, 122.7, 127.4, 144.4, 147.9, 149.0, and 167.9.

Ferulic acid-(methoxy-$d_3$) was synthesized according to a procedure similar to the one described above, using vanillin-(methoxy-$d_3$). Vanillin-(methoxy-$d_3$) was synthesized as follows. Sodium (1.3 g) was dissolved in CD$_3$OD along with 3-bromo-4-hydroxybenzaldehyde (2.25 g) in 1-methylimidazole (15 mL); CuCl$_2$ (0.7 g) was added to the CD$_3$OD solution and heated at 130 °C for 1 h. After acidification with dilute hydrochloric acid, the reaction mixture was extracted with ethyl acetate. The organic solution was washed with water and then with saturated NaCl (aq.), dried over sodium sulfate, and concentrated to obtain vanillin-(methoxy-$d_3$) with a yield of 84%. Caffeic acid was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan).

**Apparatus**

The electrochemical experiments were conducted using an Automatic Polarization System (Electrochemical Measurement System HZ-7000; Hokuto Denko Corp., Tokyo, Japan) that was connected to a personal computer (PC) for data analysis. Cyclic voltammograms were recorded using a Pt disk (1.6 mm diameter), a Pt wire, and a Ag/AgCl...
couple as the working, counter, and reference electrodes, respectively. Prior to the experiment, the working electrode was cleaned by polishing it with a slurry that contained 0.05 μm alumina particles, and by rinsing it with distilled water. The scan rate was 100 mV/s. For large-scale electropolymerization, a Pt electrode with an area of 25 cm² was used as the working electrode. The counter-electrode was a Pt mesh (50 mm × 50 mm; 80 mesh), and the reference electrode was connected to the counter-electrode. For scanning electron microscopy (SEM) analysis, small-scale electropolymerization was performed using a Pt electrode with an area of 12 mm² as the working electrode. Before the experiment, the working electrodes were burned using an ethanol lamp and washed with deionized water to remove contaminants.

**Electropolymerization**

Electropolymerization was carried out in 180 mL of 0.2 M NaOH (aqueous medium system) and in 180 mL CH₂Cl₂/MeOH (4:1 v/v) in the presence of 0.2 M LiClO₄ (organic solvent medium system). However, the film was not produced in aqueous systems.

**Cryo-SEM and SEM Analysis**

The cryo-SEM and SEM analyses were carried out using an S-3400N scanning electron microscope (Hitachi High-Technologies Corp., Tokyo, Japan). For cryo-SEM analysis, the electropolymerized ferulic acid film produced on the large-scale was released from the working electrode and washed using methanol and deionized water. The film was frozen and microtomed to obtain cross sections. For SEM analysis, the film was washed using methanol and then dried.

**Electron Ionization Mass Spectrometry (EI-MS), Nuclear Magnetic Resonance Spectroscopy (NMR), Infrared Spectroscopy (FT-IR), and Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) Analyses**

EI-MS, NMR, and FT-IR spectra were recorded using JMS-700 (JASCO, Tokyo, Japan), AVANCE 400 (Bruker BioSpin GmbH, Rheinstetten, Germany), and FT-720 (Horiba, Ltd., Kyoto, Japan) spectrometers, respectively. TOF-SIMS analysis was performed using a TRIFT III (ULVAC-PHI, Inc., Osaka, Japan) spectrometer. The spectrometer was operated with a 22-keV Au⁺ primary ion beam using a raster size of 100 μm × 100 μm, a beam current of 5 to 7 nA, and a pulse width of 13.0 ns. A low-energy pulsed electron gun (30 eV) was used for the charge compensation. The acquired data was analyzed using WinCadence 5.1.2 software (ULVAC-PHI, Inc., Osaka, Japan).

