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Effect of electron beam on thermal, morphological and antioxidant properties of kraft lignin

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ABSTRACT

Thermal, morphological and antioxidant properties of lignin irradiated with electron beam (EB) of doses 30, 60 and 90 kGy have been investigated by Electron spin resonance (ESR), Fourier transform infrared spectroscopy, Differential scanning calorimetry, Scanning electron microscope and Spectrophotometer techniques. ESR studies confirm the presence of poly-conjugated radicals in unirradiated lignin; whereas irradiated lignin posses both poly-conjugated and peroxy radicals. The peroxy radicals decay near the glass transition point on thermal heating while poly-conjugated radicals are stable even up to a temperature of 450K. Presence of conjugated structures is confirmed by the presence of 1604 cm^{-1} absorption band whose concentration increases following radiation dose. Up to a radiation dose of 60 kGy the fall in glass transition temperature (T_g) is very slow, while at 90 kGy T_g decreased abruptly. The decrease in T_g is assigned to be due to intermolecular chain interactions or plasticization effect. Granular structure of lignin is found to be effected by EB irradiation. Cracks were generated on lignin granules on EB irradiation. Due to increase in poly-conjugation, amorphous nature and granular cracks the antioxidant property is observed to increase. The current research trends in lignin materials include modification of lignin, fabrication of biodegradable thermoset/thermoplastic and use of lignin as stabilizers and dispersants. Copyright © 2015 VBRI Press.

Keywords: Electron beam; glass transition temperature; poly-conjugation; antioxidant activity.

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Introduction

Lignin, a natural polymer found in the cell walls of plants along with two major components, cellulose and hemicellulose. Lignin comprises 28% of the mass of wood-like plants. In wood, lignin consists of a family of phenyl-propane type polymers connected in a variety of structures. Lignin has a complex chemical functionality of carboxyl groups and alcohols **[1]**. Due to its non-toxic nature, renewability and extreme versatility, lignin applications have been increasing in industry. As a neutralizer or inhibitor, lignin is an effective free radical scavenger, which can reduce oxygen radicals and stabilize oxidation reactions **[2-3]**.

Lignin aromatic structures suggest that it is suited as a radical scavenger in existing commodity thermoplastics. Free radicals formed in plastic by irradiation with UV light are the major causes of quick degradation of plastics. Lignin can be substituted for more expensive UV stabilizers in polyethylene for minor effects on mechanical properties **[4]**.

Barca et al. **[5]** have reported that phenolic model compounds recognized as indigenous to the lignin structure possess significant antioxidant property. Structures with conjugated double bonds in propyl side chains have higher antioxidant activities compared to structures with saturated side chains or isolated double bonds. Lignin also contains ortho- distributed phenolic groups.

Different types of lignin samples were prepared from hybrid poplar and their antioxidant properties were determined using DPPH radical scavenging test. Results indicate that lignins with more phenolic groups and less aliphatic hydroxyl groups show high antioxidant activity. Lignin with low molecular weight and narrow polydispersity also poses high antioxidant property. Functional groups and molecular weight of lignin are influenced by processing conditions and ultimately cause a change in the antioxidant property **[6]**. The effect of oxidant dosage, oxidation temperature and time of degradation of soda lignin by microwave irradiation was investigated **[7]**. These studies revealed that microwave irradiation cause cleavage of bonds and partial degradation of aromatic rings. Compared to conventional thermo-oxidative degradation of lignin, the microwave irradiation efficiency facilitates degradation of lignin. Due to irradiation the reactivity of the lignin is enhanced. Lignin compound are known to undergo various radical initiated reactions on irradiation either in isolate state or with a composite matrix **[8]**.

Radiation induced modification of biopolymers is a feasible method to produce byproducts or to tailor their usage. From the molecular point of view, irradiation was shown to generate free radicals on polymers and then induce various reactions like scission or crosslinking depending upon both the environmental conditions and the radiation dosage. Electron beam (EB) irradiation is a promising green tool in this respect and optimized/alternative methods to utilize and to improve lignin as a precursor in a cost effective manner. The radioactive degradation of kraft lignin has been the subject of numerous investigations.

Starch films were prepared by incorporating lignin up to 30% wt. and treated with EB to a dose of 400 kGy has increased the water resistance at the surface of the film **[9]**. The increase in water resistance of the film is attributed to an increase in intermolecular covalent bonding at the surface due to EB exposure. It is most likely that the hydroxyl groups on the lignin molecule serve as the initiators of such reactions.

