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POLYMERIZATION OF WATER-SOLUBLE CONDUCTIVE POLYPHENOL USING HORSERADISH PEROXIDASE

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Dedicated to the memory of Professor Sukant K. Tripathy.

ABSTRACT

Template assisted enzymatic polymerization of phenol has been carried out in aqueous media at room temperature. The templates used to facilitate this reaction, were polystyrene sulfonate, lignin sulfonate and dodecyl benzene sulfonate. The enzyme, horseradish peroxidase, proved to be an active catalyst for the polymerization, yielding a phenolic polymer that is permanently complexed with the template used. These polyphenol complexes are high molecular weight, soluble in water and/or mixed organic solvents and show good thermal stability. Under certain conditions, physical SPS-polyphenol gels can be formed in water that have high ionic conductivity. UV-vis, FTIR and electroabsorption spectroscopy show the presence of significant backbone conjugation and NLO properties. This novel enzymatic approach is a simple, inexpen-

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sive and environmentally friendly way to prepare processable polyphenolic materials with interesting electrical and optical properties.

Key Words: Polyphenol; Horseradish peroxidase; Ionic conductivity; Enzyme polymerization

INTRODUCTION

Phenol-formaldehyde resins are widely used in high-performance engineering plastics since these polymers have excellent chemical and thermal stability and mechanical toughness [1]. These resins find numerous applications in such areas as wood composites, electrical devices, wiring enamels, abrasives, friction materials, and brake linings [2]. However, concern about the continued use of phenol-formaldehyde resins due to the high toxicity of formaldehyde, has triggered investigations into alternative routes for their synthesis [3]. There has been much interest in enzymatic polymerization as an environmentally friendly approach to polyphenol synthesis. To date however, these enzymatic reactions in aqueous media formed only phenolic dimers and trimers, which immediately precipitated out of solution, and formation of high molecular weight polymers was not possible [4].

Recently, enzymatic synthesis of polyaromatics has been extensively studied. Phenols and alkyl phenols were oxidatively polymerized by horseradish peroxidase (HRP) in organic solvents or at the air-water interface of a Langmuir trough to produce interesting polymeric materials, mainly consisting of a mixture of phenylene and oxyphenylene units [5-8]. The resulting polymers exhibited relatively high thermal stability. This process can be an alternative method for the production of conventional resins (novolak and resol resins), which involves the use of toxic formaldehyde. However, the use of solvents during polymerization in these reactions and the insolubility of the resultant high molecular weight polymers have still hindered any substantial industrial applicability.

We have recently reported on the enzymatic template polymerization of aniline using a template, such as polystyrene sulfonated (SPS) to facilitate the preferred coupling of the aniline monomers and to dope the final polyaniline/template complex to the conducting form [9-10]. The resulting high molecular weight polyaniline/SPS complex showed good electrical conductivity and water solubility for facile processability. Using a similar procedure in the present work, we have demonstrated that HRP catalyzed template polymerization of phenol results in the formation of a new class of high molecular weight polyphenol complexes that are soluble in water and/or organic-water mixtures and depending on the conditions of the reactions, can form physical gels with high ionic conductivity. Characterization of these polyphenols has been carried out using UV-vis, FTIR, electroabsorption spectroscopy, DSC, TGA, light scattering and ionic conductivity and the results will be discussed.

EXPERIMENTAL

Horseradish peroxidase (EC 1.11.1.7) was purchased from Sigma Chemical Co. (St. Louis, MO) as a salt free powder. The specific activity of the enzyme was 240 purpurogallin units/mg solid. Phenol, polystyrene sulfonated (SPS), dodecyl benzene sulfonate (DBSA), hydrogen peroxide (30% solution), phosphate buffer, and all the solvents (reagent grade) were purchased from Aldrich (Milwaukee, WI) and used as received. Lignin sulfonate (Lignosol SFX-65) was obtained from Lignotech USA (Rothschild, WI). A typical enzymatic polymerization reaction consisted of a solution of phenol (71.3 mM) with an equimolar concentration of SPS, (with respect to the repeat unit) in 10 mL of aqueous phosphate buffer (10 mM, pH 7.0). Hydrogen peroxide (71.3 mM) solution was added dropwise. The concentration of horseradish peroxidase (HRP) was 0.1-0.15 mg/mL. The reactions were carried out at room temperature and the final products were dialyzed using centricon concentrators (10,000 MW cut off, Amicon Inc., Beverly, MA). The samples were then dried under vacuum at 50°C until further analysis. The yield was typically 95% or higher. Control samples, using denatured enzyme, were prepared following a similar procedure. The enzyme was denatured in buffered water at 100°C for 30 minutes.

