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Enzymatic Synthesis of Photoactive Poly(4-phenylazophenol)

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Enzymatic synthesis, employing horseradish peroxidase (HRP), has been used to prepare a novel photoactive azopolymer, poly(4-phenylazophenol), from 4-phenylazophenol. FTIR, FT-Raman, and NMR (^{1}H, ^{13}C) spectroscopy show that the coupling reaction occurs primarily at the ortho positions with some coupling at the meta positions of the phenol ring of the monomer as well. This results in the formation of a branched polyphenylene backbone with pendant azo functionalities on every repeat unit of the macromolecules. This enzymatically synthesized azophenol polymer has an extremely high dye content (nearly 100%) and is soluble in most polar organic solvents, and good optical quality thin films are easily formed. Polymer solutions show reversible trans to cis photoisomerization of the azobenzene groups with long relaxation time. The poly(azophenol) film exhibits photoinduced absorption dichroism and large photoinduced birefringence with unusual relaxation behavior. The resulting azobenzene loaded polymer behaves as a new type of glassy “macromolecular dye”.

1. Introduction

Polymers containing azobenzene groups have been investigated for several decades, but have attracted considerable recent interest due to their potential applications in the fields of optics and optoelectronics.[1] It is well-known that azobenzene chromophores exhibit photoinduced reversible trans−cis isomerization. These photoinduced conformational changes can significantly influence the bulk and surface properties of azobenzene polymers[2] and are of interest for applications, such as optical switching,[3] command surface generation,[4] and information storage[5] among others. A large number of azobenzene-containing polymers have been designed and chemically synthesized, for example through post-azo-coupling reactions to introduce azobenzene chromophore as a pendant side chain of the polymer.[6] Approaches to synthesize main-chain azopolymers have also been developed.[7] Thin films of these polymers processed by spin-coating and layer-by-layer self-assembly have been used for the fabrication of surface relief gratings [8] and second-order nonlinear optical structures.[6]

Enzymatic, peroxidase-catalyzed, polymerization has been explored as a promising alternative approach to the synthesis of electronically and optically active polymers. Enzymatic polymerization reactions are advantageous in that they can offer simple and environmentally friendly reaction conditions and a higher degree of specificity and control over the kinetics of the reaction and higher yields of product.[9] Peroxidase-catalyzed synthesis also involves a reaction mechanism that results in a direct ring-to-ring coupling of phenol and aniline monomers.[10] Depending on the reaction conditions, the resulting polymers may have an aromatic, extended conjugated backbone structure, with interesting electrical and optical properties. The enzy-

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(1) (a) Kumar, G. S. *Azo Functional Polymer: Functional Group Approach in Macromolecular Design*; Technomic Publishing Co. Inc.: Lancaster, Basel, 1993. (b) Moerner, W. E.; Silence, S. M. *Chem. Rev.* **1994**, *94*, 127. (c) Law, K.-Y. *Chem. Rev.* **1993**, *93*, 449. (d) Willner, I. *Acc. Chem. Res.* **1997**, *9*, 3347.

(2) (a) Kumar, G. S.; Neckers, D. C. *Chem. Rev.* **1989**, *89*, 1915. (b) Xie, S.; Natansohn, A.; Rochon, P. *Chem. Mater.* **1993**, *5*, 403.

(3) Ikeda, T.; Tsutsumi, O. *Science* **1995**, *268*, 1873.

(4) (a) Liu, Z. F.; Hashimoto, K.; Fujishima, A. *Nature* **1990**, *347*, 658. (b) Berg, R. H.; Hvilsted, S.; Ramanujam, P. S. *Nature* **1996**, *383*, 505.

(5) (a) Knobloch, H.; Orendi, H.; Buchel, M.; Seki, T.; Ito, S.; Knoll, W. *J. Appl. Phys.* **1994**, *76*, 8212. (b) Knobloch, H.; Orendi, H.; Buchel, M.; Seki, T.; Ito, S.; Knoll, W. *J. Appl. Phys.* **1995**, *77*, 481.

(6) (a) Wang, X. G.; Balasubramanian, S.; Kumar, J.; Tripathy, S. K.; Li, L. *Chem. Mater.* **1998,** *10*, 1546. (b) Wang, X. G.; Li, L.; Chen, J.; Marturunkakul, S.; Kumar, J.; Tripathy, S. K. *Macromolecules* **1997**, *30*, 219. (c) Wang, X. G.; Chen, J.; Marturunkakul, S.; Li, L.; Kumar, J.; Tripathy, S. K. *Chem. Mater.* **1997**, *9*, 45.

(7) (a) Balasubramanian, S.; Kim, J.; Kim, D. Y.; Kumar, J.; Tripathy, S. K. *Macromol. Rapid Commun.* **1996**, *17*, 853. (b) Irie, M.; Hirano, Y.; Hashimoto, S.; Hayashi, K. *Macromolecules* **1981**, *14*, 262. (c) Blair, H. S.; Pague, H. I.; Riordan, J. E.; *Polymer* **1980**, *21*, 1195.

(8) (a) Kim, D. Y.; Tripathy, S. K.; Li, L.; Kumar, J. *Appl. Phys. Lett.* **1995**, *66*, 1166. (b) Kim, D. Y.; Li, L.; Jiang, X. L.; Shivshankar, V.; Kumar, J.; Tripathy, S. K. *Macromolecules* **1995**, *28*, 8835. (c) Jian, X. L.; Li, L.; Kim, D. Y.; Shivshankar, V.; Kumar, J.; Tripathy, S. K. *Appl. Phys. Lett.* **1996**, *68*, 2618. (d) Balasubramanian, S.; Wang, X. G.; Wang, H. C.; Yang, K.; Kumar, J.; Tripathy, S. K.; Li, L. *Chem. Mater.* **1998**, *10*, 1554.

(9) (a) Dordick, J. S. *Enzyme Microb. Technol.* **1989**, *11*, 194. (b) Akkara, J. A.; Kaplan, D. L.; John, V. J.; Tripathy, S. K. In *Polymeric Materials Encyclopedia*; Salamone, J. C., Ed.; CRC Press: Boca Raton, FL, 1996; Vol. 3, D−E, pp 2116−2125.

(10) Ryu, K.; McEldoon, J. P.; Pokora, A. R.; Cyrus, W.; Dordick, J. S. *Biotechnol. Bioeng.* **1993**, *42*, 807.