EB irradiation effects on lignin have received less attention in literature. Electron beam treatment of lignin compounds is reported to lead to several radical initiated reactions. These reactions leave the compound open to interaction with number of resins systems which have the capability to change the thermal, morphological properties and biodegradability of resulting composite. Therefore it is necessarily important to investigate these properties. Further effect of EB irradiation on antioxidant and radical scavenging properties of lignin is also studied. This type of work is one of the steps in modifying renewable lignin so that it is more consistent and reliable antioxidant for further applications.

Experimental

Materials

Kraft lignin in the form of powder was purchased from *Sigma Aldrich*, USA with an average molecular weight (M_w) of 10,000 and 4% sulfur impurity. Chemicals like 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Methanol, L-ascorbic acid, trichloroacetic acid (TCA), potassium ferricyanide and other reagents of 99.0% purity were purchased from HiMedia Laboratories, India to study the antioxidant activities.

Irradiation

Three lignin sample packets were prepared for electron beam irradiation with radiation doses i.e. 30, 60 and 90 kGy. The industrial electron beam accelerator is ILU-6 type, pulse linear accelerator from Board of Radiation and Isotope Technology, BARC, INDIA and can be operated up to energy of 2MeV and 20 kW power. The beam can be uniformly scanned over a length, thus constituting irradiation area of 10×100 cm. In the present studies, the dose rate chosen is 5 kGy/pass. The current of the electron pulse is 250mA and pulse frequency is 10 Hz. The speed of the roller is 15 Hz (3 cm/ sec). All samples were irradiated in the presence of air at room temperature (RT).

Methods

Electron spin resonance (ESR) spectra were recorded on JES-FA200 ESR spectrometer, JOEL at an operating frequency of 9.4 GHz (X-band) and 100 kHz modulation frequency at RT. The spectrometer was equipped with a variable temperature facility, so that irradiated samples at RT could be annealed to higher temperatures to access thermal stabilities. Fourier transform infrared spectrometer (FTIR) spectra of the sample pellet prepared by adding KBr were recorded on Perkin Elmer spectrometer that is used to characterize the chemical structure of lignin before and after irradiation. Differential scanning calorimeter (DSC) measurements were performed on a DSC-TA Q10 model calorimeter to study glass transition behavior. Approximately 4 mg of samples were sealed into the aluminum crucibles and heated from 50 to 300^{o}C at a heating rate of 20^{o}C/min in flushing Nitrogen gas. The thermograms were recorded at second heating in order to remove the thermal history of the sample. The X-ray diffractograms were recorded on Bruker D8 Advance X-ray diffractometer (XRD). The morphology was then examined under scanning electron microscope (SEM) TESCAN VEGA 3 LMU. The micrographs were captured with a magnification of 750 and 1000×.

Lignin antioxidant activity was determined by using DPPH scavenging activity **[3]**. Briefly, 0.004% w/v of DPPH radical solution was prepared in methanol and then 900 µl of this solution was mixed with 100µl of sample solution containing 50–300µg/ml of lignin. The UV absorbance of the prepared solution was measured at 517 nm (UV-2550; Shimadzu, Kyoto, Japan) on after 30 min of incubation. Methanol (95%), DPPH solution and L-ascorbic acid were used as blank, reference and control respectively.

$$\text{Scavenging activity (\%)} = \frac{(A^{\circ} - A)}{A^{\circ}} \times 100 \qquad (1)$$

Where, A^o is the absorbance of the control reaction (containing all reagents except the test sample) and A is the absorbance of the test sample.

The reducing power (RP) of lignin was determined by the method followed by Qi Lu et al **[10]** with slight modification. Sample solution was mixed with 2.5 ml of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% ($K_3Fe(CN)_6$) at 50°C, incubating for 20 min. To the solution, 2.5 ml of TCA was added and centrifuged for 10 min at 3000 g. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and 0.1% $FeCl_3$ (0.5 ml) to measure UV spectrophotometer absorbance at 700 nm. IC50 value (the concentration of the extracts required to scavenge 50% of radicals) was calculated for different radiation doses of lignin**.** For each measurement, three replicates of samples were tested and mean ± standard deviation values were reported.

Results and discussion

ESR studies

Dose dependent studies:

ESR spectrum of unirradiated lignin is shown in **Fig. 1**. The spectrum is a weak singlet with a g-value of 2.0035 and it is assigned to poly-conjugated radicals **[11-12]**.

