

# Oxidative Polymerization of Phenylenediamines by Enzyme and Magnetic Properties of the Products

DAIGO ICHINOHE, TOSHITAKA MURANAKA, HIDEO KISE

Institute of Materials Science, University of Tsukuba, Tsukuba, Ibaraki 305, Japan

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**ABSTRACT:** Oxidative polymerization of *m*-phenylenediamine was carried out using H<sub>2</sub>O<sub>2</sub> as an oxidant and horseradish peroxidase as a catalyst in mixtures of aqueous buffer solution and 1,4-dioxane or in reversed micellar solutions. When the reaction mixture was brought into contact with a stainless steel stick, the obtained polymer responded to a permanent magnet at room temperature in the air. From the ICP emission spectroscopic analysis, it was found that these polymers contained small amounts of transition metals, such as Fe and Ni. The magnetic properties of the obtained polymers were studied with a superconducting quantum interference device (SQUID). The *M* (magnetization)–*H* (magnetic field) curves showed sigmoid behavior at 300 K, but did not exhibit a residual magnetization and a coercive force. These properties resembled those of soft ferromagnetic materials. The ferromagnetic behavior was indicated even at 800 K. Polymers of *o*- and *p*-phenylenediamines exhibited lower values of saturation magnetization than polymers of *m*-phenylenediamine. © 1998 John Wiley & Sons, Inc. *J Appl Polym Sci* 70: 717–721, 1998

**Key words:** horseradish peroxidase; oxidative polymerization; phenylenediamine; magnetic property

## INTRODUCTION

Using of enzyme catalysis for polymerization is expected to have much potential for providing polymers of new structure and properties. It is known that a redox enzyme horseradish peroxidase (HRP) catalyzes the oxidative polymerization of phenol,<sup>1</sup> aromatic amines,<sup>2</sup> or their derivatives<sup>3,4</sup> with hydrogen peroxide as an oxidant in aqueous/organic solutions. Reversed micellar systems were also applicable to HRP-catalyzed polymerization of *p*-ethylphenol.<sup>5</sup> Kommareddi et al.<sup>6</sup> reported that an iron/polymer composite was prepared by HRP-catalyzed polymerization of *p*-alkylphenols in reversed micelles containing iron oxide.

Previously, we reported the oxidative polymerization of *o*-, *m*-, and *p*-phenylenediamines (PDA)

by HRP in reversed micellar systems.<sup>7</sup> It was found that the obtained polymers were attracted to a permanent magnet at room temperature in the air. Analysis of the polymers by inductively coupled plasma (ICP) emission spectroscopy revealed that the polymers contained small amounts of Fe and Ni. Therefore, it was assumed that the magnetic properties of poly(PDA)s were mainly due to transition metals, which were accidentally incorporated into the polymers. Later, however, it was considered that the spin interactions of the polymers played an important role for generation of magnetism because it was found that the magnetic properties were significantly dependent on the polymer structure; the saturation magnetization of the polymer prepared from *m*-PDA was higher than those of *o*-PDA and *p*-PDA. Similar results were reported by Araki et al.<sup>8</sup> for the materials obtained by the pyrolysis of the mixture of PDAs and triazine derivatives. Larger values of spin concentration and satura-

Correspondence to: H. Kise.

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tion magnetization have been evaluated in the samples prepared from *m*-PDA than those from *o*-PDA and *p*-PDA. These facts suggest that the topological configuration<sup>9-11</sup> of the unpaired spins is important for the ferromagnetic interaction in the organic polyradicals.

In the present work, it was found that PDA polymers could be prepared in higher yields by HRP in homogeneous systems than in reversed micellar systems. Thus, oxidative polymerization of *m*-PDA was carried out using H<sub>2</sub>O<sub>2</sub> as an oxidant and HRP as a catalyst in mixtures of aqueous buffer solution and 1,4-dioxane. This article describes the results of the measurement of magnetic properties of *m*-PDA polymers by a superconducting quantum interference device (SQUID).

## EXPERIMENTAL

### Materials

Horseradish peroxidase (HRP) (EC 1.11.17, 100 units per mg solid) was purchased from Wako Pure Chem. Ind., Ltd. (Osaka, Japan). Phenylenediamines (*o*-, *m*-, and *p*-PDA), aqueous hydrogen peroxide solution (30 wt %), and 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) were also obtained from Wako. Sodium di(2-ethylhexyl) sulfosuccinate (SESS) was a product of Tokyo Kasei Kogyo Co., Ltd. Other reagents and solvents were of guaranteed grade, and they were used as received.

