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Journal of Molecular Catalysis B: Enzymatic 44 (2007) 149–154

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

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Polymerization of bisphenol a using *Coprinus cinereus* peroxidase (CiP) and its application as a photoresist resin

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Received 20 July 2006; received in revised form 4 October 2006; accepted 10 October 2006
Available online 15 November 2006

Abstract

Bisphenol A was polymerized by *Coprinus cinereus* peroxidase in aqueous 2-propanol solution. Various polymerized products with different molecular weights and hydroxyl values were synthesized depending on the reaction compositions (the ratio of aqueous buffer to 2-propanol). Poly(bisphenol A), a polymer of bisphenol A, was mixed with a diazonaphthoquinone derivative to form a new type of photoresist. A thin photoresist film was formed on the silicon wafer and exposed to UV light for different lengths of time. Poly(bisphenol A) having a molecular weight of approximately 3000 yielded sharply contrasted patterns as compared with the other poly(bisphenol A)s having different molecular weights.

Keywords: Photoresist; Bisphenol A; Enzymatic polymerization; *Coprinus cinereus* peroxidase

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1. Introduction

Photoresist is an essential chemical in semiconductor processing, liquid crystal display (LCD) processing and many printing processes, and its consumption has steadily increased over the years [1]. In particular, positive type photoresist has been widely used, because it allows the printing pattern to be precisely controlled, as compared to negative type photoresist. Currently, various polymers are being investigated as new photoresists, because of the demand for high resolution and imagery. However, most conventional photoresists are still mainly composed of novolac resin and diazonaphthoquinone (DNQ) photoactive compound. The demand for this photoresist has sharply increased, because it is consumed on a large scale for the patterning of RGB colorant displays in the LCD manufacturing process [1]. Most conventional novolac resins are synthesized by polymerizing various *o*-cresols, such as phenolics, and formaldehyde. Novolac resin shows excellent film-forming properties, good adhesion, etch resistance, and high solubility in alkaline solution. However, the toxic properties of the formaldehyde used in novolac resin have led to a considerable research effort to find alternative ways to polymerize novolac like resins.

The enzymatic polymerization of phenolics using peroxidase has been intensively studied [2–4] and several advantages of enzymatic polymerization have already been reported, as compared to the conventional polymerization process used for novolac resin. Especially, poly(bisphenol A) synthesized by horseradish peroxidase (HRP) showed excellent performance as a positive photoresist [5]. However, research into enzymatic polymerization has been deterred, since the use of peroxidases such as HRP is not economically viable. Recently, we reported a successful enzymatic polymerization using the fungal peroxidase, *Coprinus cinereus* peroxidase (CiP), for the synthesis of polycardanol [6]. This fungal peroxidase can be readily produced in large quantity in a bioreactor, which would make it economically viable if it can polymerize the target phenolics in high yield.

In this paper, we report the synthesis of poly(bisphenol A) using CiP and the effects of the reaction conditions on the properties of the photoresist.

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doi:10.1016/j.molcatb.2006.10.004

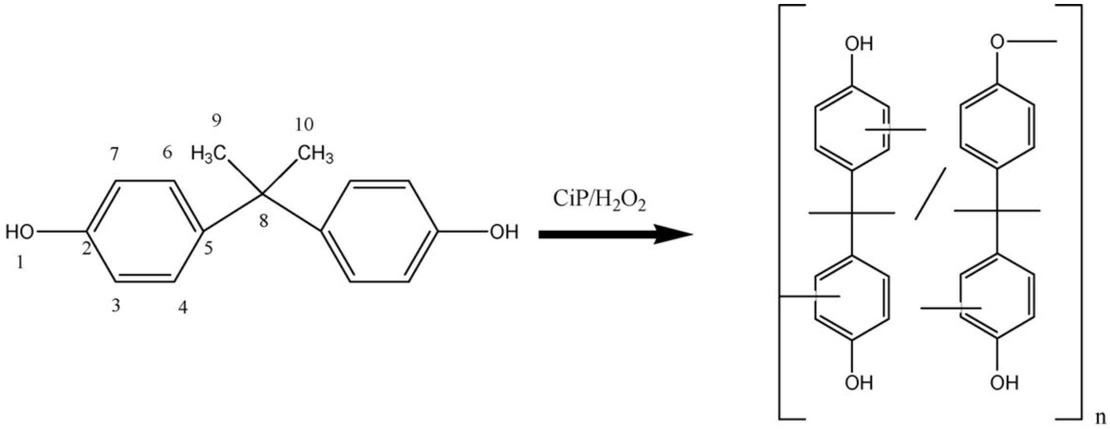

Fig. 1. Scheme of polymerization of bisphenol A using CiP.