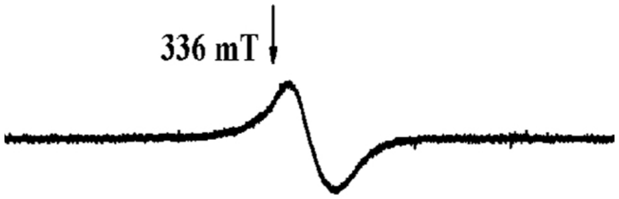

Fig. 1. ESR spectrum of unirradiated lignin.

ESR spectra of lignin irradiated with EB to different doses are shown in **Fig. 2**. The g-values are in between 2.002 to 2.004.

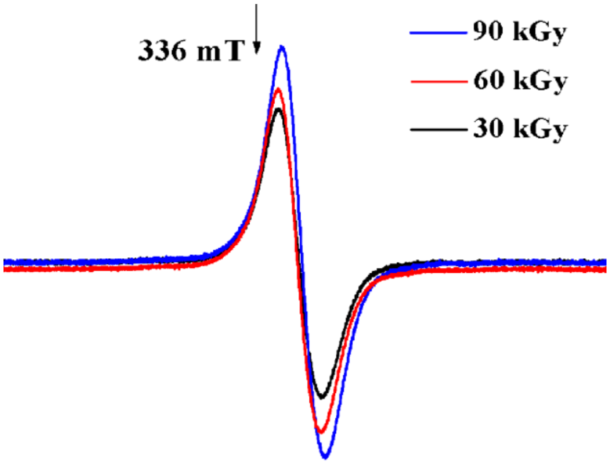

Fig. 2. ESR spectra of electron beam treated lignin of doses 30, 60 and 90 kGy.

The spectrum of irradiated lignin is assigned to two types of radicals (i) poly-conjugated radical (ii) peroxy radical. Lignin contains phenols, alcohol, glycosidic and acetal oxygen groups. When lignin is irradiated with electron beam dissociation of these groups takes place producing primary radicals. As the glass transition temperature (T_g) of the lignin is around 403K **[13, 14]**, the radicals are supposed to be trapped in the frozen lignin matrix at RT. Therefore very few radicals are accessible to atmospheric oxygen and get converted to as peroxy radicals. Some of the primary free radicals initiate secondary process like dehydration and water elimination process leading to the formation of poly-conjugated structures **[15]**.

Radicals produced on EB irradiation of lignin gives ESR singlet spectrum with narrow line width. The peroxy radical in lignin is characterized by its g-value (g = 2.0033); while poly-conjugated radical have g-value of 2.0030 **[12]**. Kuzina et al **[12]** have used D-band ESR spectroscopy to resolve the singlets and detected the existence of poly-conjugated radicals in lignin. In the present studies, the authors have recorded temperature dependent ESR spectra to resolve the singlets as described in the later section. Therefore overall ESR spectrum of irradiated lignin is a superposition of singlets arising due to the poly-conjugated radicals and peroxy radicals. There is a gradual increase in intensity of ESR signal with the increase of radiation dose as shown in **Fig. 3**. Formation of more number of free radicals increases of radiation dose might have occurred in lignin as observed for many polymeric systems **[16]**.

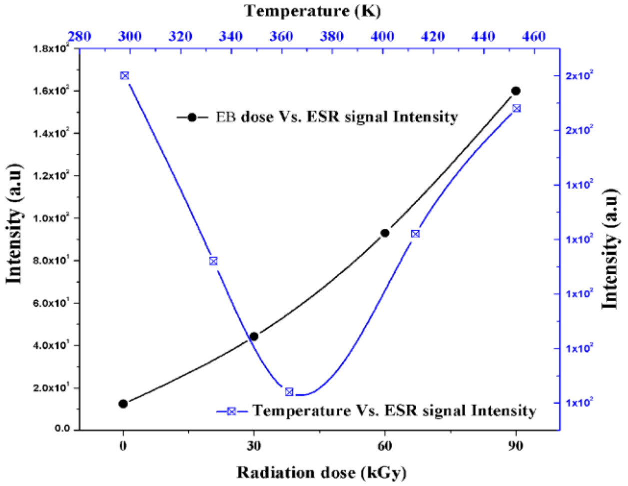

Fig. 3. Variation of ESR intensity with (●) electron beam dose and (☒) temperature of lignin.