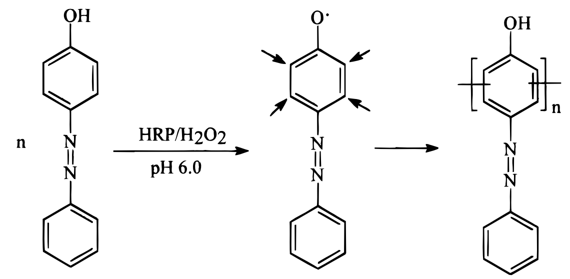
10.1021/cm000072p CCC: $19.00

matic synthesis of polyphenols using the enzyme, horseradish peroxidase (HRP) in organic solvents was first demonstrated by Dordick and co-workers.[11] Since then, a variety of modified enzymatic reactions such as the use of mixed-solvent systems,[12] Langmuir monolayers,[13] reversed micelles,[14] and the presence of template,[15] have been investigated to optimize these reactions for polyphenol and poly(aromatic amine) synthesis.

Enzymatic polymerization of phenols proceeds primarily through ortho coupling with possibility of some para linkages.[10] Blocking of the para position leads to linear macromolecules, although only modest molecular weights are realized. The possibility of enzymatic polymerization of azo-functionalized anilines and phenols offers an approach to build in substantial chromophore density in the polymers. In this case, polymerization is expected to occur through the aniline or phenol ring, making the azo benzene chromophore part of each repeat unit in the polymer. This provides a significantly higher azobenzene dye content than those polymers that are chemically post modified with azobenzene groups. In addition, this direct coupling of the azobenzene side chain to a polyphenylene backbone may provide interesting optical and electronic properties.

We have recently reported the enzymatic polymerization of diaminoazobenzene.[16a] Although the polymer showed interesting photoisomerization behavior in solution, it was not easily processable. Polymerization was expected to proceed via an ortho coupling reaction. The presence of amine groups at both ends and their participation in the coupling reaction leads to its poor processability. However, this polymer was processed into thin films with modest optical quality for photofabrication studies.[16b] In the present work, an azo-functionalized phenol, 4-phenylazophenol, is polymerized by the catalysis of HRP. NMR (^{1}H and ^{13}C) and FTIR show that coupling occurs primarily at the ortho positions on the phenol ring of 4-phenylazophenol with some meta couplings as well, as shown in Scheme 1 and results in an azo-functionalized polyphenol which has a branched polyphenylene backbone. This poly-azophenol exhibits significantly better solubility and processability than the enzymatically synthesized poly-diaminoazobenzene. Optical quality films of this poly-azophenol may be prepared with ease. Since both ortho and meta couplings occur through the phenol ring and the degree of polymerization is modest, a highly articulated macromolecular dye is realized. Photophysical properties involving photoinduced mass transport of this new class of dye macromolecules are presented in the following paper of this series. In this paper, details of synthesis, molecular structural characterization and relevant photochemical and physical properties are presented.

Scheme 1



2. Experimental Section

2.1. Materials. Horseradish peroxidase (EC 1.11.1.7) (200 units/mg) was purchased from Sigma with RZ > 2.2. A stock solution of 10 mg/mL in pH 6.0, 0.1 M phosphate buffer was prepared. The monomer, 4-phenylazophenol, was obtained from Aldrich and used as received. All other chemicals and solvents used were commercially available, of analytical grade or better, and used as received.

2.2. Enzymatic Polymerization. Enzymatic polymerization of 4-phenylazophenol was carried out at room temperature in a 100 mL, 50% acetone and 50% 0.01 M sodium phosphate buffer mixture, which contained 2.0 g of 4-phenylazophenol. To this solution, 2.0 mL of HRP stock solution was added. The reaction was initiated by the addition of H_2O_2. To avoid the inhibition of HRP due to excess H_2O_2, a diluted stoichiometric amount of H_2O_2 (0.2 M) was added incrementally under vigorous stirring over a 3 h time period. After the addition of H_2O_2, the reaction was left stirring for one more hour. The yellow precipitates formed during the reaction were then collected with a Buchner funnel, washed thoroughly with the mixed solvent of 20% acetone and 80% water (v/v) to remove any residual enzyme, phosphate salts, and unreacted monomers, and then vacuum-dried for 24 h. The final yield of polymer was ~80%. The GPC analysis shows a molecular weight of the order of 3000 (M_w) with a polydispersity of 3.5, indicating a degree of polymerization of roughly 15.

2.3. Characterization. The UV–vis absorption spectra were determined with a Perkin-Elmer Lambda 9 spectrophotometer. Infrared spectra for both the monomer and polymer were measured by casting thin films on a KCl crystal window using a Perkin-Elmer 1720 FT-IR spectrometer. Raman spectra of monomer and polymer in powder form were recorded on a Perkin-Elmer 1760 FT-Raman spectrometer. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra of monomer and polymer in methyl sulfoxide-d_6 (DMSO-d_6) were obtained on a Bruker ARX-500 MHz FT-NMR spectrometer. The thermal properties of the polymer were measured with a Du Pont thermal analyzer, TGA 2950 (TA Instrument Inc.), and DSC 2910 (TA Instruments Inc.). Molecular weight was measured using a Waters HPLC system equipped with a model 510 HPLC pump, model 410 differential refractometer, model 441 absorbance detector, on-line multiangle laser light scattering (MALLS) detector (MiniDawn, Wyatt Technology Inc.), model 712 sample processor, and five Ultrastyragel GPC columns connected in the following series: 500, 10^3, 10^4, 10^5, and 100 Å. THF was used as a carrier solvent in a flow rate of 1 mL/min. Polystyrene standards are used as references in the molecular weight measurements.

2.4. Optical Quality Polymer Film Preparation. Polymer solution in spectroscopic grade dioxane was prepared and filtered through a 0.45 μm membrane. The solution was then

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(11) Dordick, J. S.; Marletta, M. A.; Klibanov, A. M. *Biotechnol. Bioeng.* **1987**, *30*, 31.

(12) (a) Akkara, J. A.; Senecal, K. J.; Kaplan, D. L. *J. Polym. Sci., Part A: Polym. Chem.* **1991**, *29*, 1561. (b) Akkara, J. A.; Salapu. P.; Kaplan, D. L. *Indian J. Chem.* **1992**, *31B*, 855. (c) Wang, P.; Dordick, J. S. *Macromolecules* **1998**, *31*, 941. (d) Ikeda, R.; Uyama, H.; Kobayashi, S. *Macromolecules* **1996**, *29*, 3053.

(13) Bruno, F.; Akkara, J. A.; Samuelson, L. A.; Kaplan, D. L.; Marx, K. A.; Kumar, J.; Tripathy, S. K. *Langmuir* **1995**, *11*, 889.