**Analysis of the Solution after Electropolymerization**

The organic solvent medium was removed under reduced pressure from the solution after electropolymerization. The residue was acidified using dilute hydrochloric acid to obtain the reaction products as precipitates. The precipitates were separated into two fractions by preparative thin layer chromatography (PLC Silica gel 60 F₂₅₄ 0.5 mm; EMD Millipore Corp., Billerica, MA, USA) using acetone/hexane (1/1, v/v) as the eluent. The early eluted fraction contained only (3,6-bis(4-hydroxy-3-methoxyphenyl)tetrahydrofuro[3,4-c]furan-1,4-dione (compound I), which has the following spectra: EI-MS: /m/z = 386 [M⁺]; ¹H NMR (Acetone-d₆) δ: 3.85 (6H, s), 4.09 (2H, br), 5.77 (2H, br), 6.86 (2H, d, J = 8.2 Hz), 6.92 (2H, dd, J = 8.2, 1.8 Hz), and 7.05 (2H, d, J = 1.8 Hz); and ¹³C NMR (Acetone-d₆) δ: 49.1, 56.4, 83.2, 110.4, 116.0, 119.6, 13.8, 148.2, 148.7, and 176.0. The later fraction was subjected to GC-MS analysis (ITQ 900 connected to TRACE 1300GC;
ThermoFischer Scientific, Waltham, MA, USA) after trimethylsilylation in order to detect sinapic acid (confirmed by authentic data) and 4,4’-dihydroxy-3,3’-dimethoxy-β,β’-bicinnamic acid (compound 2). The identity of compound 2 was confirmed by reference data of Renger and Steinhart (2000).

RESULTS AND DISCUSSION

Cyclic Voltammetry

Figure 1 shows the cyclic voltammograms of 20 mM ferulic acid in aqueous and organic solvent media at a scan rate of 100 mV/s. In the aqueous system, the oxidation broad peak was observed at a potential of 300 to 500 mV (vs. Ag/AgCl electrode) in the first cycle. However, no peaks were observed when repeating the cycle more than two times; this phenomenon has been observed in the case of many other phenolics (Gattrell and Kirk 1993a, 1993b; Ciszewski and Milczarek 2001; Ezerskis and Jusis 2001; Milczarek and Ciszewski 2003; Ferreira et al. 2006).

During electrooxidation, many phenolic compounds in an aqueous medium undergo rapid film passivation on the electrode surface, leading to a drastic decrease in the current, whereas in an organic solvent medium, the oxidation peaks are observed at ~800 mV after repeating the cycle more than twice. This implied that electrons tunneled through the oxidized polymer film formed on the electrode surface, or that the film was degraded.
or ruptured from the electrode, which allowed ferulic acid to reach the electrode surface (Gattrell and Kirk 1993a, 1993b; Ciszewski and Milczarek 2001).

**Electropolymerization**

Figure 2 shows the change in the electrode potential versus time at constant-current (0.025 mA/cm²) electrolysis with the apparatus. In the aqueous medium, the oxidation potential remained at 250 to 350 mV initially (*i.e.*, 0 to 30 s). However, after 30 s, the potential increased drastically to about 1000 mV. In the organic solvent medium, the oxidation potential remained constant at about 300 mV for 5 min. This observed result was in agreement with the cyclic voltammetry results, which showed that the film generated by oxidative polymerization of ferulic was coated on the electrode to passivate in the aqueous system, whereas the oxidation of ferulic acid occurred continuously in the organic solvent system.

![Figure 2: Electrode potential change during constant current electrolysis](image)

**Fig. 2.** Electrode potential change during constant current electrolysis: (a) aqueous medium (0.2 M NaOH (aq)); and (b) organic solvent medium (CH₂Cl₂/MeOH solution containing 0.2 M LiClO₄)

Large-scale electropolymerizations, which used two different reaction media (aqueous and organic solvent), were conducted in the constant-current mode. The reaction conditions and yields are shown in Table 1. A thin polymer film was obtained in the organic solvent medium (Fig. 3a), but was not produced in the aqueous medium. These results are consistent with the earlier results from cyclic voltammetry and from electrode potential changes at constant current conditions. In the organic solvent medium, the yield increased as the ferulic acid dosage and the reaction time increased. The formation rate of the electropolymerized polymer film decreased as the reaction time increased. This is likely attributable to the reduction of the ferulic acid concentration due to its electropolymerization onto the electrode’s surface.

The cryo-SEM analysis showed that the polymer film had a porous lamellar structure of about 10 μm thickness (Figs. 3b and 3c). Electropolymerization was performed for a short time period on a small scale to investigate the growth process of the film on the electrode surface; the electrode was subjected to SEM analysis after electropolymerization.
Figures 3d and 3e show the SEM images of the electrodes after 5 min and 30 min from the start of the current, respectively. The images showed that small granules of the polymer were generated on the electrode surface at the beginning of the electropolymerization. As the reaction progressed, the granules gathered together to form the porous film.