Temperature dependent studies

To access the thermal stability of radicals produced on EB irradiation of lignin, ESR spectra are recorded at different temperatures i.e. at 300K (RT), 333K, 363K, 413K and 453K are as shown in **Fig. 4**. All the spectra are singlets with slight change in g-values and poses narrow line widths as reported in literature **[12]**. On thermal heating, the broad singlet is stable up to 333K, while a doublet like structure is appeared at 363K. Beyond 363K a singlet was again observed and it continued to appear even up to a temperature of 450K. The RT spectrum is assigned to be due to both peroxy radicals and poly-conjugated radicals. Both the radicals give component singlet spectra with little variation in g-value. As the temperature is increased, the radicals gain thermal energy and begin to interact with themselves or with other radicals or other polymeric chains. When the temperature of observation is nearer to the glass transition temperature, the radical reactions are optimum **[17]**. It is evident that the doublet like structure appeared around 363K is disappeared at 403K i.e. around T_g of lignin. The decayed component is assigned to peroxy radicals which usually decay at this temperature. Since poly-conjugated radicals are thermally more stable **[18]** the singlet appearing at 450K corresponds to poly-conjugated radicals. Variation of ESR intensity against temperature is shown in **Fig. 3**. Non-linearity of the radical decay curve in **Fig. 3** is an indication for heterogeneous thermal stability of free radicals.

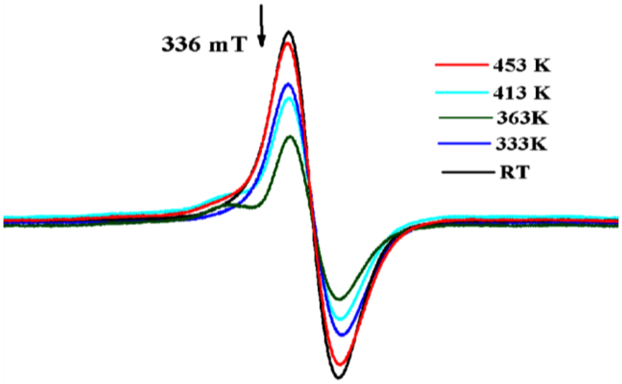

Fig. 4. ESR spectra of irradiated lignin at different temperatures RT, 333K, 363K, 413K and 453K.

FTIR studies

Fig. 5 shows the FTIR spectra of unirradiated and irradiated lignin. The spectrum poses various absorption bands which characterize different chemical groups in lignin.

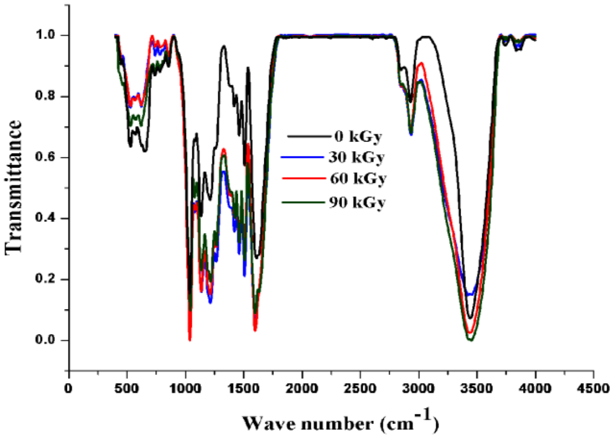

Fig. 5. FTIR spectra of unirradiated and electron beam irradiated lignin of doses 30, 60 and 90 kGy.

A wide absorption band focused at 3440 cm^{-1} is attributed to the stretching vibration of hydroxyl group in phenolic structure **[19]**. The absorption bands appearing at 2928-2937 cm^{-1}, 1715-1712 cm^{-1}, 1605-1598 cm^{-1}, 1506 cm^{-1} and 1328 cm^{-1} are attributed to the CH stretching in aromatic, methylene/methoxy/methyl groups, conjugated carbonyl (C=O) groups, ring stretching of aromatic groups, and bending vibrations of OH groups respectively **[19]**. The absorption band at 1604cm^{-1} is assigned to conjugated structures **[20-21]**. On irradiation, intensity of the 1604cm^{-1} absorption band is increased with increase of radiation dose. The FTIR study confirms the enhancement in conjugation of lignin on irradiation. These studies suggest that cleavage of phenoxy groups on irradiation and subsequently led to the formation of conjugated groups in lignin.

