VALORIZATION OF WASTE RICE STRAW: A GREEN APPROACH TO PREPARATION OF LIGNIN NANOPARTICLES FROM WASTE BIOMASS

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Rice straw is an abundant source of essential biopolymers such as cellulose, hemicelluloses, and lignin; however, the majority of global rice straw production remains underutilized. This study presents a novel approach to valorize waste rice straw through the extraction of lignin and subsequent preparation of lignin nanoparticles (LNPs). Alkali lignin (AL) was first extracted via alkaline treatment, followed by LNPs preparation through a simple acid precipitation method. The average yields of AL and LNPs were 10.72% and 21.08%, respectively. The nanoparticles were characterized using UV-Visible spectroscopy, FT-IR, DLS and TEM. Results revealed that the LNPs were discrete, spherical, and well-dispersed, with an average particle size of approximately 201 nm and a polydispersity index (PDI) of 0.284 – well within the acceptable range for drug delivery systems.

Keywords: lignin nanoparticles, nanocarrier, rice straw, valorization, waste biomass

INTRODUCTION

Rice is a universally grown cereal food crop that comes from the grass family of the plant kingdom.[1] Nearly two-thirds of the world's population consume rice (*Oryza sativa* L.) as staple food. There are approximately 110,000 varieties of rice with different qualities and nutritional profiles cultivated worldwide.[2] Apart from Antarctica, rice is cultivated on every continent.[3,4] However, only the rice producing countries of Asia, such as China, India, Indonesia *etc*., account for 89% of the total production of rice worldwide. Bangladesh is also among these countries, producing 7% of the global production of rice.[5,6]

Every year, 34.7 million metric tons of rice is produced in Bangladesh, which covers 91% of the total food grain production of the country.[7] Rice production engrosses about 78% of the total cultivated areas in Bangladesh, and bolsters the overall gross domestic product (GDP) of Bangladesh.[8] The rice production is naturally associated with the side-production of large amounts of crop residues, predominantly, rice straw. Rice straws are the remnants of the rice plants that are left in the fields after harvesting the rice grains. For every ton of rice grain harvested, almost 1.35 tons of rice straw remain the fields. These straws are primarily composed of cellulose (33-47%), hemicelluloses (19-27%) and lignin (5-24%), and are one of the globally abundant lignocellulosic materials.[9–11] Nevertheless, most of the rice straw is either used merely as cattle feed or burnt as a waste for disposal.[10,12] If properly utilized, rice straws possess the potential to become an invaluable asset, being a source of various important biopolymers, such as cellulose, hemicelluloses and lignin.

Lignin is a three-dimensional amorphous biopolymer, consisting of phenylpropanoid units (cumaryl, coniferyl and sinapyl alcohol) cross-linked on a random manner).[13,14] It is the second most abundant biopolymer on earth after cellulose, accounting for almost 30% of the total organic carbon in the biosphere.[15–17] It is one of the major constituents of plant cell walls, along with cellulose and hemicelluloses.[18] Lignin is associated with several essential properties like biocompatibility, biodegradability, antioxidant activity, low cytotoxicity and many more.[19–22] Moreover, when converted into nanoparticles, lignin exhibits enhanced properties, such as larger surface area, good stability, sustainability and non-hemolytic activity.[23–26] Currently, lignin nanoparticles are being given utmost attention throughout the scientific community and are being extensively used in several high value added materials, such as as reinforcing agents in composites, as antioxidants, as UV-absorbents and most importantly, as drug delivery vehicles for anticancer and oral drugs.[27–30] The versatility and ease of preparation are other factors that boost the interest among researchers in working with lignin nanoparticles. Numerous simple yet effective approaches are already in use for lignin nanoparticles. Approaches like acid precipitation,[31] anti-solvent precipitation[32,33] and solvent exchange[34,35] are being applied to prepare lignin nanoparticles, owing to their simple methodology. Various other methods such as ultrasonication,[36,37] interfacial-crosslinking[38,39] *etc*. are also used extensively for lignin nanoparticles preparation. Some authors have also utilized different approaches to prepare lignin microparticles, such as self-assembly[40] and mechanical treatment.[41]

Since a large volume of rice straw is produced in Bangladesh every year and almost all of it is underutilized, various approaches should be developed to take full advantage of the untapped potential of rice straws. The extraction of lignin from the waste rice straw to produce lignin nanoparticles for advanced applications in various areas may create such an avenue for valorization of rice straw waste.