2. Experimental

2.1. Production and purification of the fungal peroxidase

C. cinereus IFO 8371 was used as the peroxidase-producing strain. The medium used for the production of the peroxidase contained 30 g/L glucose, 5 g/L peptone (Difco Lab., USA), and 3 g/L yeast extracts (Difco Lab.). The details of the production and purification of the fungal peroxidase were reported previously [6]. Finally, the purified CiP was concentrated to a final concentration of 20,000 U/mL for the polymerization of bisphenol A.

2.2. Enzymatic polymerization of bisphenol A

The reaction scheme for the polymerization of bisphenol A (Aldrich, USA) is shown in Fig. 1. Bisphenol A was used without further purification. The enzymatic polymerization of bisphenol A was carried out as follows: 2.28 g of bisphenol A (10 mmol, Mw 228.3) was dissolved in a mixture of 12.5 g 2-propanol and 12.5 g phosphate buffer (100 mM, pH 7.0). Twenty-thousand units of CiP was then added to the reaction mixture, which was stirred for 5 min. The reaction was started by adding 15% H_2O_2 solution continuously at a rate of 2.0 mmol/h at 20 °C with gentle stirring for 5 h. Then, the reaction mixture was centrifuged at 5000 rpm and the supernatant was decanted. The settled materials were washed three times with aqueous methanol solution (50:50, v/v) to remove the unreacted monomer and buffer. The washed material was dried in a vacuum to give poly(bisphenol A).

2.3. Evaluation of peroxidase-catalyzed poly(bisphenol A) as photoresist resin

The photoresist thin film was prepared on a silicon wafer by spin-coating from a 10 wt.% ethylcellosolve solution of the poly(bisphenol A) and a photosensitizer (PS-105, Koyo Chemicals, Japan) (70:30 wt.%). The resulting film placed under a photomask pattern was exposed to UV light with constant light doses (20 mW/cm^2) for variable time periods. The UV-irradiation was performed using a MDA4000 (Midas system, Korea) with a metal halide lamp (3 kW, GL-30201BF). After the UV irradiation, the film was developed in an alkaline developer (AZ300 MIF developer, Clariant Corp., Switzerland) and rinsed in water. After the development of the test film, the developed patterns were observed using optical microscopy (Nikon, Japan). A resolution on the 2 μm scale was used for the examination of the various poly(bisphenol A) polymers, in order to evaluate their potential application as a photoresist resin. The depth profile on the developed pattern was obtained by employing a depth profiler (Alpha-step 500, KLA-Tencor, USA) and the aspect ratio of the developed pattern was calculated.

2.4. Analytical methods

The peroxidase activity (U/mL) was measured as follows. Several microliters of reaction solution were added to 2 mL of a 0.18 mM solution of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) in 50 mM phosphate-citrate buffer (pH 5.0). One microliter of a 15% H_2O_2 solution was added to initiate the color generation reaction. One unit of peroxidase was defined as the amount of enzyme required to catalyze the conversion of 1 μmol of ABTS ($\varepsilon = 34{,}700\ \mathrm{cm}^{-1}\mathrm{M}^{-1}$) per min at 25 °C. The bisphenol A concentrations during the enzymatic polymerization were analyzed by HPLC using a μBondapak C18 column (Waters, USA). The mobile phase was composed of acetonitrile:distilled water:acetic acid (8:2:1, v/v/v) at a flow rate of 0.1 mL/min. The absorbance was measured at 280 nm. The molecular weights of the poly(bisphenol A) polymers were determined by gel permeation chromatography (GPC). GPC analysis was carried out using a refractive index detector under the following conditions: PL4 mixed BB columns (TOSOH, Japan) and tetrahydrofuran as a solvent at 1.0 mL/min. The calibration curves for the GPC analysis were obtained using polystyrene standards. FT-IR spectra were recorded on a Perkin-Elmer FT-IR 2000 to confirm the polymer structure.