DSC studies

Glass transition temperature is one of the important factors in processing of polymers **[22]**. The glass transition behavior of lignin is studied by differential scanning calorimetry. DSC thermograms of unirradiated and irradiated lignin are shown in **Fig. 6**. Unirradiated lignin exhibit a first order transition around 130°C (403K) corresponding to the T_g of lignin. Unlike the other polymers, determination of T_g in lignin is very difficult due to its complex structure and the changes it undergoes on thermal heating **[14, 23]**. The fall in the specific heat at glass transition point is gradual for lignin instead of a sharp fall as observed for other polymers **[4]**. Due to this gradual fall in specific heat, selection of midpoint is difficult. A small reduction in T_g was observed for lignin irradiated to a dose of 60 kGy. Beyond this radiation dose i.e. at 90 kGy, the T_g decreased markedly from 130° to 112°C as shown in **Fig. 6**. The decrease in T_g is assigned to a decrease in molecular weight and plastification phenomena in lignin which arise due to the intermolecular interaction of hydroxyl groups **[24]**.

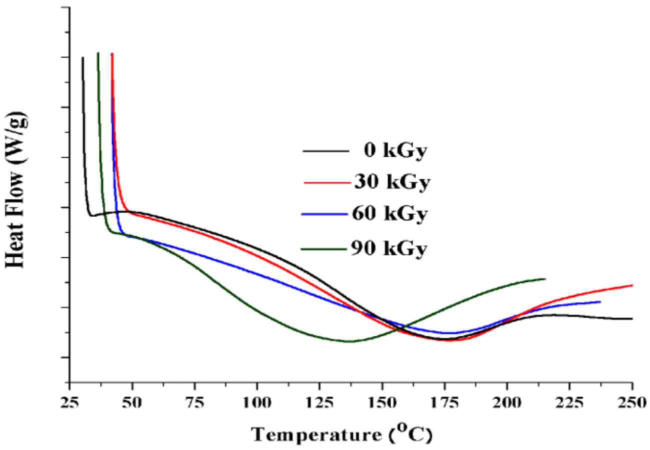

Fig. 6. DSC thermograms of unirradiated and electron beam irradiated lignin of doses 30, 60 and 90 kGy.

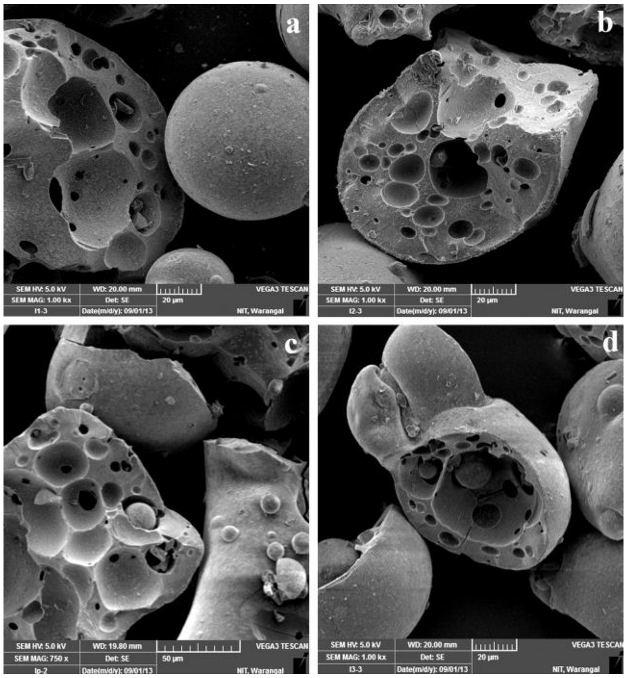

Fig. 7. SEM micrographs of unirradiated (a) and electron beam irradiated lignin of doses 30 (b), 60 (c) and 90 kGy (d).

SEM micrographs

The SEM micrographs of unirradiated and irradiated lignin are shown in **Fig. 7**. The granules of the unirradiated lignin are irregular with different sizes and having smooth surfae. Most of the lignin granules are broken which may be due to shear stress or temperature variation during the processing of lignin. There are pits on the cross-section surface of lignin granule. When the lignin is treated with EB, cracks were developed on the surface of the cross-section view as shown in **Fig. 7(b)** and **7(c)**. The magnitude of the cracks increased with the increase of EB dose. At a dose of 90 kGy, cracks are very prominent when compared to lignin irradiated to low dose and native lignin as shown in **Fig. 7(d).**