In this work, lignin has been extracted from waste rice straw via alkaline treatment and sulfuric acid treatment. Afterwards, lignin nanoparticles were prepared from the extracted lignin at room temperature via a simple acid precipitation method, using a sodium hydroxide solution and dilute nitric acid solution (Fig. 1). To the best of our knowledge, no similar approach has been reported to valorize waste rice straws. This work thus attempted to uplift the rank of rice straw among waste biomass and create a new avenue for green preparation of lignin nanoparticles.

EXPERIMENTAL

Materials

Sodium hydroxide (NaOH) was acquired from Amresco, LLC, Ohio; sulfuric acid (H_2SO_4) was purchased from Merck, Germany; and nitric acid (HNO_3) was purchased from PT. Smart Lab, Indonesia. Freshly harvested waste rice straw was collected from a paddy farmer in Khandokia, Chattogram, Bangladesh.

Rice straw pretreatment and alkaline extraction of lignin

The rice straws were initially rinsed three times with deionized water to eliminate any residual soil or dirt. The cleaned straws were then chopped into small segments measuring approximately 2-2.5 inches in length and then dried in a hot air oven overnight at moderate temperature with regular observation of moisture content reduction. When the rice straws were visibly void off any moisture and appeared crisp and brittle, drying was ended and the straws were milled into powder using a grinder and filtered through a 400-mesh sieve. Following sieving, the fine rice straw powder was kept in an air-tight sample bottle for later use.[42]

For the extraction of lignin, 5 g of rice straw powder was suspended in 250 mL of 5% NaOH (1:50 solid: liquid ratio) and thermally treated for about 3 hours at 80 °C in a hotplate magnetic stirrer (160 rpm). After thermal treatment, the dark brown mixture was cooled for 2 hours, and the bottom layer of insoluble rice straws were separated via filtration. Next, 5M H_2SO_4 was gradually added to the filtrate to lower its pH to 2~3. The pH-change triggered and facilitated the precipitation of alkali lignin (AL) from the filtrate solution. The lignin was then collected via centrifugation (6000 rpm, 15 mins), rinsed repeatedly with deionized water to wash off any residual acid, dried in hot air oven and stored in a desiccator (Fig. 2).[43–45] To determine the yield percentage of the extracted lignin, the following formula was employed:

$$\%Lignin\ yield = \left(\frac{Weight\ of\ Lignin\ extracted}{Weight\ of\ rice\ straw\ taken}\right) \times 100 \quad (1)$$

Preparation of lignin nanoparticles

Lignin nanoparticles (LNPs) were prepared from the extracted AL via the acid precipitation approach adopting the methodology from our previously published work.[46,47] First, 10 mg of AL was dissolved in 20 mL of NaOH to prepare a 0.5 mg/mL solution. The solution was stirred at 500 rpm for 30 minutes to allow the lignin to dissolve completely.[27] Subsequently, the undissolved substance was discarded through filtration, and 4-5 mL of diluted acid (dilute HNO_3) was quickly added to the solution. Within minutes, the solution turned cloudy, substantiating the successful formation of nanoparticles (Fig. 3). The LNPs were then separated by centrifugation, washed with deionized water until neutral pH, dried in hot air oven at 60 °C for 6 hours, milled into fine powder and kept in an air-tight vial in a desiccator for characterization studies.[48] The following formula was utilized to calculate the yield percentage of the LNPs:

$$\%LNPs\ yield = \left(\frac{Weight\ of\ LNPs}{Weight\ of\ Lignin\ taken}\right) \times 100 \quad (2)$$