### Polymerization

A typical polymerization procedure in a homogeneous system was as follows: a solution of PDA (0.27 g, 2.5 mmol) and HRP (8 mg) in a mixture of 7.5 mL of 1,4-dioxane and 42.5 mL of 0.1M HEPES buffer solution (pH 7.1) was placed in a 300-mL Erlenmeyer flask. The reaction was initiated by adding 0.256 mL of H<sub>2</sub>O<sub>2</sub> solution to the above solution. The same volume of H<sub>2</sub>O<sub>2</sub> solutions were added 3 times at an interval of 30 min (total 0.768 mL). The mixture was stirred magnetically at 30°C for 24 h. After the reaction, a stainless steel stick (about 2 mm  $\phi$   $\times$  40 mm) was immersed into the reaction mixture, and the whole mixture was kept standing with occasional stirring for several hours. Then the polymer was separated by filtration, washed with distilled water and methanol, and then dried under vacuum.

The reactions in reversed micelles were carried out using SESS as a surfactant and isooctane as a solvent. A reversed micellar solution was pre-

pared by dissolving SESS and pure water in isooctane. Then, HRP (8 mg) in a 0.1M HEPES buffer solution (pH 7.1) and PDA were added to the micellar solution, and the mixture was magnetically stirred at 30°C overnight. The reaction was initiated by adding H<sub>2</sub>O<sub>2</sub> solutions as described above for homogeneous reactions (0.768 mL total). The total reaction volume was 50 mL, and final concentrations of SESS, water, and PDA were 0.3, 4.5, and 0.05M, respectively. The reaction temperature was 30°C, and the products were obtained by the same method as above.

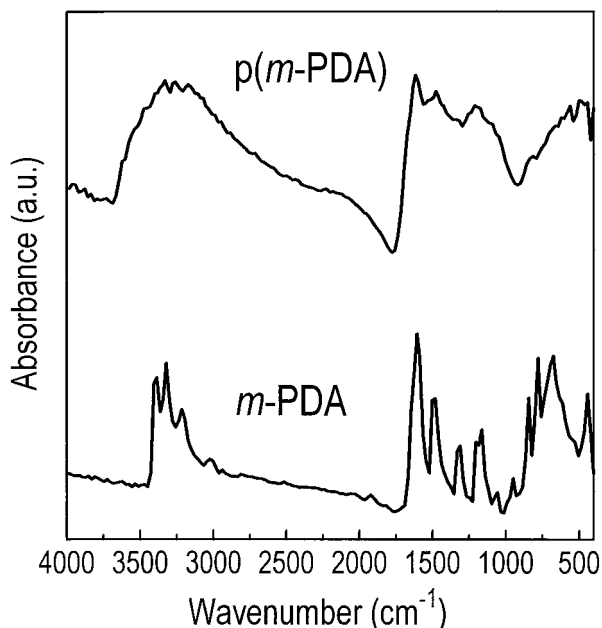
## ANALYSIS

Infrared (IR) spectra were recorded on a JASCO FT-IR 300 spectrophotometer at 4-cm<sup>-1</sup> resolution on KBr pellets. Electron spin resonance (ESR) measurements were performed at the X band with 100 kHz field modulation on a JEOL JES-TE200. Mn<sup>2+</sup>-MgO was used as a reference for measurement of the *g* value. The spin concentration was measured using CuSO<sub>4</sub> · 5H<sub>2</sub>O as a reference. The magnetic properties of the polymer were measured on a SQUID (Quantum Design, MPMS-5SPL). The contents of Fe, Co, and Ni in polymers were measured with an ICP emission spectrophotometer (Nippon Jarrell-Ash, ICAP-575).