Spectral characterization was performed with a Perkin-Elmer Lambda-9-UV-Vis-Near IR spectrophotometer (Norwalk, CT). FTIR spectra, of the samples deposited on ZnSe, were obtained from a Perkin Elmer FTIR 1720X. Thermogravimetric analysis (TGA) and differential scanning calorimetric (DSC) analysis was conducted using a TA instrument 2950 and a DSC instrument 2910 (New Castle, DE), respectively. The TGA and the DSC were performed under nitrogen at a heating rate of 10°C/min.

Static light scattering (SLS) measurements of SPS, SPS-phenol (prior to polymerization) and SPS-polyphenol (after enzymatic polymerization) were performed using a Brookhaven instrument. A stock solution of SPS was prepared by dissolving an appropriate amount of polymer in 0.01 M filtered sodium phosphate aqueous solution buffered at pH 7.0. The stock solution was sonicated for one hour at 25°C and then re-dialyzed for 24 hours and finally filtered multiple times using a 0.45 μm filter. A similar procedure was followed for SPS-phenol and SPS-polyphenol. However SPS-polyphenol was dissolved in 50:50 DMSO/water instead of pure water. Subsequent concentrations required for the measurements were achieved by diluting the stock solution, using filtered buffer, and adding directly into the scattering cell. A similar procedure was followed for the DBSA/polyphenol. All the measurements were made in solutions where NaCl was present. The excitation source, a linearly polarized 35 mW He-Ne laser (Melles-Griot, Model No 05-LHP-927) operating at 632.8 nm, was housed on the goniometer fixed arm. Finally the data was analyzed using Zimm-plot software supplied by Brookhaven Instruments (Vers. 2.04).

The ionic conductivity of all solid polyphenols, synthesized with HRP in the presence of lithium trifluoromethanesulfonate (0.128M Alfa Aesar Word Hill,

MA), was measured using a dielectric analyzer (DEA TA instrument 2970, New Castle, DE). Typically, a water solution of the polyphenol complex was deposited and dried onto the DEA probe disc. An electrode chamber (EC) was used to measure the ionic conductivity of the polyphenol in the gel state. The measurements were made using a Hewlett-Packard 4284A LCR meter (Palo Alto, CA).

Electro-absorption spectroscopy was carried out on a high molecular weight SPS-polyphenol film. The film was spin coated onto an indium tin oxide (ITO) glass substrate and a layer of aluminum was evaporated as the top electrode. A sinusoidal electric field (f =500Hz, 5V rms) was applied to the sample. A light beam, from a tungsten lamp passing via a monochromator, was incident normally on the sample. The electro-absorption signal ΔI, which is defined as the change in the output intensity I, was detected by a lock-in amplifier (Stanford Research System SR830) set at twice the electrical modulation frequency (2f). The output intensity, I, (without the electric field) was measured by using a chopper. A computer was used to synchronize the wavelength change of the monochromator and the data reading for the lock-in amplifier.

RESULTS AND DISCUSSION

The UV-Vis spectra for the monomer and the polymer formed by the DBSA-phenol system are shown in Figure 1. The presence of a large, broad absorption