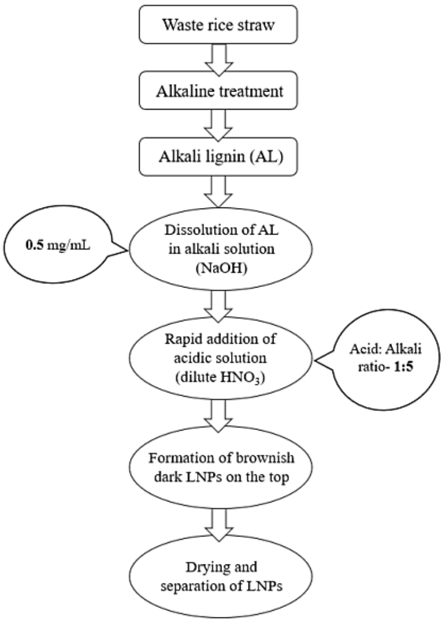

Figure 1: Diagram of the LNPs preparation procedure

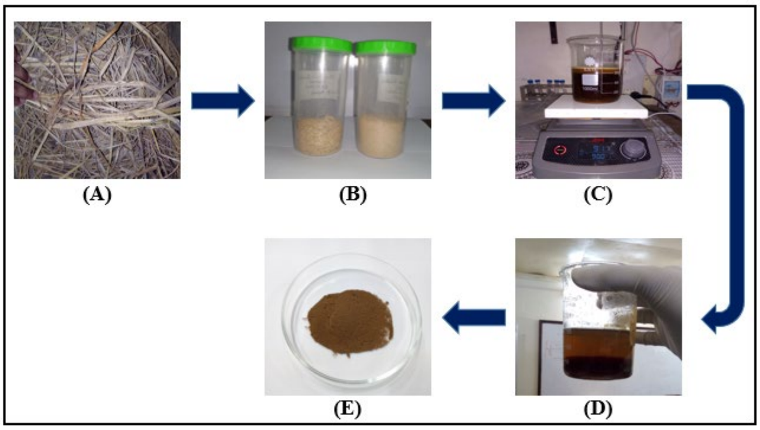

Figure 2: Extraction of alkali lignin from waste rice straw: (A) fresh rice straw; (B) finely ground rice straw powder; (C) alkaline treatment of rice straw powder; (D) precipitation of alkali lignin and (E) AL powder

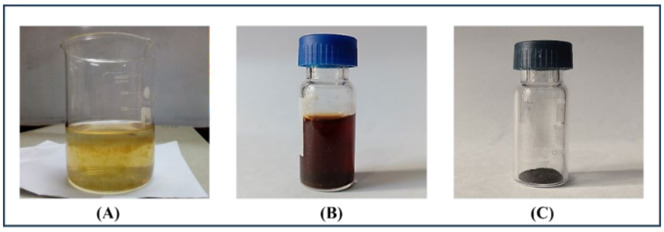

Figure 3: (A) Formation of LNPs after rapid acid addition; (B) LNPs solution in isopropyl alcohol (IPA) and (C) Dried LNPs powder

Characterization of LNPs

UV-visible spectroscopy

UV-visible spectroscopy was employed to verify the successful extraction of AL from the rice straw. AL was dissolved in dimethyl sulfoxide (DMSO, 0.05%, w/v) and the absorbance from 200 to 600 nm was recorded with a UV-visible spectrophotometer (UV-1800, Shimadzu, Japan).[47,49]

FT-IR spectroscopy

Fourier transform infrared spectroscopy (FT-IR) was employed to identify the functional groups within the LNPs. The FT-IR spectra were obtained in the range of 4000-400 cm^{-1} using a PerkinElmer Spectrum Two UATR Fourier transform infrared spectrometer (PerkinElmer Inc., MA, USA).[47]

Dynamic light scattering technique

The average particle size (hydrodynamic diameter, Z-average) and size distribution (polydispersity index, PDI) were determined using the dynamic light scattering technique (DLS). The LNPs were suspended in distilled water (0.02 mg/mL) and sonicated for approximately 30 minutes prior to performing measurements with a Malvern Zetasizer-ZEN3600 (Malvern Instruments Ltd., Malvern, U.K.).[47]