(14) (a) Rao, A. M.; John, V. T.; Gonzalez, R. D.; Akkara, J. A.; Kaplan, D. L. *Biotechnol. Bioeng.* **1993**, *41*, 531. (b) Premachandran, R.; Banerjee, S.; John, V. T.; McPherson, G. L.; Akkara, J. A.; Kaplan, D. L. *Chem. Mater.* **1997**, *9*, 1342. (c) Premachandran, R. S.; Banerjee, S.; Wu, X.-K.; John, V. T.; McPherson, G. L.; Akkara, J. A.; Ayyagari, M.; Kaplan, D. L.; *Macromolecules* **1996**, *29*, 6452.

(15) (a) Samuelson, L. A.; Anagnostopoulos, A.; Alva, K. S.; Kumar, J.; Tripathy, S. K. *Macromoleclues* **1998**, *31*, 4376. (b) Liu, W.; Kumar, J.; Tripathy, S. K.; Senecal, K. J.; Samuelson, L. A. *J. Am. Chem. Soc.* **1999**, *121*, 71.

(16) (a) Alva, K. S.; Lee, T. S.; Kumar, J.; Tripathy, S. K. *Chem. Mater.* **1998**, *10*, 1270. (b) Tripathy, S. K.; Kim, D. Y.; Nirmal, K.; Balasubramanian, S.; Liu, W.; Wu, P.; Bian, S.; Samuelson, L. A.; Kumar, J. *Synth. Met.* **1999**, *101*, 738.

spin-coated onto glass slides. The film thickness was controlled to 0.2–5.0 μm by adjusting the solution concentration (0.2–0.8 M) and spin speed (3000–7000 rpm). The spin-coated films were then dried under vacuum for 24 h at 40–50 °C and stored in a desiccator until further studies.

2.5. Photoisomerization. The solution of 4-phenylazophenol and poly(4-phenylazophenol) in dioxane (30 μg/mL) in a sealed quartz cuvette was exposed to UV light at 360 nm. UV–vis absorption spectra were sequentially recorded after every 6 s of exposure for the monomer solution and after 12 s of exposure for the polymer solution, respectively. The changes in absorption spectra during the process of thermal relaxation at room temperature were sequentially recorded as well at an interval of 20 min for the monomer and 40 min for the polymer, respectively, immediately following irradiation by UV light at 360 nm for 3 min. To minimize the change in the absorption characteristics due to the relaxation of the polymer during the scan period, a scan speed of 480 nm/min was usually used in these measurements. Similar investigations were also performed with UV light at 254 nm and Ar$^+$ laser at 488 nm as the pump lights. Experiments were repeated in other solvent systems such as acetone and DMF as well.

2.6. Photoinduced Absorption Dichroism. To investigate photoinduced dichroism in poly-azophenol, absorption changes in a spin-coated film sample (0.26 μm thick) were measured for a low-power, linearly polarized probe laser beam at 488 nm when the sample was exposed to another linearly polarized pump laser beam at the same wavelength. To avoid the possible interference effects that could influence the measurements, the probe and the pump light beams were from two argon ion lasers, respectively, and pass through the sample in nearly opposite directions (normal to the sample surface). The probe laser beam has a diameter much smaller than that of the exciting laser beam and was centered in the central area of the pump beam on the sample. A quarter wave plate was put in the path of the pump light such that linearly or circularly polarized light could be obtained by adjusting the orientation of the quarter wave plate. Circularly polarized light was used for erasing the induced absorption dichroism. The intensity of the pump laser beam was 130 mW/cm^2. The intensity of the probe laser beam was kept low enough such that its influence on chromophore orientation was negligible.

2.7. Photoinduced Birefringence. To measure the induced birefringence, a polymer film sample (0.26 μm thick) was placed between two crossed polarizers in the path of a low-power He–Ne laser beam at 633 nm. The absorption of the polymer at 633 nm is much smaller relative to that at 488 nm. Since the polymer film is initially isotropic no light can pass through the second polarizer. When the sample was irradiated by a laser beam at 488 nm with a linear polarization direction making a 45° angle with respect to the polarization direction of the polarizers, a 633 nm laser signal appeared behind the second polarizer. This signal is a result of the photoinduced birefringence in the polymer medium. The probe beam intensity transmitted at steady state in this experimental configuration is described by the equation[17]

$$I = I_{\mathrm{i}} \sin^2(\delta/2) \qquad (1)$$

where $\delta = 2\pi\Delta n d/\lambda$. Δn, d, and λ are photoinduced birefringence, sample thickness, and wavelength of probe light, respectively. I_{i} is the intensity of transmitted light when phase difference δ is equal to π.

3. Results and Discussion

3.1. Enzymatic Polymerization. Peroxidase-catalyzed polymerization of phenols and aromatic amines has been extensively studied in recent years. Initially, a major limitation of enzymatic polymerization was that only low molecular weight polymer could be formed from pure aqueous solutions.[18] Further research has shown that by introducing organic solvents into the reaction media, although the activity of the HRP is decreased, the solubility of monomer and molecular weight of the final polymer is increased significantly.[11,12a] Usually the molecular weight of the resulted polymer increases with the content of organic solvents. It is now well-known that HRP is an effective biological catalyst in mixed water–organic solvent systems, even when the organic solvent content is higher than 85%.[11] In this work, a mixture of 50% acetone and 50% aqueous buffer (v/v) was found to be an effective reaction medium for the enzymatic polymerization of 4-phenylazophenol and was used for all reactions.

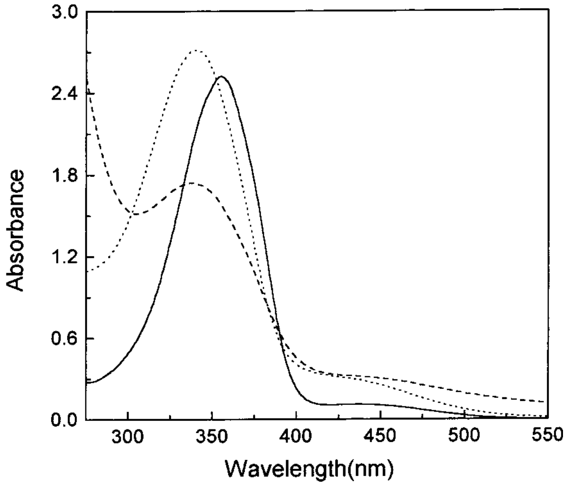

Figure 1. UV–vis absorption spectra of (—) monomer in dioxane, (···) polymer in dioxane, and (- - -) thin film of polymer on glass slide.