**Table 1. Electropolymerization in Organic Solvent Medium on the Large Scale**

<table>
<thead>
<tr>
<th>Ferulic Acid (mg)</th>
<th>Reaction Time (h)</th>
<th>Polymer Film Yield (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>320</td>
<td>1</td>
<td>8.5</td>
</tr>
<tr>
<td>320</td>
<td>3</td>
<td>12.2</td>
</tr>
<tr>
<td>640</td>
<td>3</td>
<td>20.6</td>
</tr>
<tr>
<td>640</td>
<td>6</td>
<td>28.0</td>
</tr>
<tr>
<td>1296</td>
<td>3</td>
<td>33.1</td>
</tr>
</tbody>
</table>

* Constant current at 62.5 mV in CH₂Cl₂/MeOH medium containing 0.2 M LiClO₄

![Fig. 3](image1.jpg)

**Fig. 3.** Electropolymerized film using ferulic acid: (a) photographic image after 3 h electropolymerization; (b) SEM image of the film at cross section; (c) SEM image of the surface of the film; (d) SEM image of the electrode surface after 5 min electropolymerization; and (e) SEM image of the electrode surface after 30 min electropolymerization

In this study, NaOH aqueous solution and organic solvent were chosen as a medium according to the solubility of the ferulic acid. The dissociation of COOH and phenolic OH of ferulic acid occurred in NaOH aqueous solution, however it does not in organic solvent.
For further development of the electropolymerization, we will study using other solvents with concerning the effect of the dissociation on the electropolymerization, for instance pyridine as a basic organic solvent.

**Analysis of the Reaction Media after Electropolymerization for Organics**

Oxidized organic compounds of low-molecular-weight are expected reaction products from electrochemical reactions, along with ferulic acid, and are expected to be found in the reaction media. To better understand the reaction mechanism of electropolymerization and the polymer film structure, the low-molecular-weight reaction products where identified. After the reaction media were acidified, the resulting precipitates were separated by silica gel column chromatography and analyzed by GC-MS; four compounds, caffeic acid, sinapic acid, compound 1 and compound 2, were identified (Scheme 1). The presence of caffeic and sinapic acid implied that there were demethylation and methoxylation reactions occurring with ferulic acid, respectively, at the electrode; additionally, the presence of compounds 1 and 2 implied radical coupling reactions of ferulic acid at the electrode. The presumed reaction mechanisms are shown in Scheme 1.

![Scheme 1. Postulated reaction mechanisms of ferulic acid during electropolymerization](image)

The electrochemical behaviors of phenols are complicated. In a previous study, radical coupling was the dominant reaction that occurred at the electrode surface when
using coniferyl alcohol (Matsushita et al. 2009). In this study, compounds 1 and 2 were detected in the reaction medium after electropolymerization, which suggested that radical coupling occurred at the electrode surface. Several researchers have examined the electropolymerization of methoxyphenols and they have suggested that two-electron oxidation followed by a nucleophilic substitution occurred, which resulted in demethylation to yield o-quinones (Petek et al. 1973; Ueda et al. 1982; Ciszewski and Milczarek 2001). An o-quinone unit could be reduced to a catechol unit by reversible electrochemical reactions (Ciszewski and Milczarek 2001). In this study, we confirmed the existence of a catechol unit in the reaction solution after electropolymerization in the organic solvent medium. The reaction mechanism to form o-quinone unit will be discussed in detail later. Furthermore, the addition of a methoxy group to ferulic acid was also detected. The mechanism for this reaction was not clear. Postulated mechanisms for this reaction are: (1) one-electron oxidation for both ferulic acid and methanol, which is followed by radical coupling the two generated radicals; or (2) two-electron oxidation of ferulic acid, which is followed by methanol addition.

**FT-IR**

FT-IR spectra for ferulic acid and electropolymerized polymer are shown in Fig. 4. Peaks appeared at 1600 to 1700 and at 1770 cm\(^{-1}\), which suggested that the film contained o-quinone groups, as well as carboxyl and ester groups. These results are consistent with detecting caffeic acid, which could have formed from the o-quinone intermediate (Scheme 1) after a reduction at the counter electrode, and detecting compound 1 in the reaction medium after electropolymerization. The broad peak at 3440 cm\(^{-1}\) of the electropolymerized polymer was larger than that of ferulic acid, which implied that the electropolymerized film contained numerous hydroxyl groups.