Transmission electron microscopy

Transmission electron microscopy (TEM) was utilized to examine the surface structure of the LNPs. For this, a suspension of LNPs in isopropyl alcohol (IPA) was created and subjected to sonication for 30 minutes. A droplet of the LNPs suspension was placed on a carbon-coated copper grid and allowed to dry before observation using a Talos F200X G2 STEM (Scanning-TEM), Thermo Fischer Scientific Inc., MA, USA.[47]

RESULTS AND DISCUSSION

Yield percentages of AL and LNPs

The yield percentages of the extracted alkali lignin from rice straw and prepared lignin nanoparticles from extracted AL are presented in Table 1. According to the table data, the average %yield of AL from rice straw was 10.72%, and the average %yield of LNPs from AL was 21.08%. The data indicates that the preparation of LNPs from AL was relatively more fruitful than extraction of AL from waste rice straw.

UV-Vis absorbance studies

Proper extraction of AL from waste rice straw was confirmed by measuring the UV-Vis absorption spectra of AL using DMSO as reference. Figure 4 represents the UV absorbance spectra of AL. From the figure, it is evident that the extracted AL exhibited maximum absorbance between 250 nm and 350 nm.[49] According to previous reports, this absorbance could be ascribed to the presence of chromophores in lignin molecules.[50,51]

FT-IR analysis

FT-IR analysis was aimed at studying the chemical structure of the LNPs prepared from the extracted alkali lignin. Figure 5 displays the FT-IR spectrum of the LNPs, and it represents the absorption bands typical for AL. The broad absorption band within the range from 3000 to 3600 cm^{-1} is due to the phenolic and aliphatic hydroxyl groups present.

Table 1
Yield percentage data of AL and LNPs

Alkali lignin (AL)				Lignin nanoparticles (LNPs)			
Rice straw (g)	AL obtained (g)	%Yield	Avg. %Yield	AL used (mg)	LNPs obtained (mg)	%Yield	Avg. %Yield
10	1.068	10.68		200	43	21.5	
10	1.076	10.76	10.72	200	41	20.5	21.08
10	1.071	10.71		200	42.5	21.25	