The enzymatic polymerization of 4-phenylazophenol was studied using UV–vis spectroscopy. Figure 1 shows the absorption spectra of solutions of the monomer and polymer in dioxane and a cast thin film of the final polymer. The monomer spectrum of 4-phenylazophenol is similar to that known for other azo benzene derivatives[19] where a maximum absorption at 355 nm, characteristic of *trans*-4-phenylazophenol and a weak broad peak at about 440 nm due to *cis*-4-phenylazophenol are present. Comparison of these solution spectra shows that a significant absorption change occurs as a result of polymerization. The trans absorption at 355 nm in the monomer, blue shifts to 345 nm in the polymer and the cis absorption at 440 nm becomes stronger in the polymer. Also, the thin film of the polymer shows a stronger and broader absorption in the wavelength range of 400–600 nm.

A more detailed investigation of the conformational changes of the azo chromophore during the reaction was carried out spectroscopically. Figure 2 shows the change in the UV–vis absorption spectra as H_2O_2 is added incrementally to the acetone–water reaction medium. Slight solvatochromic shifts in the absorbance maxi-

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[17] Born, M.; Wolf, E. *Principles of Optics*; Pergamon Press: New York, 1980.

[18] Saunders, B. C.; Holmes-Siedle, A. G.; Stark, B. P. In *Peroxidase*; Butterworths: London, 1964.

[19] (a) Fujiwara, H.; Yonezawa, Y. *Nature* **1991**, *351*, 724. (b) Song, X.; Perlstein, J.; Whitten, D. G. *J. Am. Chem. Soc.* **1997**, *119*, 9144. (c) Ross, D. L.; Blanc J. In *Photochromism*; Broen, G. H., Ed.; Wiley-Interscience: New York, 1971; pp 471–557. (d) Gabor, G.; Frei, Y. F.; Fischer, E. *J. Phys. Chem.* **1968**, *72*, 3266.

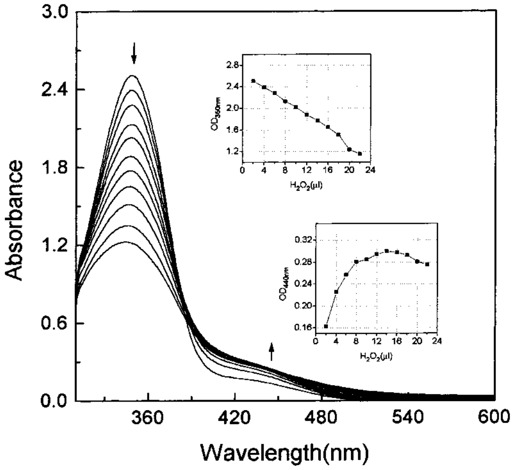

Figure 2. Absorbance change during enzymatic polymerization of 0.1 μM 4-phenylazophenol in 3 mL of a 50% acetone and 50% buffer mixture at pH 6. After each addition of 2 μL of H_2O_2 (0.02 M) a spectrum is taken. The insets show the change of absorbance at 350 and 440 nm as a function of the amount of H_2O_2 added.

mum of 4-phenylazophenol are observed in the acetone–water solutions when compared to the previous dioxane solutions. The strong peak at 350 nm in the monomer spectrum shows that the monomer exists primarily in the trans form in the reaction mixture. However, as the polymerization proceeds, the trans peak at 350 nm decreases, broadens, and shifts to shorter wavelengths and the broad, cis absorption band around 440 nm increases. These results show that a trans to cis isomerization of 4-phenylazophenol occurs during enzymatic coupling of the monomer. A similar transition was observed during the enzymatic polymerization of diaminoazobenzene.[16a]

The changes in the absorbance at 350 and 440 nm during the course of the reaction are plotted as a function of the amount of H_2O_2 added and are shown as insets in Figure 2. These plots show that the decrease in absorbance at 350 nm is linear, while the increase in absorbance at 440 nm levels off after addition of less than half of the stoichiometric amount of H_2O_2. One possible explanation for this trans to cis isomerization during polymerization is that incorporation of the phenol ring into the backbone of the polymer causes strong steric hindrance to the trans form. The growing polymer has significant conformational constraint, and a blue shift of the trans absorption is observed.[16a] This blue shift is accompanied by a partial isomerization to the cis form to help accommodate the structural constraints of the growing macromolecule.

3.2. Polymer Characterization. *FTIR.* Structural and mechanistic information on the enzymatic polymerization was determined using FTIR. The assignments for the major vibrational bands in the FTIR spectra are summarized in Table 1. The FTIR spectra of 4-phehylazophenol and poly(4-phenylazophenol) show broad peaks from 3100 to 3600 cm^{-1} and are assigned to OH stretch and internal hydrogen bond formation.[20] The retention of a strong OH stretch region in the polymer suggests that most of the OH groups in the monomer are not involved in the coupling reaction and that the resulting polymer has significant phenol functionality. The appearance of the peak at 3068 cm^{-1} in the polymer spectrum may be indicative of the C=O···H–OC stretch of a quinoidal intramolecular hydrogen-bonding structure. The OH groups present in the polymer could be oxidized into a terminal quinoid structure which would readily be available for the formation of hydrogen bonding. The bands at 650–900 cm^{-1} are assigned to C–H out-of-plane bending and are very characteristic of substitution patterns in an aromatic ring.[20] The bands at 771 and 686 cm^{-1} correspond to C–H out-of-plane bending from the monosubstituted benzene ring of 4-phenylazophenol.[20] The relative intensities of these two bands in the polymer are similar to that observed in the monomer, indicating that few substitution changes have occurred on this aromatic ring. The peaks from 840 to 1139 cm^{-1} are due to the para-bisubstituted (phenol) aromatic ring of the monomer. After polymerization, these peaks become broad and weak, indicating that significant substitution is occurring on the phenol ring. Meanwhile, the broadening of the aromatic C=C stretch at around 1600 cm^{-1} in the polymer spectrum indicate a dispersity of stretches which would be associated with the complicated articulated structure of the backbone (further discussed later in the NMR section).