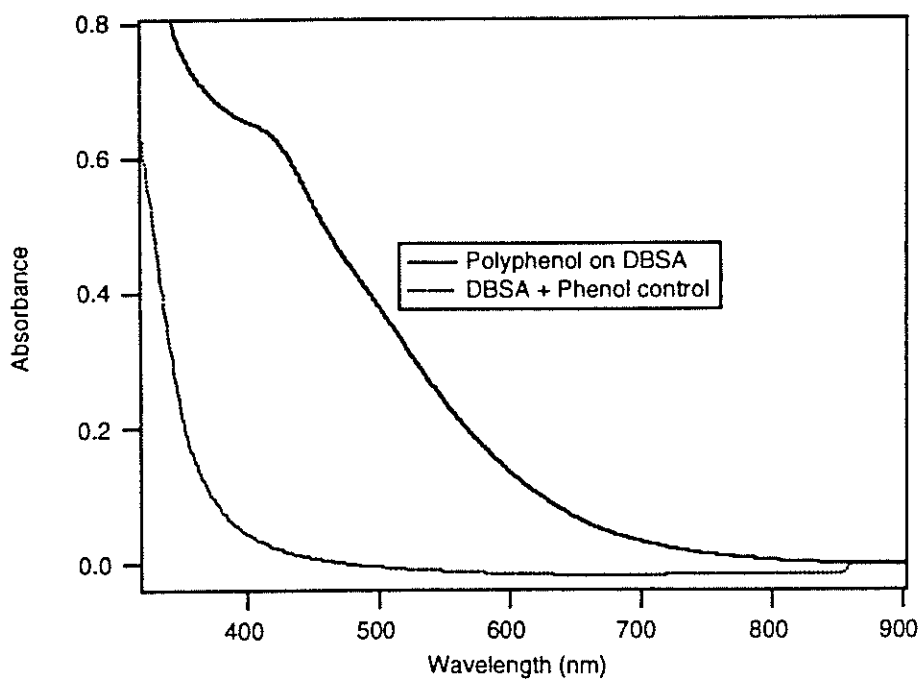

Figure 1. UV-Vis of DBSA-phenol (control) and of DBSA-polyphenol. Similar results were found for templates such as SPS and LGS.

tail for the polymer spectrum from 300-800 nm, which is absent in the control sample, indicates an extended degree of conjugation in the polymer [7]. In a parallel experiment, different aliquots of H_2O_2 were injected into the reaction media. The UV-Vis spectra for the final products were measured and are shown in Figure 2. These spectra for SPS/polyphenol show the presence of a higher degree of conjugation with increasing amounts (100 μL and 200 μL) of H_2O_2. In the latter cases, gel formation in the aqueous solutions was also observed. The gels showed thermal stability over a temperature range of 50-90°C and did not show any phase transition triggered by temperature, pH or salt concentration. Beyond 90°C, solvent evaporation prevents an accurate measurement of the gel stability. Moreover, this physically crosslinked gel, after dialysis and water evaporation, was not soluble in water. The dried polymer gel however was soluble in water/DMSO solution (ratio 50:50). It is believed that the nature of the crosslinking in the gels is most likely due to hydrogen bonding and physical entanglements between the SPS and the polyphenol.

Similar UV-Vis spectra were obtained for the LGS-polyphenol and the DBSA-polyphenol. Each of these polymers was deposited on quartz slides for spectral measurements and in both cases, the presence of conjugation in the polymeric chain backbone was observed. However, gel formation was not observed for any of these alternative templates.

FTIR spectra were measured for each of the polymer systems and are shown in Figure 3. A significant shift to lower frequency was observed for the polyphe-