## RESULTS AND DISCUSSION

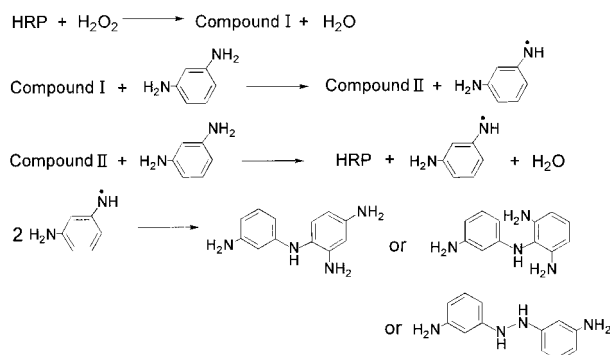
The reaction of *m*-PDA with H<sub>2</sub>O<sub>2</sub> using HRP as a catalyst in HEPES buffer solution–1,4-dioxane afforded black precipitates as products. These products were only partly soluble in common solvents, such as tetrahydrofuran (THF) and N,N-dimethylformamide (DMF). No precipitates were obtained without HRP, which suggests that the reaction was catalyzed by HRP. The polymer yield was markedly affected by the solvent composition. Maximum polymer yield was obtained in 85% HEPES buffer solution–15% 1,4-dioxane by volume. Yield decreased sharply at 40% or higher contents of 1,4-dioxane, and at 90% 1,4-dioxane, the product yield was about 10%. These results may be the consequence of the decrease of catalytic activity of HRP due to denaturation by 1,4-dioxane.<sup>12</sup>

The structure of the polymer was studied by Fourier transform infrared (FTIR) spectroscopy.



**Figure 1** IR spectra of *m*-PDA (lower) and poly(*m*-PDA) (upper).

As shown in Figure 1, the characteristic N—H stretching bands of  $\text{NH}_2$  groups in *m*-PDA were observed at  $3200\text{--}3400\text{ cm}^{-1}$ . In the spectrum of the polymer, these absorption bands changed to broad bands, which suggests the formation of secondary amino groups. It is known that the initial step of HRP-catalyzed oxidation with  $\text{H}_2\text{O}_2$  as an oxidant is the formation of HRP–oxygen complex (compound I).<sup>13</sup> This will subsequently react with *m*-PDA to form a monomer radical and HRP–OH (compound II), as illustrated in Scheme 1. The monomer radicals may be formed also by reaction of compound II with *m*-PDA. Coupling of the monomer radicals can occur by N—N, N—C, or C—C bondings. In the IR spectrum of *m*-PDA, characteristic bands appeared at  $900\text{--}600\text{ cm}^{-1}$ ,

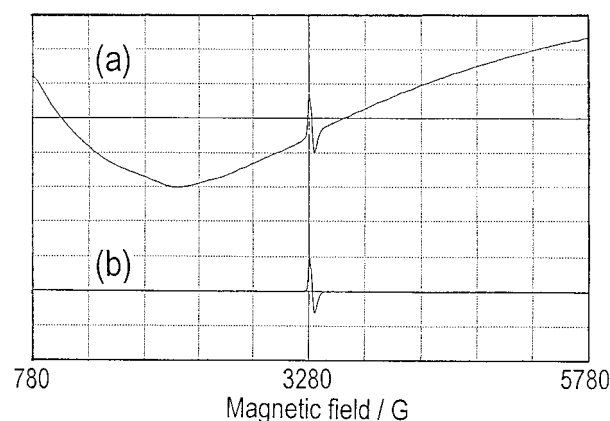


**Scheme 1**

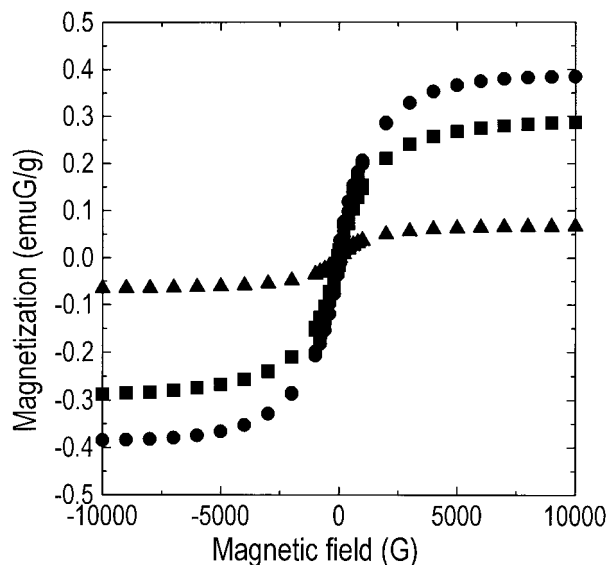
which are due to C—H out-of-plane deformation of metasubstituted benzene nuclei. In contrast, poly(*m*-PDA) did not exhibit distinct bands in this region. This seems to indicate that the polymerization proceeded irregularly by C—N, N—N, or C—C bondings, leading to the formation of branched or crosslinked polymers.