Table 1. Assignments of Major Vibrational Bands of Monomer and Polymer in the FTIR Spectra

description	4-phenylazophenol	poly(4-phenylazophenol)
OH stretch	3315(s)	3306(s)
C=C stretch	1592(s)	1591(s)
(aromatic)	1500(s)	1491(s)
C=O stretch		1700(w)
C–O stretch	1239(s)	1230(s)
out of plane	771(m)	769(m)
CH bend	686(m)	691(m)
	840(m)	
	1139(m)	

The weak peaks in the polymer spectrum at ~1700 cm^{-1} are believed to be due to C=O vibrations from quinoid and ketonic structures.[20] John and co-workers[14c] observed similar peaks for the enzymatic polymerization of 2-naphthol and 4-ethylphenol and found these structures to be present in both the main chain and end groups of the polymer. In comparison, the weakness of these peaks in the poly(4-phenylazophenol) suggests that there is a minimal amount of these structures formed in this reaction. It is also possible that there are phenoxy (C–O) linkages present since these have been observed previously in the HRP-catalyzed polymerization of phenol.[12d] However, the characteristic bands[20] at ~1270–1280 cm^{-1} for these functionalities are not clearly observed in these FTIR spectra. These spectral features provide indirect evidence that the polymerization proceeds primarily on the phenolic ring of 4-phenylazophenol through carbon–carbon coupling and not through the phenol functionality. More direct information, which supports this enzymatic coupling mechanism, is provided by NMR studies and is described as follows.

NMR. ^{1}H NMR spectra of the 4-phenylazophenol and poly(4-phenylazophenol) are given in Figure 3. The monomer spectrum shows as expected sharp peaks and

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(20) (a) Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Identification of Organic Compounds*, 5th ed.; John Wiley & Sons: New York, 1991. (b) Socrates, G. *Infrared Characteristic Group Frequencies*; John Wiley & Sons: New York, 1994.

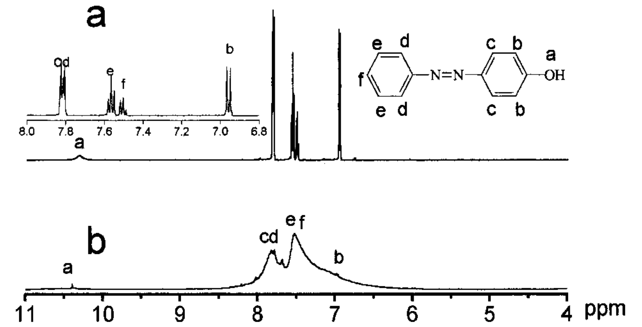

Figure 3. ^{1}H NMR spectra of (a) monomer and (b) polymer of 4-phenylazophenol in DMSO-d_6. The inset in a is the expanded region from 6.8 to 8 ppm. The assignments for the various resonances in the monomer are made as shown.

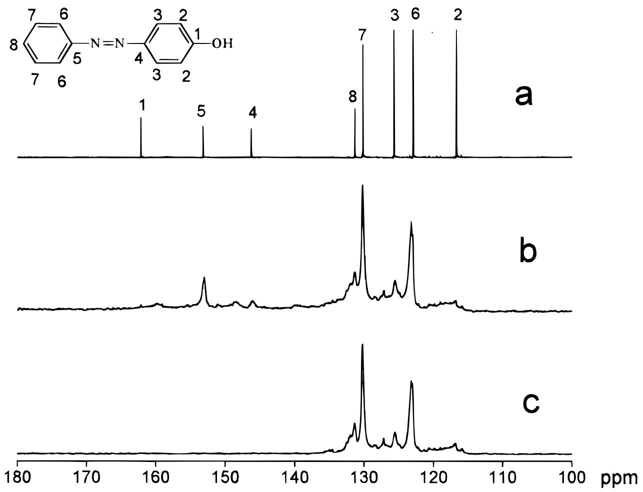

Figure 4. ^{13}C NMR of (a) monomer, (b) polymer, and (c) DEPT 90 spectrum of the polymer of 4-phenylazophenol in DMSO-d_6. The assignments for the monomer are made as shown.

the individual proton assignments for these peaks are given. After polymerization, however, only two broad peaks, centered at 7.5 and 7.8 ppm are observed. A similar broadening of proton NMR has been observed for other enzymatically prepared polyphenol systems and is attributed to the polymerization.[12a,14c] A more detailed look at these spectra shows that the polymer peaks at 7.5 and 7.8 ppm originate from the resonance of the e, f and c, d protons respectively, that is, found in the monomer. Upon polymerization, the resonance at 6.95 ppm for proton b (the ortho proton of the phenol ring) decreases significantly and is nearly gone in the spectrum of the polymer. Additionally, an apparent decrease in the intensity of the c, d protons in the polymer spectra is observed, indicating that some coupling is also occurring at these positions. Little change is observed in the peak from the OH proton at 10.4 ppm. These spectra provide direct evidence that the ortho positions of the phenolic ring are the most favored for coupling. This dominance of ortho–ortho coupling has also been observed for the enzymatic synthesis of polyphenol in monophasic organic solvents.[12a,14c]

Figure 4 shows the ^{13}C NMR spectra of 4-phenylazophenol and poly(4-phenylazophenol). The monomer spectrum has eight sharp peaks that are assigned as shown in Figure 4a. The ^{13}C spectrum of the polymer in Figure 5b is more resolved than the ^{1}H polymer spectrum and more informative. Comparison of the most significant differences in these spectra show that the C2 peak at 116.7 ppm decreases dramatically and almost disappears in the polymer spectrum. This is consistent with the ^{1}H NMR and confirms that the ortho positions of the phenolic ring are extremely reactive coupling sites. Also, the C3 peak of the phenolic ring at 125.6 ppm shows a significant reduction in intensity and confirms that some coupling also occurs at the meta positions. The C6, C7, C8, and C5 peaks however show little change in the polymer spectrum, other than broadening, which suggests that coupling is not occurring on this aromatic ring. To assist the assignment of the observed ^{13}C NMR resonance peaks of the polymer, DEPT pulse sequence is used for spectral editing. The DEPT-90 experiment of the polymer provides a spectrum for ^{13}C resonance of the protonated carbons as shown in Figure 4c. The disappearance of the downfield resonance peaks in the region from 136 to 165 ppm in Figure 4c compared to Figure 4b indicates that these peaks are from nonprotonated aromatic carbons. The peaks at 162.2 and 145.7 ppm are due to the two ipso carbons (C1 and C4) on the phenol ring. The new peaks