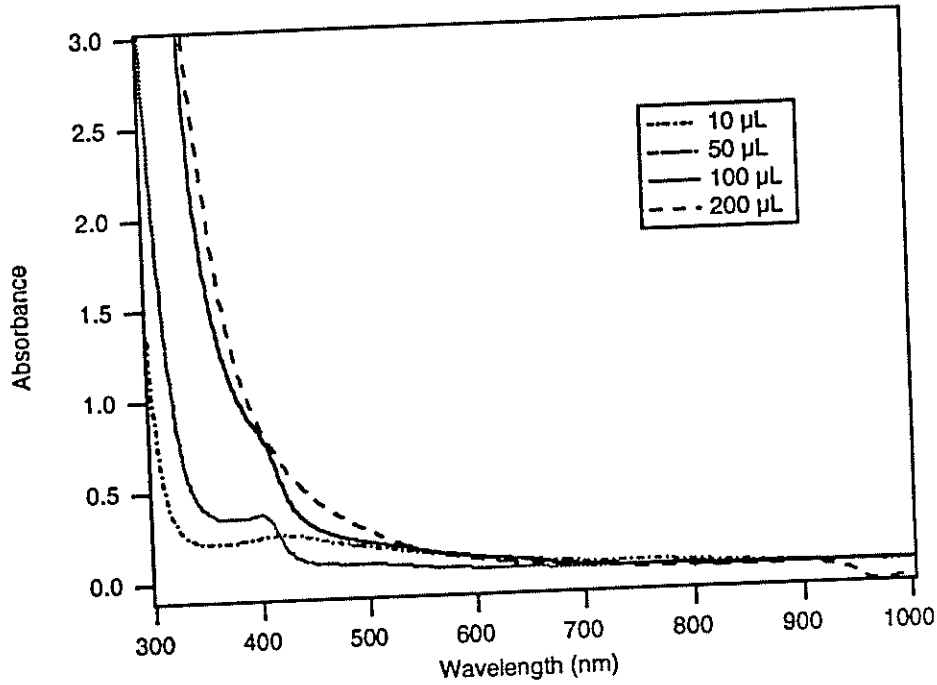

Figure 2. UV-Vis of polyphenol with SPS synthesized with different concentrations of hydrogen peroxide.

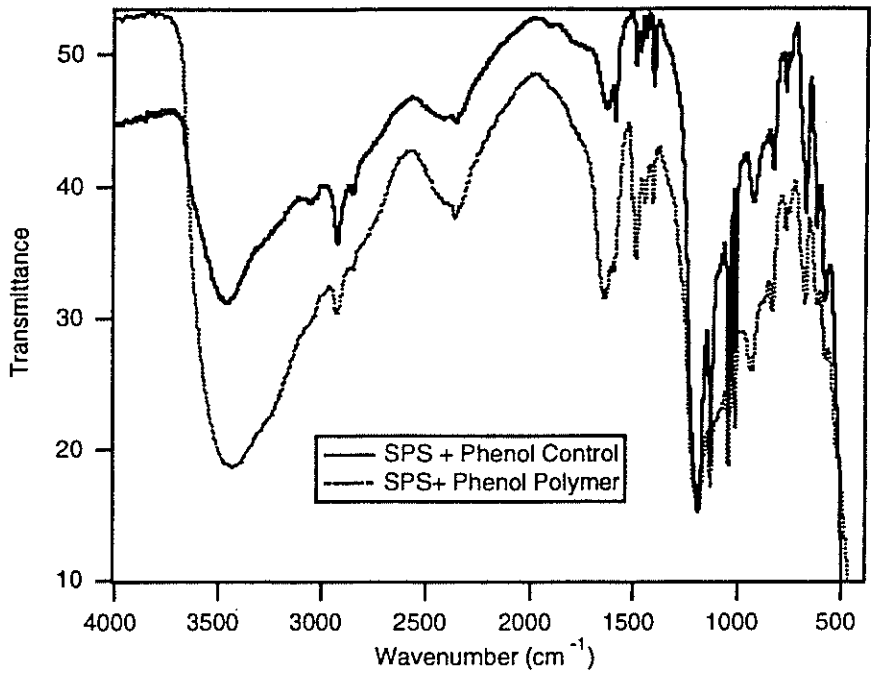

Figure 3. FTIR spectra of SPS/phenol control and polyphenol with SPS after enzymatic reaction. Similar results were found for templates such as DBSA and LGS.

nols in the OH stretch region (3000-3500 cm^{-1}) with respect to the monomer. This shift is believed to be due to stronger hydrogen bonding in the polymer complex that is formed during the enzymatic reaction. Another important feature noted in the spectra is the emergence of a peak in the 1550-1690 cm^{-1} region, which is attributed to the presence of conjugated moieties in the complex [11]. Similar results were observed for all systems studied.