It was found that when a reaction mixture was brought into contact with a stainless steel stick, the obtained polymer was attracted to a permanent magnet. This magnetically active polymer was produced in good reproducibility. The analysis by ICP emission spectroscopy revealed that the polymer contained small amounts of Fe and Ni; in a typical polymer, the contents of Fe and Ni were 0.1 and 0.5 wt %, respectively, but Co was not detected. Poly(*m*-PDA)s prepared by HRP/ $\text{H}_2\text{O}_2$  under the same conditions as above but without contact with stainless steel sticks were not magnetically active. Furthermore, poly(*m*-PDA)s prepared by oxidative polymerization by ammonium peroxodisulfate and treated with  $\text{H}_2\text{O}_2$  in the presence of a stainless steel stick were not attracted to a permanent magnet. Only the poly(*m*-PDA)s prepared by HRP/ $\text{H}_2\text{O}_2$  and treated with a stainless steel stick exhibited the magnetic activities. Therefore, the magnetism of the polymers was most probably derived from spin interactions between oxidation product of poly(*m*-PDA) prepared by HRP and Fe or Ni. The Fe and Ni were probably dissolved in the solution and then incorporated into the polymer. The amounts of the transition metals in  $\text{H}_2\text{O}_2$ , HRP, and PDA, which were used for polymerization, were much less than the amounts of the metals found in the polymer, thus ruling out the possibility of incorporation of metals from these compounds.

The ESR spectra of magnetically active poly(*m*-PDA)s were taken on powder samples in the mag-



**Figure 2** ESR spectra of poly(*m*-PDA)s (a) treated with and (b) not treated with a stainless steel stick.

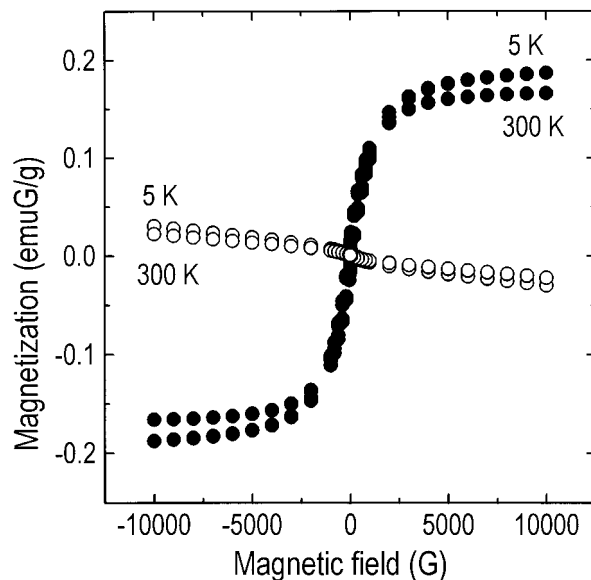


**Figure 3**  $M$  (magnetization)– $H$  (magnetic field) plots at 300 K for poly(PDA)s prepared in reversed micellar solutions: (■) poly(*o*-PDA); (●) poly(*m*-PDA); (▲) poly(*p*-PDA).

netic field of  $3280 \pm 2500$  G at room temperature (Fig. 2). The  $g$ -values of the polymers were about 2.004. The spin concentrations in the polymers were determined to be in the range of  $10^{18}$ – $10^{19}$  spins  $\cdot$   $g^{-1}$ . Broad ESR bands due to ferromagnetic metals<sup>14</sup> were observed for magnetically active poly(*m*-PDA)s [Fig. 2(a)]. On the contrary, the magnetically inactive polymers did not show bands due to transition metals.