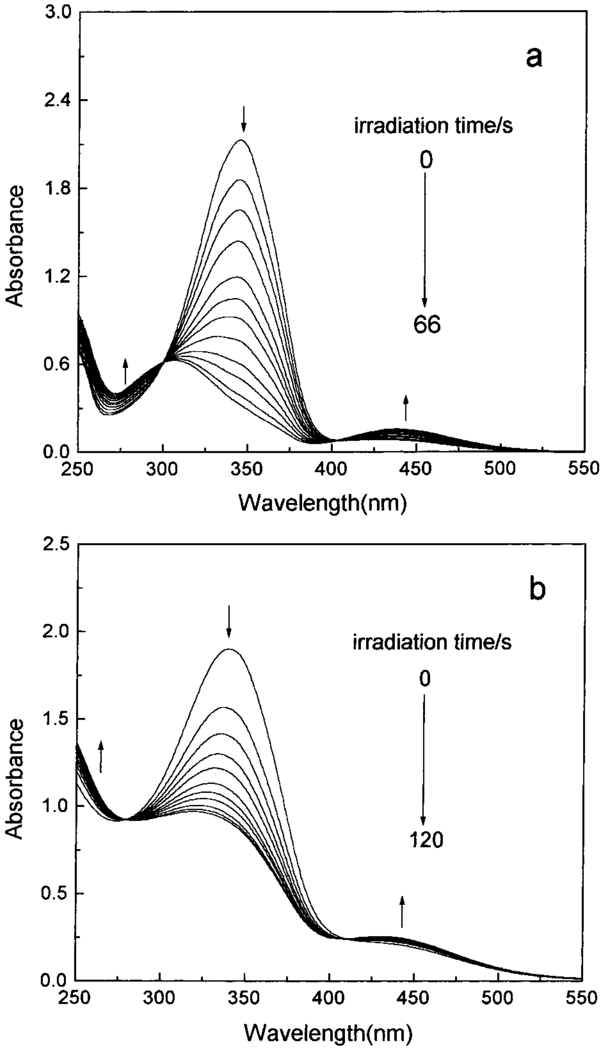

Figure 5. Changes in the absorption spectra of (a) 4-phenylazophenol and (b) poly(4-phenylazophenol) in dioxane solution (30 μg/mL) during irradiation with UV light at 360 nm. Each spectrum was recorded after 6 s irradiation for the monomer, and 12 s irradiation for the polymer solution.

are observed in the polymer spectrum at 148.7 and 151.7 ppm that are believed to be due to the formation of nonprotonated carbon during the coupling reaction.

The NMR studies confirm that coupling is most favorable at the ortho (C2) positions of the phenol ring, with some reaction also taking place at the meta (C3) positions. The ortho coupling is consistent with previous studies on the enzymatic polymerization of *p*-phenylphenol[12a] and 4-ethylphenol.[14] The coupling at the meta position however is unusual and may be due to the substitution of the aromatic-N═N at the para position of the phenol ring. This group is a strong electron donor that may serve to activate the meta position of phenol ring for coupling. The involvement of ortho as well as meta carbons in the coupling reaction may lead to a macromolecule with a dendritic structure.

FT-Raman. Additional structural and mechanistic information was provided by FT-Raman spectroscopy. The comparison of the FT-Raman spectra of the monomer and polymer shows that the C−H in-plane bending vibration of monosubstituted benzene, at 1006 cm^{-1} is relatively unchanged, while a decrease in the ring vibration band at 1191 cm^{-1} for para-disubstituted benzene[21] is observed in the polymer spectrum. These results are consistent with what was observed for both FTIR and NMR studies where it was shown that coupling occurs primarily on the phenolic aromatic ring.

FT-Raman spectroscopy provides further insight into the trans−cis isomerization during the polymerization reaction and the nature of the cis structure in the final polymer. The vibrations of the azo band (N═N) are strongly Raman active. Two Raman vibrations of the N═N are observed in the monomer spectrum. The strong band at 1420 cm^{-1} is due to the *trans*-4-phenylazophenol, and the weak band at 1472 cm^{-1} is due to the cis form.[21] As previously observed in the UV−vis absorption studies, dominance of the trans form over the cis is observed in the monomer. After polymerization, however, the vibrations of the azo groups become more complicated and two new Raman bands emerge in the polymer spectrum. The band at 1442 cm^{-1} is assigned to the vibration of trans N═N, and the band at 1491 cm^{-1} to the cis form.[21] It is known that the vibration frequencies and intensities of the azo band may significantly be affected by molecular conformation.[21] The N═N vibration spectrum of the polymer can be explained in light of increased cis content and shifts due to various ortho and meta substitution patterns on the phenol ring of the 4-phenylazophenol and other structural constraints.

Thermal Properties. Thermogravimetric analysis (TGA) of poly(4-phenylazophenol) was performed under nitrogen. Like other enzymatically synthesized polyphenols,[12a] poly(4-phenylazophenol) shows very good stability. There is less than 15% mass loss up to 300 °C and less than 40% to 600 °C. The differential scanning calorimetry (DSC) measurement shows that the polymer has a T_g around 115 °C. This measurement was carried out in nitrogen atmosphere with a scan rate of 10 °C/min. The samples were cooled at the same rate after the first scan and T_g value was taken to be the inflection position on the second scan trace. No other thermal transition is observed either in the cooling or heating cycles, confirming only a glassy state.

3.3. Optical Properties. *Photoisomerization*. Figure 5 shows the changes in the absorption spectra of 4-phenylazophenol and poly(4-phenylazophenol) in dioxane during irradiation by UV light at 360 nm. As shown, the absorption band at around 350 nm due to the $\pi-\pi^*$ transition of the trans form of the azo bond decreases gradually, while the absorption band at 440 nm due to the $n-\pi^*$ transition of the cis form of the azo bond increases with light irradiation time for both the monomer and the polymer.[19] Two apparent isosbestic points are seen at 300 and 405 nm in the monomer spectra (a), and at 275 and 410 nm in the polymer spectra (b), respectively. The photostationary state of 4-phenylazophenol attained here is similar to that previously observed in other solvents at low temperature. Gabor and co-workers[19d] have shown that the cis to trans thermal isomerization of 4-phenylazophenol is strongly solvent dependent. The slow thermal isomerization rate in dioxane makes it possible to record the photoisomerization process at room temperature as we described. The absorption change of the monomer at around 350 nm due to the trans form of the azo chromophore is more dramatic during photoexcitation than that of the polymer. An absorbance decrease of about 90% (from 2.25 to 0.3) for the monomer at 350 nm was observed, while only 60% (from 1.8 to 0.8) was measured for the polymer at 345 nm. The smaller absorption change suggests that some trans azo chromophores in the polymer may not be isomerized to the cis state by photoexcitation due to the constrained structure. Another interesting observation is that the rate of the photoinduced trans to cis isomerization of the monomer is significantly faster than that of the polymer. It takes ~120 s of total exposure to complete the photoinduced trans to cis isomerization for the polymer solution, while only 66 s of exposure is needed for the monomer under the same conditions.