Table 1
Effects of solvent on the peroxidase-catalyzed polymerization of BPA

Solvents[a]	Yield[b] (%)	Molecular weight (Mn, Mw)	Polydispersity	Log *P*
DMSO	70.1	(1000, 1840)	1.84	−0.69
Methanol	5	(1400, 1820)	1.30	−0.32
1,4-Dioxane	93.6	(1960, 3190)	1.63	−0.234
ethanol	84.5	(2690, 5350)	1.98	0.056
2-Propanol	59.4	(18,600, 60,900)	3.27	0.42
2-Propanol (HRP)	–	–	–	0.42
Methanol (HRP)	93	(1400, 1820)	1.30	−0.32
Methanol (SBP)	80	(1700, 2550)	1.50	−0.32

[a] The solvents are a 50:50 mixture of water and the solvent.

[b] The yield mentioned is actual isolated yield.

3. Results and discussion

3.1. Peroxidase-catalyzed polymerization of bisphenol A

In this study, peroxidase derived from *C. cinereus* (CiP) and hydrogen peroxide were used as a catalyst and oxidizing agent, respectively. The polymerization of bisphenol A was performed in an equiweight mixture of organic solvent and phosphate buffer (pH 7.0) for 5 h at 20 °C. During the reaction, the formation of powdery precipitates was observed. After the reaction, the precipitates were collected by centrifugation and washed with an equivolume mixture of methanol and distilled water. The isolated polymer was completely soluble in ethylcellosolve. As shown in Table 1, the effects of the aqueous organic mixture on the CiP-catalyzed polymerization of bisphenol A were studied. In the very hydrophilic aqueous solvent mixture (low log *P*), the peroxidase-catalyzed polymerization of bisphenol did not take place at all. On the other hand, CiP yielded the polymerized product having a higher molecular weight in the relatively hydrophobic aqueous solvent mixture (high log *P*), as compared to that produced in the other solvents. Fig. 2 shows the relationship between the log *P* of the organic solvent and the molecular weight of the poly(bisphenol A) whose polymerization was catalyzed by CiP in an equiweight mixture of organic solvent and phosphate buffer. An organic solvent which is too hydrophilic can penetrate and replace the water bound on the protein, which may disturb the authentic three dimensional protein structure [14]. In addition, a more hydrophobic solvent can dissolve the higher molecular weight polymer, which may result in higher molecular weight poly(bisphenol A) being produced in an aqueous 2-propanol mixture. However, the polymerization of bisphenol A was also dependent on the type of peroxidase. Although HRP and SBP yielded a polymerized product in an aqueous methanol mixture with a high yield of over 80%, CiP yielded poly(bisphenol A) with very low yield. Since CiP produced poly(bisphenol A) having a higher molecular weight in an aqueous 2-propanol mixture, the subsequent experiments were performed in this solvent.

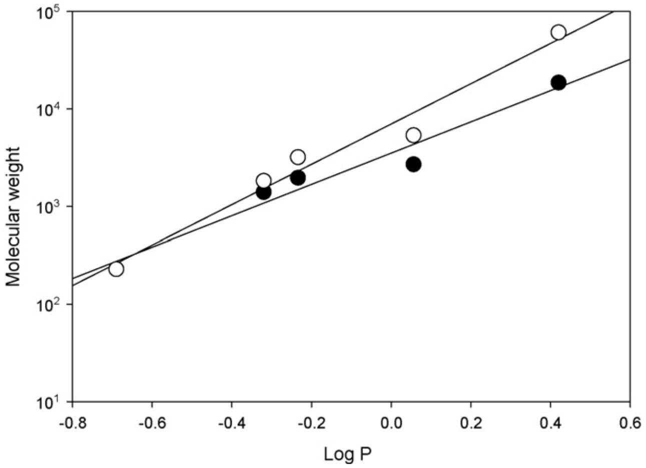

Fig. 2. The relationship between the log *P* of the organic solvent and molecular weight of the CiP-catalyzed poly(bisphenol A) in an equiweight mixture of organic solvent and phosphate buffer; (●) Number-averaged molecular weight (Mn); (○) Weight-averaged molecular weight (Mw).

3.2. Effects of solvents composition on polymerization of bisphenol A

Table 2 summarizes the results of the polymerization of bisphenol A in a mixture of 2-propanol and buffer. In the case where the 2-propanol content was less than 30%, the monomer was partially insoluble in the medium. Even at this compo-

Table 2
Effects of solvent composition on the peroxidase-catalyzed polymerization of BPA

Ratio of 2-propanol and buffer	Yield[a] (%)	Molecular weight (Mn, Mw)	Polydispersity	Hydroxyl value (meq/mmol)	Phenylene bond:oxyphenylene bond
20: 80	88.0	(1130, 1560)	1.381	1.30	65:35
30: 70	82.5	(1560, 2950)	1.553	1.29	64:36
40:60	70.3	(6450, 8310)	1.228	1.19	60:40
50:50	59.4	(18,600, 60,900)	3.247	1.16	58:42

[a] The yield mentioned is actual isolated yield.