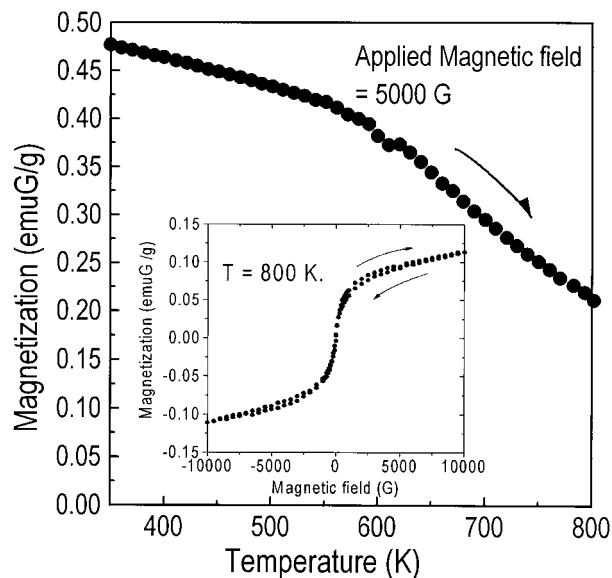
The magnetic properties of poly(PDA)s were investigated by using a SQUID. Figure 3 shows plots of magnetization ( $M$ ) against the magnetic field ( $H$ ) for poly(PDA)s prepared by HRP/ $H_2O_2$  in reversed micellar systems. The  $M$ – $H$  plots of poly(*m*-PDA) and poly(*o*-PDA) clearly exhibited S-shaped curves at 300 K. These magnetic profiles resemble those of soft ferromagnetic materials<sup>15</sup> which, in general, are easily magnetized and demagnetized. Also, they are characterized by low values of a residual magnetization and a coercive force.<sup>16</sup> It was found that magnetization behavior of poly(PDA)s is almost independent of temperature;  $M$ – $H$  curves at 5 K were almost the same as those at 300 K (data not shown). The saturation magnetization at 300 K of poly(*m*-PDA) ( $0.38 \text{ emuG} \cdot \text{g}^{-1}$ ) was higher than those of poly(*o*-PDA) ( $0.29 \text{ emuG} \cdot \text{g}^{-1}$ ) and poly(*p*-PDA) ( $0.07 \text{ emuG} \cdot \text{g}^{-1}$ ), which suggests that the structure of PDA polymers affects the inter- or intramolecular spin interactions.

As shown in Figure 4, the  $M$ – $H$  plots of the magnetically active poly(*m*-PDA)s prepared in ho-



**Figure 4**  $M$ – $H$  plots at 5 and 300 K for poly(*m*-PDA)s prepared in homogeneous solutions: (●) treated with a stainless steel stick; (○) not treated with a stainless steel stick.

mogeneous systems also indicated S-shaped curves. Similarly to the polymers obtained in reversed micelles, the magnetization was almost independent of temperature. The  $M$ – $H$  plots of magnetically inactive polymers did not indicate S-shaped curves at 5 and 300 K. The saturation magnetization at 300 K was about  $0.2 \text{ emuG} \cdot \text{g}^{-1}$ .



**Figure 5** Temperature dependence of magnetization of poly(*m*-PDA) at magnetic field of 5000 G. Inset shows  $M$ – $H$  plots for poly(*m*-PDA) at 800 K.



Figure 5 shows a temperature dependence of magnetization at higher temperatures for magnetically active poly(*m*-PDA) at the magnetic field of 5000 G. Although the magnetization gradually decreased with an increase in temperature, it remained nonzero even at 800 K. Besides, the sample showed S-shaped curves in the *M*-*H* plots at 800 K (Fig. 5, inset). The transition temperatures from ferromagnetism to paramagnetism (Curie temperature  $T_c$ ) of Ni and Fe are 631 and 1043 K, respectively. Therefore, the above result suggests that the magnetism of this polymer at higher temperatures is mainly due to Fe. However, the *M*-*H* curves of this polymer did not show a hysteresis loop like a ferromagnetic metal.

In summary, we synthesized magnetically active PDA polymers by HRP-catalyzed polymerization of PDAs in homogeneous or reversed micellar solutions followed by treatment of the polymers with stainless steel sticks. The magnetic properties are considered to be affected by spin interactions of these polymers and Fe or Ni. The *M*-*H* curves of these polymers resembled those of soft ferromagnets, and the ferromagnetic properties were maintained at 800 K. Soft ferromagnetic materials are expected to have application to a wide range of electronic instruments and devices.<sup>17</sup> The present method is considered to be promising for a novel preparation of magnetically active polymers under mild conditions in good reproducibility.

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