The thermal relaxation processes of the monomer and the polymer after photoexcitation were also monitored at room temperature by UV−vis spectroscopy. The changes in absorption spectra of 4-phenylazophenol and poly(4-phenylazophenol) after photoexcitation with UV light at 360 nm are shown in Figure 6a and b, respectively, together with the spectra before photoexcitation. It was expected that in the relaxation process both, the monomer and the polymer will undergo a series of cis−trans isomerization. The molecules tend to relax back to the conformations that have the spectral features identical to those of the one before irradiation. The relaxation process for the monomer is significantly faster than the polymer. Further, it appears that for the polymer even after long-term relaxation (tens of hours) the original trans population may not be recovered. The same pattern of behavior was observed for polymer solutions in acetone and DMF with slightly different kinetics. However, for monomer solutions in acetone and DMF the cis−trans thermal isomerization is quite rapid, and nearly complete recovery of the trans state is achieved after photoexposure is stopped.[19d] The longer time needed for the relaxation process of the polymer in this case can be ascribed to the conformational

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(21) Lin-Vien, D.; Colthup, N. B.; Fateley, W. G.; Grasselli, J. G. *The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules*; Academic Press: San Diego, 1991.

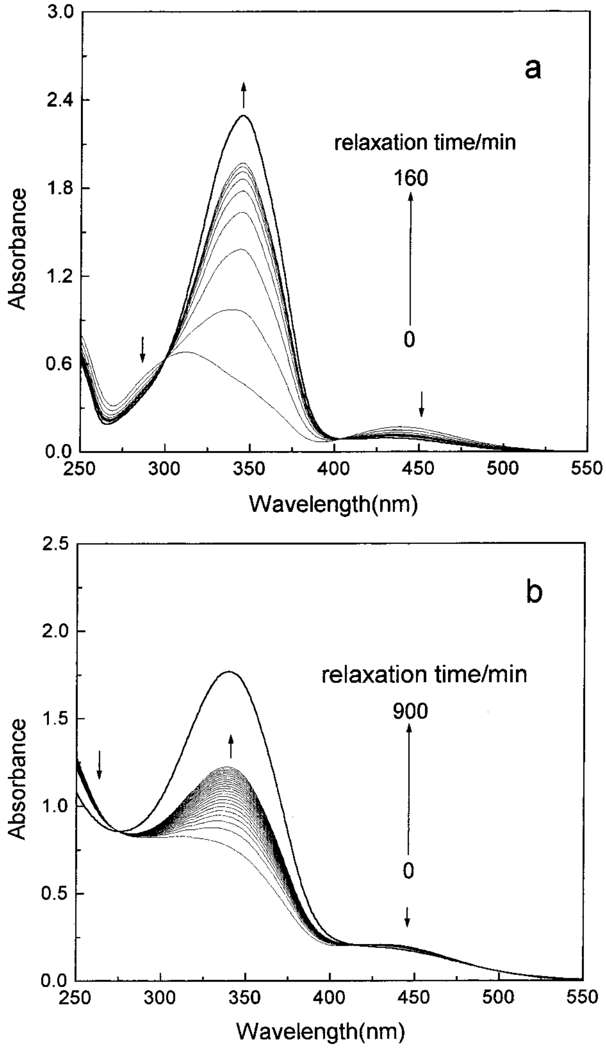

Figure 6. The absorption spectra of the relaxation process of (a) 4-phenylazophenol and (b) poly(4-phenylazophenol) after photoexcitation at 360 nm. The solution of the polymer (30 μg/mL) in dioxane was excited by UV light at 360 nm for 5 min and immediately after that the spectra were recorded every 20 min for the monomer solution, and 40 min for the polymer solution. The spectra of the monomer and polymer solutions before photoexcitation are also shown as the thick lines.

changes associated with the isomerization in a constrained geometry.[16a] The decrease of the photoisomerization and thermal isomerization rate further confirms that the azo chromophores in the polymer are strongly constrained compared to the monomer chromophore.

The photoisomerization of poly(4-phenylazophenol) in dioxane was also carried out by irradiation with UV light at 254 nm and Ar^+ laser at 488 nm (cis absorption bands). The changes in absorption spectra due to irradiation at 254 and 488 nm show similar trends as described above, but are much smaller (data not shown here). The increase at trans absorption band and decrease at cis absorption bands upon excitation at 254 nm previously reported in poly(diaminoazobenzene)[16a] were not observed in this case. The pump beams at 254 and 488 nm are still within the trans absorption envelope although significantly in the wings. The constrained cis states may not be accessible for cis to trans isomerization in the present case.

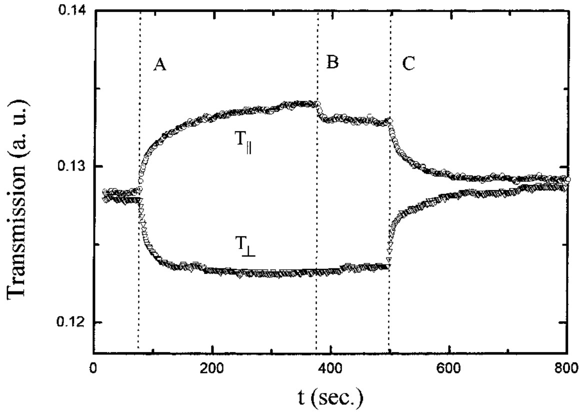

Figure 7. Time evolution of the transmission for light polarized parallel ($T_{\|}$) to and perpendicular ($T_{\perp}$) to the pump light polarization. Dotted lines marked by A, B, and C, respectively, indicate the moment of turning the pump laser on, of turning the pump laser off, and of starting of erasure with a circularly polarized light.