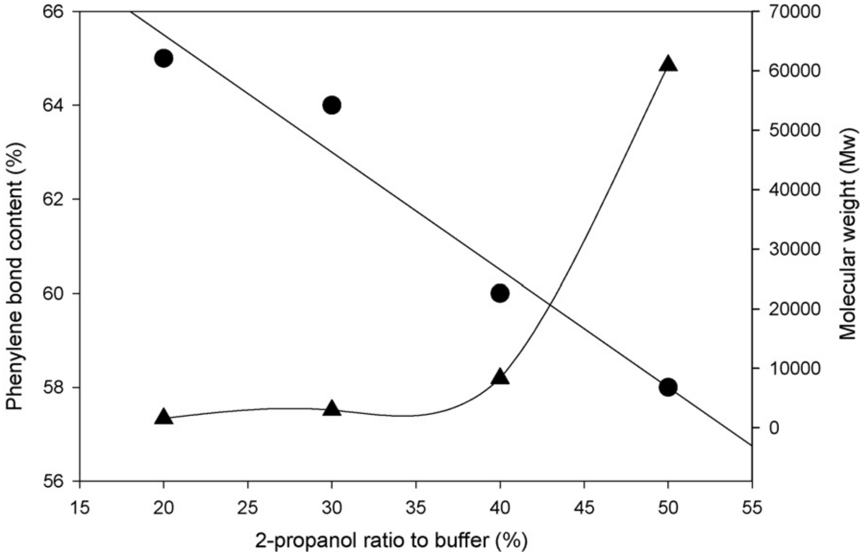

Fig. 3. Relationship between 2-propanol content and polymer structure in the CiP-catalyzed polymerization of bisphenol A in a mixture of 2-propanol and buffer; (●) phenylene bond content, (▲) molecular weight (Mw).

sition, the CiP-catalyzed polymerization yield was relatively high, as shown in Table 2. A lower content of 2-propanol in the reaction medium can increase the stability of CiP, because less water bound on CiP can be replaced by 2-propanol molecules. Although the dissolution of bisphenol A is limited in a solvent mixture having a lower content of 2-propanol, a higher yield of poly(bisphenol A) was, nevertheless, achieved, because the polymerized product escaped from the reaction medium by precipitation and the vacant space could therefore be refilled with monomer.

Changing the hydrophobicity of the solvent makes it possible to control the regioselectivity [8]. The phenylene unit content linearly decreased as the 2-propanol content increased, varying in the range from 65 to 58% (Fig. 3, correlation coefficient = 0.96).