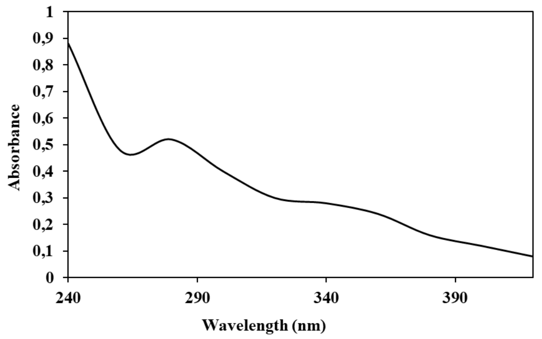

Figure 4: UV-visible absorption spectrum of AL in DMSO

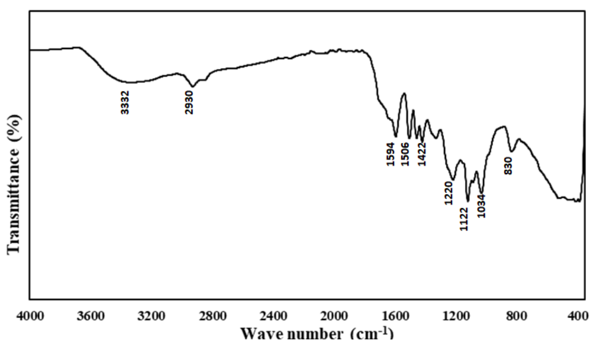

Figure 5: FT-IR spectrum of prepared LNPs

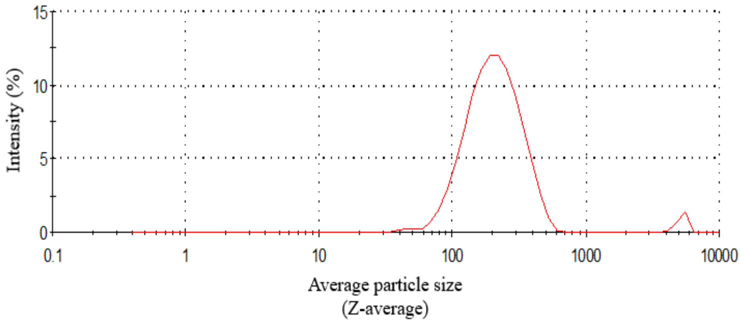

Figure 6: Size distribution of LNPs by dynamic light scattering (DLS)

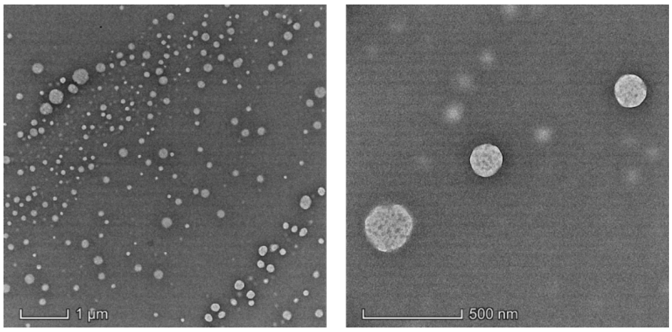

Figure 7: TEM images of prepared LNPs

The bands observed at 2850 and 2930 cm^{-1} can be attributed to the C-H stretching of methyl and methylene groups. The bands at 1422, 1506 and 1594 cm^{-1} are assigned to aromatic skeletal vibration typical of lignin, which confirms that the basic lignin structure remained intact during nanoparticle preparation.[48,52] Additionally, several other absorption bands were also observed at various other wavenumbers, for example, the bands at 1122, 1220 and 1330 cm^{-1} occurred due to C-O stretching of ether groups. The band at 1034 cm^{-1} can be assigned to primary O-H stretching or aromatic C-H deformation. In addition, the C-H in-plane deformations in aromatic rings at 830 cm^{-1} are also observed in the FT-IR spectrum.[53]

DLS analysis

The average particle size (expressed as hydrodynamic diameter, Z-average) and size distribution (expressed as PDI) of the prepared LNPs were determined via dynamic light scattering technique (DLS). The results obtained from DLS analysis showed that the LNPs have an average particle size of 201.37 ± 1.20 nm (Z-average) and PDI of 0.284 ± 0.012 (Fig. 6). These values indicated that the average particle size and polydispersity index falls well within the acceptable limit for a biomaterial suitable for drug delivery application (size below 250 nm and PDI in the range of 0.100–0.300.[54–57]

TEM analysis

Surface morphology of the LNPs was observed using transmission electron microscopy (TEM). The images obtained from TEM (presented in Fig. 7) clearly indicate that the nanoparticles formed were discrete, spherical, uniformly shaped and highly dispersed with smooth outer surfaces. The appearance of the LNPs observed in the TEM is also in coherence with earlier reports of the preparation of LNPs.[58–60]

CONCLUSION

Despite being a rich source of many important biopolymers, most of the globally produced rice straw is either used as cattle feed or discarded as waste. In this paper, we have attempted to valorize this waste by extracting alkali lignin from it. The extracted AL was then used to prepare lignin nanoparticles (LNPs). The average yield percentages were 10.72% for AL and 21.08% for LNPs respectively. The LNPs were characterized via UV-Vis spectroscopy, FT-IR, DLS and TEM. The results showed that the LNPs were discrete, spherical particles, with an average particle size of ~200 nm and a PDI value of 0.284 – these values are well within the limit for a biomaterial to be employed as a drug delivery vehicle. According to these findings, rice straws could be transformed into a highly significant bio-based resource required for drug delivery applications by extracting lignin nanoparticles from it. Such an approach could ultimately have a significant ameliorating impact on the economic growth of a country.

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