Photoinduced Absorption Dichroism. Figure 7 shows the measured time evolution of the transmission for probe light polarized parallel ($T_{\|}$) and perpendicular ($T_{\perp}$) to the pump light polarization in a polymer thin film. The moments of switching the pump laser on, of switching the pump laser off, and of starting of erasure with a circularly polarized laser are indicated by dotted lines marked A, B, and C, respectively. The photoinduced absorption variation is described in the context of the photoinduced trans–cis–trans isomerization. Upon excitation by a linearly polarized light, the azobenzene groups will undergo repeated trans–cis–trans isomerization until a fraction of the chromophores orient themselves in a plane perpendicular to the polarization of the pump light. Thus, the optical transmission for light polarized parallel to the pump light ($T_{\|}$) increases and for light polarized perpendicular to the pump light ($T_{\perp}$) decreases as shown.[22] After the pump laser is switched off (from B) the transmission for light polarized parallel to the pump light decreases only slightly. A detailed study shows that this decrease is composed of at least a fast and a slower decay component. The faster one is from the cis-to-trans isomerization. The slower one originates from thermal randomization of oriented azo-dye molecules. Study on other azobenzene-containing polymers recently also confirms the observed establishment of excess cis population when the solid polymer films are photoexcited and decay of cis population as photoexcitation is terminated.[23] The partial slower decay indicates that only a small part of oriented azo-dye molecules lose the induced orientation and restore to a random orientation and the rest of photooriented azo-dye molecules remain frozen. This is again explained in terms of the constrained nature of the azobenzene chromophores discussed earlier. For light having orthogonal polarization to the pump laser the transmission change is not remarkable after the pump laser is turned off. This interesting observation can be explained in light of the above discussion. The absorption changes caused by the

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(22) Todorov, L.; Nikolova, L.; Tomova, N. *Appl. Opt.* **1984**, *23*, 4309.

(23) (a) Sekkat, Z.; Dumont, M. *Synth. Met.* **1993**, *54*, 373. (b) Birabssov, R.; Landraud, N.; Galstyan, T. V.; Ritcey, A.; Bazuin, C. G. Rahem, T. *Appl. Opt.* **1998**, *37*, 8264.

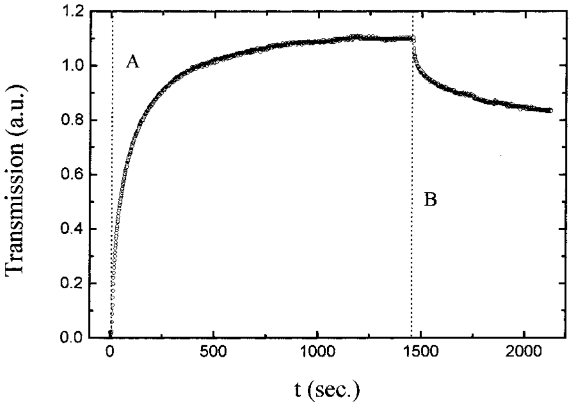

Figure 8. Kinetics of transmission of probe beam through crossed polarizers. Dotted lines marked by A and B indicate the moment of switching the pump laser on and off, respectively.

cis-to-trans isomerization and thermal randomization of oriented azo-dye are in the opposite direction for this polarization. The transmission behavior after stopping photoexcitation will depend on the speed and the amplitude of these two decay modes. For poly-azophenol used in our experiment these two processes appear to have comparable amplitudes in the initial stage of decay and cancel each other. This is the reason an obvious decay was not observed in the initial stages starting at B. A slower decay is observed in the long time scale. Chromophores that have been forced into an orthogonal alignment to the polarization of the pump beam are so constrained that their relaxation time constants are long.

When the sample is irradiated by a circularly polarized laser beam, the induced orientation is erased almost completely as shown by the last step (from C). Further, from Figure 7 it can be seen that the transmission for both polarized probe beams at stationary state of erasure is higher compared to the un-irradiated sample. This is due to the fact that the circularly polarized light induced orientation of an excess of azo-dye molecules in the direction of propagation of the laser beam. Such oriented azo chromophores do not absorb the probe beam (since the probe laser is antiparallel to the acting laser beam). Therefore, the transmission of the probe beam became slightly increased. A small amount of azobenzene chromophores may also be trapped in a long-lived cis state. The photoinduced orientation process shown in Figure 7 is reversible and can be repeated many times without the film showing any apparent fatigue. The steady-state magnitude of photoinduced dichroism was dependent on the intensity of the pump light. A transmission change of 4% was measured at pump light intensity of $I_a = 130$ mW/cm^2 (corresponding to Figure 7). This corresponds to an absorption coefficient change of 0.15 mm^{-1} for the sample used for linearly polarized light at 488 nm.

Photoinduced Birefringence. Figure 8 shows the kinetics of transmission of the probe laser beam at $\lambda = 633$ nm when the sample was illuminated by a 488 nm laser. (The pump beam was turned on at $t = 5$ s as indicated by the first dotted line marked A.) A partial orientation decay was observed after the pump light was turned off. (Turning off moment is indicated by the second dotted line marked B.) The shown time evolution demonstrates that the photoinduced orientation and decay process do not exhibit a single exponential characteristic. Exponential functions with two different time constants, one in tens of seconds, and the other in hundreds of seconds, fit the recording data well. It was experimentally found that the saturation value of the photoinduced birefringence and the time required to reach saturation birefringence are dependent on the intensity of the pump light. At intensity $I_a = 130$ mW/cm^2, evaluation of the measured data for a sample 0.26 μm thick yields a light induced birefringence $\Delta n = 0.03$.

4. Conclusions

A novel azopolymer, poly(4-pheneylazophenol), has been synthesized using HRP-catalyzed polymerization. The coupling reaction occurs at ortho as well as meta positions on the phenol ring of 4-phenylazophenol, accompanied by an interesting trans to cis isomerization of the azo chromophore. This results in the formation of a branched polyphenylene backbone with pendant azo functionalities on every repeat unit of the macromolecule, which may be difficult to synthesize by traditional chemical methods. Certain azo chromophores may be conformationally constrained in the shape-retentive macromolecule. These constraints of the azo chromophores in poly(azophenol) result in an interesting photoinduced isomerization behavior with long relaxation time. The photoinduced birefringence with long relaxation time indicates that this type of macrodye molecule is promising in volume holographic recording. This biologically derived polymer behaves like a “glassy macromolecular dye” with good solubility in most polar organic solvents, suggesting new opportunities with regard to the design and synthesis of processable, optically active macromolecular systems for photofabrication. A wide variety of other azo-functionalized phenols and anilines may be polymerized and copolymerized by this simple and versatile enzymatic approach, to generate a new class of photoactive polymers.

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Supporting Information Available: Discussion on the modeling of the polymer structure, Figure S1 (FTIR spectra of (a) monomer and (b) polymer of 4-phenylazophenol), Figure S2 (the computer modeled structure of poly(4-phenylazophenol) with 15 repeat units), and Figure S3 (FT-Raman Spectra of (a) monomer and (b) polymer of 4-phenylazophenol in the powder form). This material is available free of charge via the Internet at http://pubs.acs.org.

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