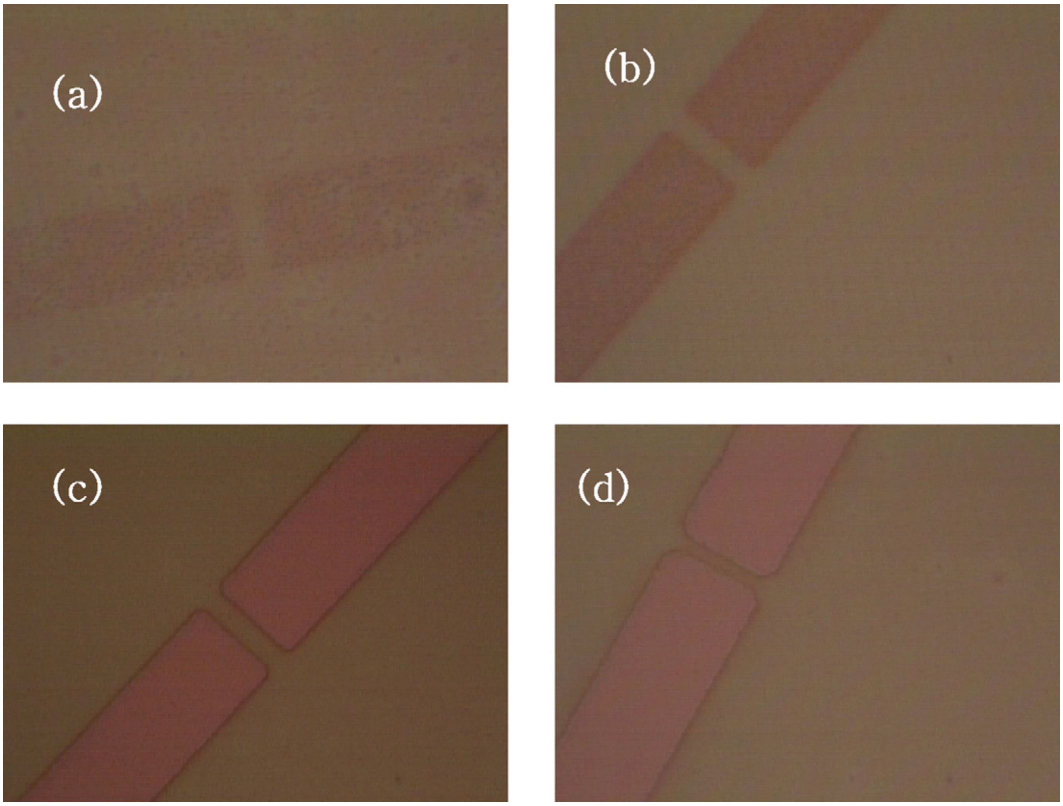

Fig. 4. Photograph of the developed test pattern on the silicon wafer observed by optical microscopy (magnification 1000×) for the various poly(bisphenol A) polymers. The minimum line space is 2 μm; (a) 2-propanol wt% = 50%, (b) 2-propanol wt% = 40%, (c) 2-propanol wt% = 30% and (d) 2-propanol wt% = 20%.

A similar tendency was observed in the laccase-catalyzed polymerization of phenol in an aqueous alcohol [7]. Molecular modeling provides hints as to how phenoxy radicals tend to be coupled [9,11]. According to the study of Huang et al. [9,12], a single electron is likely to associate, with almost equal probability, with atoms 1, 3, 5 and 7 of the bisphenol radical, as shown in Fig. 1. However, atom 5 of the bisphenol radical will have a lower probability of single electron association, due to the bulky dimethylmethane group. The presence of the bulky dimethylmethane group sterically hinders the para position from coupling [10,13]. This leads to radical coupling between the 1–3, 1–5, 3–3, 3–5, and 5–5 atoms of the bisphenol radical, which results in a theoretical degree of formation of the phenylene unit of 60%. This theoretical degree of formation of the phenylene unit is within the range of experimental values (58–68%, Fig. 3).

The experimental results shown in Table 2 allow us to draw the following conclusion: a more hydrophilic solvent mixture will yield the poly(bisphenol A) polymer having a higher content of phenylene units, while its molecular weight will be decreased, because of its limited solubility. This implies that the phenoxyl radical placed on atom 1 has less stability or a shorter lifetime in more hydrophilic solvent mixtures.

3.3. *Evaluation of peroxidase-catalyzed poly(bisphenol A) as photoresist resin*

A photoresist thin film was prepared on a silicon wafer by spin-casting from an ethyl cellosolve solution containing the poly(bisphenol A) and a photoactive compound (DNQ derivatives) (70:30 wt.%). The film thickness was adjusted to about 0.25 μm. For the evaluation of the photosensitivity, the film was exposed to UV light and developed in an alkaline developer and rinsed in distilled water. The developed patterns were observed using optical microscopy and a depth profiler, as shown in Figs. 4 and 5, respectively. Most sharply contrasted pattern was obtained from a poly(bispenol A) polymer (Mw: 2950) synthesized in aqueous 2-propanol solution (2-propanol:buffer = 30:70 w/w). The poly(bisphenol A) with lower molecular weight than 2950 was too sensitive to UV radiation and resulted in the over-developed pattern as shown in Fig. 4. On the contrary, poly(bisphenol A) with higher molecular weight showed resistant property to developing solution, which yielded an unclear pattern. A depth profiler was employed to evaluate quantitatively the developed pattern as shown in Fig. 5. As shown in Fig. 5, the depth of developed