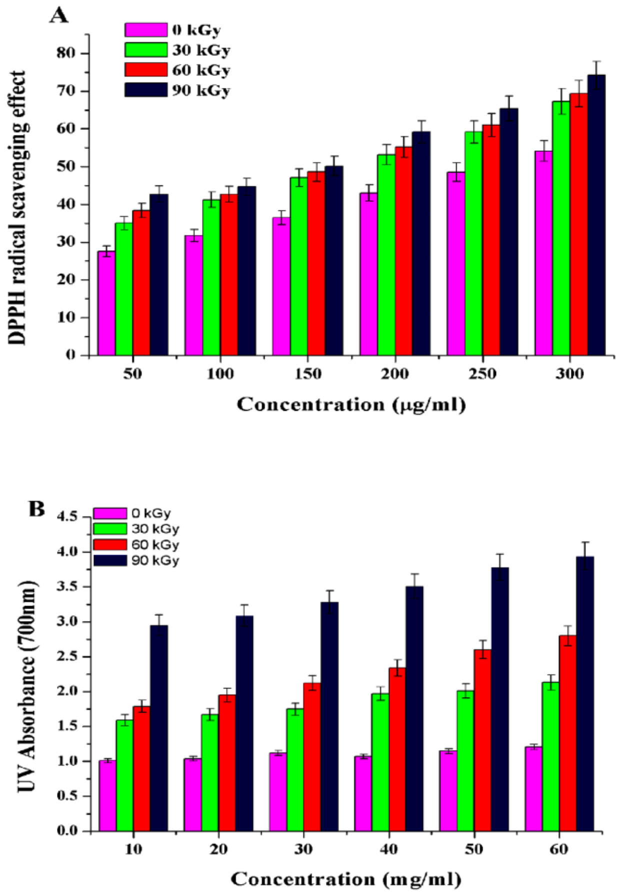

Fig. 8. DPPH radical scavenging capacity of unirradiated and irradiated lignin (A), Reducing power of unirradiated and irradiated lignin (B).

DPPH radical scavenging activity and reducing power

DPPH assay provides the scope of antiradical activity of many plant extracts and foods due the presence of phenolic and flavonoid compounds present in them **[25]**. **Fig. 8A** depicts the change in DPPH % inhibition values of unirradiated and irradiated lignin of various concentrations. The scavenging activities of unirradiated and irradiated lignin were found to be concentration dependent and there is an increase in the % inhibition values with the increase of radiation dose. The IC50 values of 0(unirradiated), 30, 60 and 90 kGy lignin are 221±0.63. 177±0.25, 153±1.02 and 140±0.83 μg/mL respectively. A decrease in IC50 values means an increase in antioxidant activity of irradiated lignin.

Tatiana et al **[25]** have studied the radical scavenging activity of lignins and observed some structural property relations. According to their studies, non-esterified OH groups, phenolic groups, hydroxyl groups and double bonds between the outer most carbon atoms in side chain increase the radical scavenging activity (RSA). On the other hand high molecular weight, heterogeneity in terms of component composition i.e. carbohydrate admixture and polydispersity decrease RSA.

In reducing power (RP) assay, the presence of reductants (antioxidants) in samples would result in the reduction of Fe^{3+} to Fe^{2} by donating an electron. RP is associated with antioxidant activity and serve as a significant reflection of the antioxidant property **[27]**. From **Fig. 8B**, it is observed that UV absorbance value of lignin is concentration dependent and it increased with the dose of irradiation. The sample with higher UV absorbance has stronger RP **[2]**.

Unirradiated lignin poses many intermolecular chain interactions and high molecular weight when compared to irradiated lignin. But on irradiation a decrease in molecular weight might have occurred increasing RSA of lignin. Due to development of the cracks in the lignin granules, the solvent can easily diffuse thus increasing its solubility and RSA. The SEM studies also reflect the same aspect.

Conclusion

EB irradiation induces both chemical and physical changes in lignin. Due to cleavage of chemical groups, primary radicals are produced in lignin. The primary radicals undergo secondary reactions producing peroxy and poly-conjugated radicals which are detected by ESR technique. Peroxy radicals decay at lower temperature (403K) while poly-conjugated radicals are stable up to elevated temperature. Evidence for enhancement in poly-conjugated structures is proven from FTIR results. T_g of lignin is found to decrease on irradiation. Granular structure of lignin is severely affected due to EB radiation. Antioxidant activity of lignin is enhanced due to EB irradiation. Though lignins RSA and antioxidant properties are established, its role as stabilizer is to be investigated in future.

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