![Fig. 4. IR spectra of electropolymerized film of ferulic acid (red) and ferulic acid (black)](image)

**TOF-SIMS Analysis**

TOF-SIMS analysis is a very useful technique for the investigation of macromolecular structures. After the bombardment of primary ions (Au\(^+\)) onto the sample surface, the generated secondary ions are detected by TOF-MS. The data provided evidence of the partial structure of the polymer sample. Recently, bio-polymers such as
lignin, cellulose and human hair were subjected to the TOF-SIMS analysis to study their structures (Saito et al. 2005a, 2005b; Fardim and Holmbom 2005; Matsushita et al. 2008, 2012; Kojima et al. 2011). Figure 5 shows the mass spectra of the electropolymerized polymers using ferulic acid, ferulic acid-(methoxy-d₃), ferulic acid with the CH₂Cl₂/CD₃OD solvent medium, and caffeic acid.

![Mass spectra of electropolymerized polymers](image)

**Fig. 5.** TOF-SIMS spectra of electropolymerized film and fragment ions: (a) from ferulic acid; (b) from ferulic acid-(methoxy-d₃); (c) from ferulic acid reacted in CH₂Cl₂/CD₃OD solvent medium; and (d) from caffeic acid

The spectra of the electropolymerized polymer film of ferulic acid showed major peaks at m/z 167 and 149. On the other hand, those obtained using ferulic acid-(methoxy-d₃) and ferulic acid under CH₂Cl₂/CD₃OD solvent medium had a peak at m/z 170; this
indicated that the \( m/z \) 167 peak had shifted. This suggested that the methoxy group of ferulic acid was not removed and that the methanol from the reaction medium was added to the ferulic acid. From these results, the presumable structures at \( m/z \) 167 are shown in Fig. 5 (fragment ion I and II). Fragment ion I corresponded to the existence of sinapic acid in the reaction medium after electropolymerization. Fragment ion II has also been observed by other researchers using TOF-SIMS analysis to analyze a substance produced from enzymatic polymerization of sinapyl alcohol (Saito et al. 2005b). Fragment ion II is expected to form by addition of methanol at benzylic position after radical coupling for ring rearomatization (Freudenberg 1965).

The peak at \( m/z \) 149 did not shift in either case. These results suggest that the methyl group in the methoxy group of ferulic acid was removed during electropolymerization; additionally, the methanol in the solvent medium was not involved in the formation of this fragment ion during TOF-SIMS analysis.

This \( m/z \) 149 peak also appeared in the spectra of the electropolymerized film using caffeic acid, which implied that the peak was closely related in the catechol unit. The presumed fragment ion is shown in Fig. 5 (fragment ion III).

As mentioned above, some researchers have reported that compounds containing \( o \)-quinones were produced by electro-oxidation of guaiacyl-type groups in aqueous solution (Petek et al. 1973; Ueda et al. 1982; Ciszewski and Milczarek 2001), which are formed by the cleavage between oxygen and aryl carbon (i.e., the substitution of the \( CH_3O^- \) group with the HO- group); this is referred to as the argentide oxide oxidation mechanism (Synder and Rapoport 1972). However, in this study using a non-aqueous medium, \( o \)-quinones were detected in the electropolymerized film, which suggested that demethylation had occurred during electropolymerization (Scheme 1).

**CONCLUSIONS**

1. A polymer film was obtained by electropolymerization of ferulic acid using an organic solvent medium, which had a porous lamellar structure of \( \sim 10 \mu m \) thickness, and possessed high amounts of carboxyl, hydroxyl, and \( o \)-quinone units.
2. From the analysis of the organic medium after electropolymerization, caffeic acid, sinapic acid, and compounds 1 and 2 were detected.
3. Using deuterium-labeled compounds, it was found that the \( o \)-quinones were generated by demethylation of the methoxy group of ferulic acid during electropolymerization; additionally, radical coupling and addition of methoxyl group occurred.

**ACKNOWLEDGMENTS**

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REFERENCES CITED


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