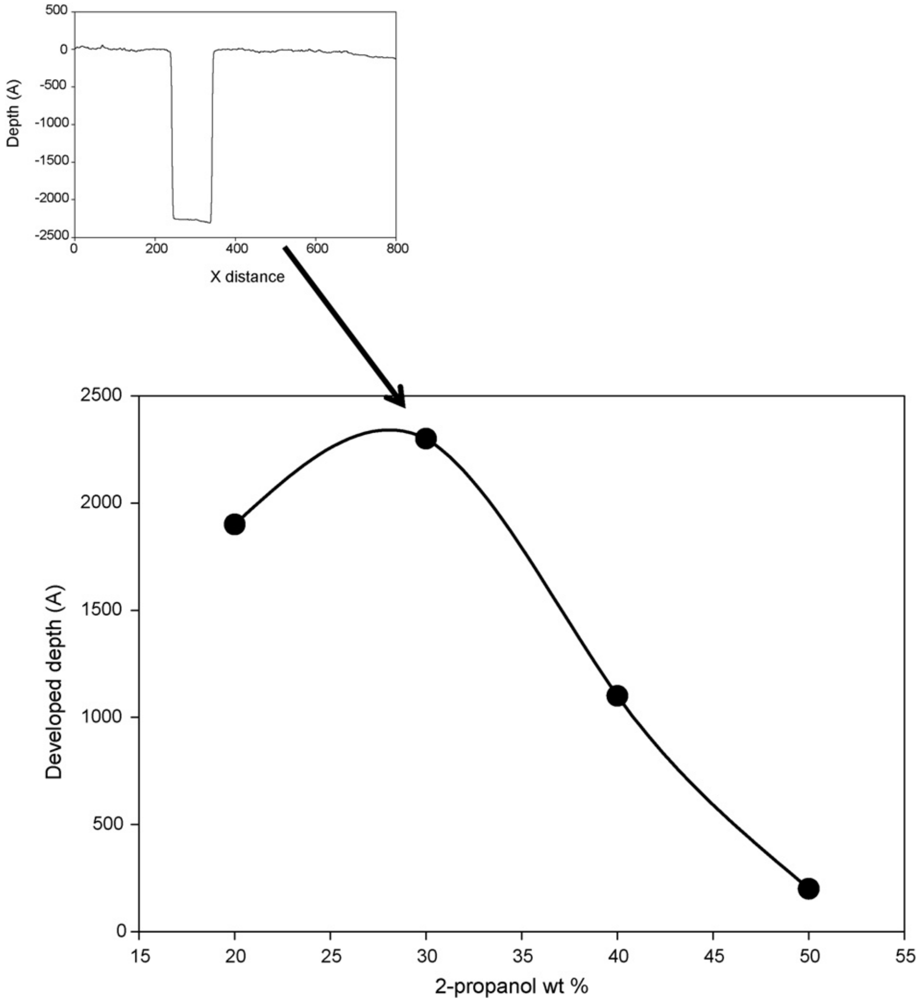

Fig. 5. Depth of developed test pattern on silicon wafer for the various poly(bisphenol A) polymers.

pattern was the highest for a poly(bispenol A) polymer (Mw: 2950) and approached about 240 nm. This result confirmed again that a poly(bisphenol A) (Mw: 2950) can be a superior photoresist compared to others. Although the poly(bisphenol A) with lower molecular weight than 2950 was developed fast, it showed a lower depth because unexposed part was also dissolved in developing solution. A depth profiler measured the depth difference between exposed and unexposed part. On the while, poly(bisphenol A)s with higher molecular weights yielded much lower depth. Since those polymers have higher molecular weight, they are reluctant to be dissolved. In addition, lower content of hydroxyl values of these polymers could be a cause because hydroxyl groups are known to form the chemical bond with DNQ. Polymers having fewer hydroxyl groups have less probability to form the bonds with DNQ, which could deter the dissolution of polymers in developing solution [15].

4. Conclusions

Bisphenol A was polymerized by *C. cinereus* peroxidase in aqueous 2-propanol solution. Various polymerized products with different molecular weights and hydroxyl values were synthesized depending on the reaction compositions (the ratio of aqueous buffer to 2-propanol). A new positive type photoresist was developed by mixing poly(bisphenol A) and a photoactive compound such as DNQ. The hydroxyl value and molecular weight of the poly(bisphenol A) are the critical factors determining the photosensitivity of the formulated photoresist. The optimized poly(bisphenol A) obtained by varying the hydroxyl value and molecular weight showed excellent performance as a photoresist.

Acknowledgement

This work was financially supported by Microbial Genomics and Application 21st Frontier Program (Contract No. MG05-0308-1-0) and authors acknowledge the support.

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