Thermal properties for the polymers were determined by TGA and the thermograms of the SPS/polyphenol are given in Figure 4. The TGA analysis indicated that a significant amount of material (89%) remains after heating the polymer to 400°C. A significant degradation was first observed at 420°C where it is believed the SPS begins to degrade. The polymer complex retained 78% of its mass residue at 600°C. Thermal analysis carried out on the polyphenol/DBSA and polyphenol/LGS showed the final residue retained 61% and 80% of the mass at 600°C, respectively. The lower thermal stability for the polyphenol/DBSA is attributed to the low molecular weight of the surfactant DBSA, in comparison the other high molecular weight templates. These results were very similar to previous thermograms obtained from polymers enzymatically synthesized in organic media [6]. The chemical and physical properties of these residual materials have not been studied. DSC of all samples did not show any Tg or Tm before onset of degradation.

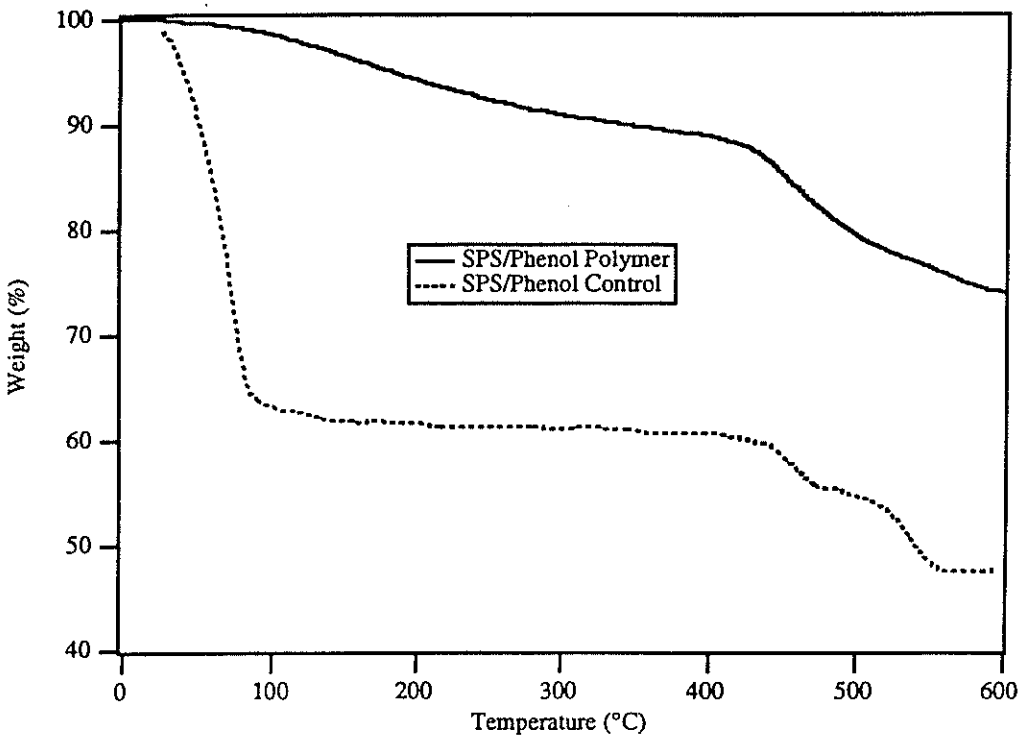

Figure 4. TGA of SPS/phenol before and after enzymatic polymerization.

SLS measurements on an SPS (only) control sample gave a molecular weight of 1.08×10^6 g/mol, whereas the sample of SPS with phenol monomer prior to polymerization gave a molecular weight of 1.16×10^6 g/mol. An increase in radius of gyration was observed from 81 nm to 87 nm between SPS and the SPS-phenol (before polymerization), respectively. In comparison, the enzymatically polymerized SPS-polyphenol showed an increase in molecular weight to 1.29×10^7 g/mol and a decrease in radius of gyration to 56 nm (Table 1). This significant increase in molecular weight upon polymerization is most likely due to both polymerization of phenol in the presence of the SPS template and aggregation and entanglement of some of the SPS polymer chains as the complex forms.

Table 1. SLS Data for Polyphenols

System (conc. Of H_2O_2)	Mw (g/mol)	Rg (nm)	A_2 [(cm^{3}mol)/g^2)]	FH
SPS	$1.08 * 10^6$	81.7	$2.98 * 10^{-3}$	0.47
SPS+Phenol (control)	$1.16 * 10^6$	86.7	$2.53 * 10^{-3}$	0.47
SPS+Phenol (10μL)	$3.60 * 10^6$	38.0	$1.40 * 10^{-4}$	0.49
SPS+Phenol (100μL)	$1.13 * 10^7$	56.1	$7.83 * 10^{-4}$	0.47
DBSA+Phenol (100μL)	$6.07 * 10^6$	47.1	$3.16 * 10^{-4}$	0.49

It is important to note here that strong hydrogen bonding is also believed to be occurring in each of these systems, as supported by the FTIR data. The significant decrease in the radius of gyration for the polymerized complex further supports the presence of strong hydrogen bonding in the final polymer complexes.

Although the molecular weight of the SPS-polyphenol complex is as high as 10 million daltons, it is not possible to differentiate the molecular weight of the polyphenol from that of the entire complex. The second virial coefficient, which describes the polymer-polymer interaction, was determined to be 2.98×10^{-3}, 2.41×10^{-3} and 1.26×10^{-3} for SPS, SPS-phenol and SPS-polyphenol, respectively. The Flory-Huggin's interaction parameter is 0.472, 0.466, and 0.485 for the SPS, SPS-phenol and SPS-polyphenol respectively and the 'χ' value is below 0.5 for all three cases. This indicates that the solvent is good for each of the polymers [13].

SLS is known to have poor resolution for low molecular weight compounds, consequently, the molecular weights of the DBSA/phenol system were not measurable.

However, the molecular weight of the DBSA/polyphenol could be measured by SLS and was found to be 3.5×10^6 g/mol. This high molecular weight is not attributed to molecular aggregation of the high molecular weight template such as with the SPS system, but to possible aggregation of the polyphenol with the micelles.

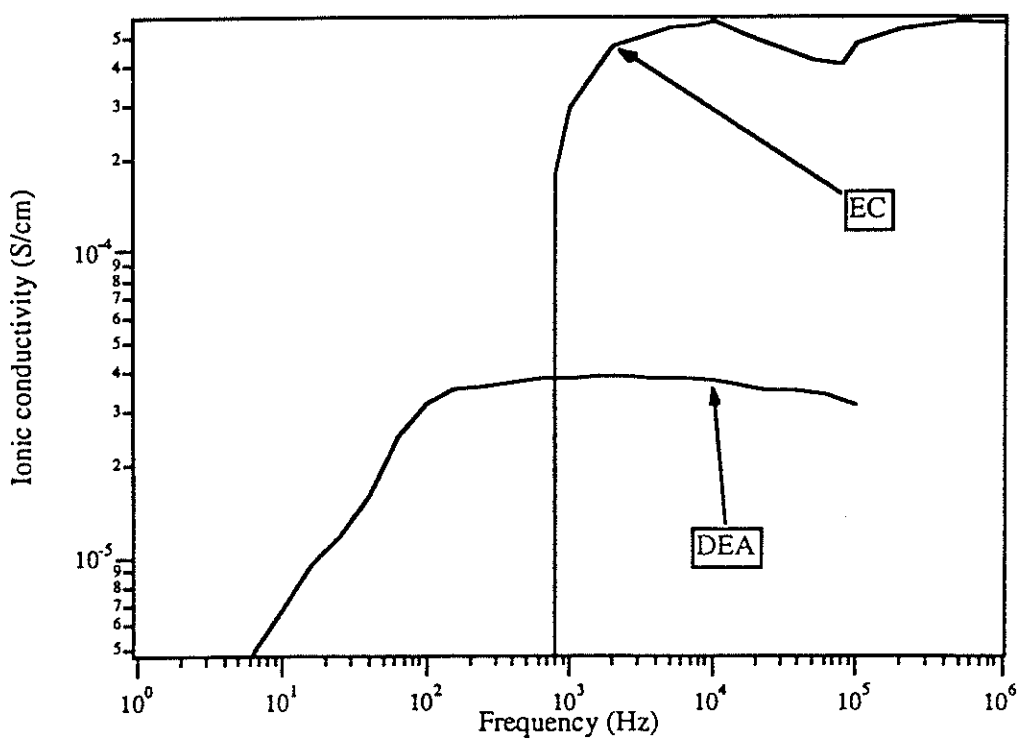

Figure 5. DEA and EC measurements for the SPS/polyphenol complex.

The ionic conductivity values measured with DEA and EC are reported in Figure 5. Conductivity in the 10^{-5} S/cm range was observed for the solid film, and a maximum of 3.9×10^{-5} S/cm was found at 398 Hz. The ionic conductivity for the SPS-polyphenol complex in the gel form was measured in the range of 10^{-4} S/cm with a peak of 5.5×10^{-4} S/cm at 8×10^{5} Hz.

The $\chi^{(3)}$ value, obtained by electro-absorption, for the SPS-polyphenol system, was 1.0×10^{-12} esu. However, enzymatically polymerized phenol on SPS shows an interesting electro-absorption behavior (Figure 6). The film has modest absorption (optical density < 0.003) from 300 nm to 860 nm. The electro-absorption has a broad response behavior throughout the visible range [14-16]. Usually, electro-absorption only exists near the linear absorption region and the width of the electro-absorption peak is close to the width of the linear absorption. The linear absorption shows no well defined peaks in the visible area. We therefore, attribute this behavior to either the electric field induced transitions or to the presence of a distribution of conjugation lengths in the polyphenol [17].

CONCLUSION

Phenol was oxidatively polymerized through enzymatic catalysis with three different template systems, SPS, LGS, and DBSA to produce high molecular weight polyphenol complexes. The final polymer complexes are soluble in water

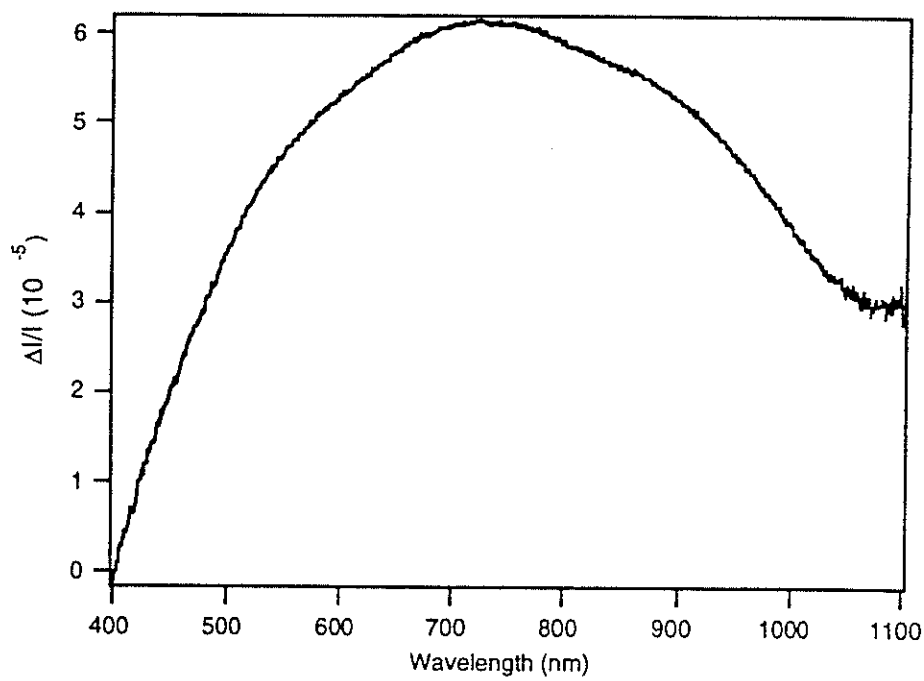

Figure 6. Electro-absorbance vs. wavelength of SPS/polyphenol.

and/or organic solvent mixtures. Furthermore, SPS-polyphenol, exhibited the highest molecular weight, ranging in the millions, and formed a physical gel in water, which has good thermal stability in the range of 60–90°C. This procedure alleviates impediments to processing found with similar polyphenols formed in bulk organic solvents, synthesized either enzymatically or chemically. Using this approach, polymeric materials with novel electronic and optical properties can be synthesized for a wide variety of industrial applications.

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