

# Synthesis of Lignin Nanoparticles: Top-Down and Bottom-Up Approaches †

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**Abstract:** Lignin, a main byproduct from paper manufacturing and biorefineries, is now emerging as a new low-cost, renewable starting material for new product development. Its biocompatibility and safety make it valuable for creating novel and value-added products. Lignin, a polymer with many hydrophilic and active groups, confers many useful properties. However, there are several challenges to overcome due to its complex chemical structure and heterogeneous self-assembly behavior. Nanostructured systems using lignin could address these challenges, finding applications in food science, cosmetics, and healthcare. This study explores two main green synthesis approaches for lignin nanoparticles: bottom-up based on the self-assembly in a solvent–antisolvent system and top-down based on the ultrasonication. These nanoparticles are evaluated for morphology, estimation of phenolic content and antioxidant effects. Specifically, the antisolvent nanostructures show a spherical conformation with a higher antioxidant activity due to a better organization of phenolic hydroxyl groups. Obtained results have been exploited to draw an efficient and cheap technological route for lignin valorization.

**Keywords:** lignin; biowaste; lignin nanoparticles; green synthesis approach



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## 1. Introduction

Lignin represents a significant byproduct of both the pulp and paper industry and the biorefinery sector. It is currently gaining recognition as an economical and sustainable raw material for creating novel products and processes [1,2]. Lignin is a complex polymer with interconnected structures, featuring numerous functional hydrophilic and active groups like hydroxyls, carbonyls, and methoxyls. These groups impart a range of valuable properties, including the ability to adsorb organic pollutants in soil and water treatment, antioxidant capabilities, and redox reactivity [3]. Typically, lignin properties are strictly correlated to various factors such as the extraction method and molecular weight. In order to establish a versatile and reproducible system, lignin undergoes conversion into nanoparticles utilizing distinct techniques including the bottom-up and top-down approaches [4]. The bottom-up strategy leverages lignin's hydrophobicity and involves anti-solvent precipitation, resulting in the production of lignin nanoparticles through a mechanism of nucleation and growth of nuclei [5]. The top-down approach, conversely, utilizes high-pressure homogenisation to fragment and reduce the lignin polymer until it reaches the nanoscale. In this study, an overview is presented on the impact of synthesis routes on the physicochemical properties of lignin nanoparticles (LNPs) [6]. The resulting

nanostructures were examined in-depth using SEM to assess morphology, DLS to characterize size and UV-Vis analysis to determine light absorption properties. Functional characterization was even conducted, which demonstrated that the nanoparticles had a greater amount of phenolic content and displayed strong antioxidant properties. The production of high-quality lignin nanoparticles draws a novel pathway to utilize the potential of lignin derivatives in many applications including bio-nanocomposites, adsorbent materials, and controlled drug release formulations.

## 2. Materials and Methods

### 2.1. Materials

Sigma Aldrich (Milan, Italy) provided the alkali lignin (Kraft Lignin), acetone (99.8%), Folin–Ciocâlțeu reagent, 2,2-diphenyl-1-picryl-hydrazil (DPPH), methanol (99.9%), and sodium carbonate. All chemicals were used exactly as received, with no further purification.

### 2.2. Synthesis of Lignin Nanoparticles

Bottom-up approach: according to previously reported methods [7], the solvent–antisolvent route was exploited. In detail, two stock solutions were obtained by dissolving 100 or 600 mg of alkali lignin in a 7:3 (*v/v*) mixture of acetone/water at room temperature and under constant magnetic stirring for three hours. The solutions were then mixed with distilled water in a 1:9.2 (*v/v*) ratio and stirred at room temperature for 24 h to allow the formation of AS-1LNP and AS-6LNP samples. Top-down approach: US-1LNP and US-6LNP samples were obtained using the same amount of reagents as the previous procedure. Thus, by adding 10 mg or 60 mg of alkali lignin to an acetone/water mixture in a volume ratio of 0.7:9.5 (*v/v*), lignin nanoparticles were then formed by sonication for 10 min in a probe tip sonicator at 20 kHz frequency and 40% amplitude.

### 2.3. Characterization Techniques

Morphological analysis of LNPs was carried out by using Scanning Electron Microscopy (SEM) with a Field Emission Ultrapluss ZEISS Microscope. In a Cressington sputter coated 208HR, a drop of NPs suspension was deposited onto the surface of an aluminum stub and sputter-coated with a Pt/Pd layer (5 nm). Hydrodynamic diameter was evaluated with Dynamic Light Scattering (DLS) using particle size analyzer (Zetasizer ZS, Malvern Instruments Ltd., Malvern Panalytical, Malvern, UK,  $\lambda = 632.8$  nm) and five runs for each measurement.

### 2.4. Total Phenolic Content of LNPs

The Folin–Ciocâlțeu reagent (FCR), also known as the gallic acid equivalence method (GAE), is a phosphomolybdate and phosphotungstate mixture used for the colorimetric *in vitro* assay of phenolic and polyphenolic antioxidants. When the phenol moiety is added to a solution containing FCR, the concentration of this phenolic compound can be estimated using UV spectrophotometry [8]. Gallic acid is commonly used as a reference standard, and results are expressed in milligrams of equivalent gallic acid per gram of sample.

The sample was added at a final concentration of 25  $\mu\text{g/mL}$  to a solution containing 1:3:14 *v/v/v* Folin–Ciocâlțeu reagent, 75 g/L of  $\text{Na}_2\text{CO}_3$ , and  $\text{H}_2\text{O}$ . The absorbance at 765 nm was measured after 30 min at 40 °C using a UV-VIS spectrophotometer, 230 V (Shimadzu, Milan, Italy). Experiments were carried out in triplicate.

### 2.5. Antioxidant Activity of LNPs

LNPs' antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method. The DPPH exists as a purple solution in the stable radical form and as a yellow solution when neutralized by the antioxidant, according to the literature [9,10].

In brief, 2 mL of 100 M DPPH methanol solution was mixed with 0.1 mg/mL of each aqueous sample. After 5 min of incubation in the dark, the absorbance at 517 nm was

measured. The percentage of DPPH free radical scavenging activity was calculated as follows (Equation (1)):

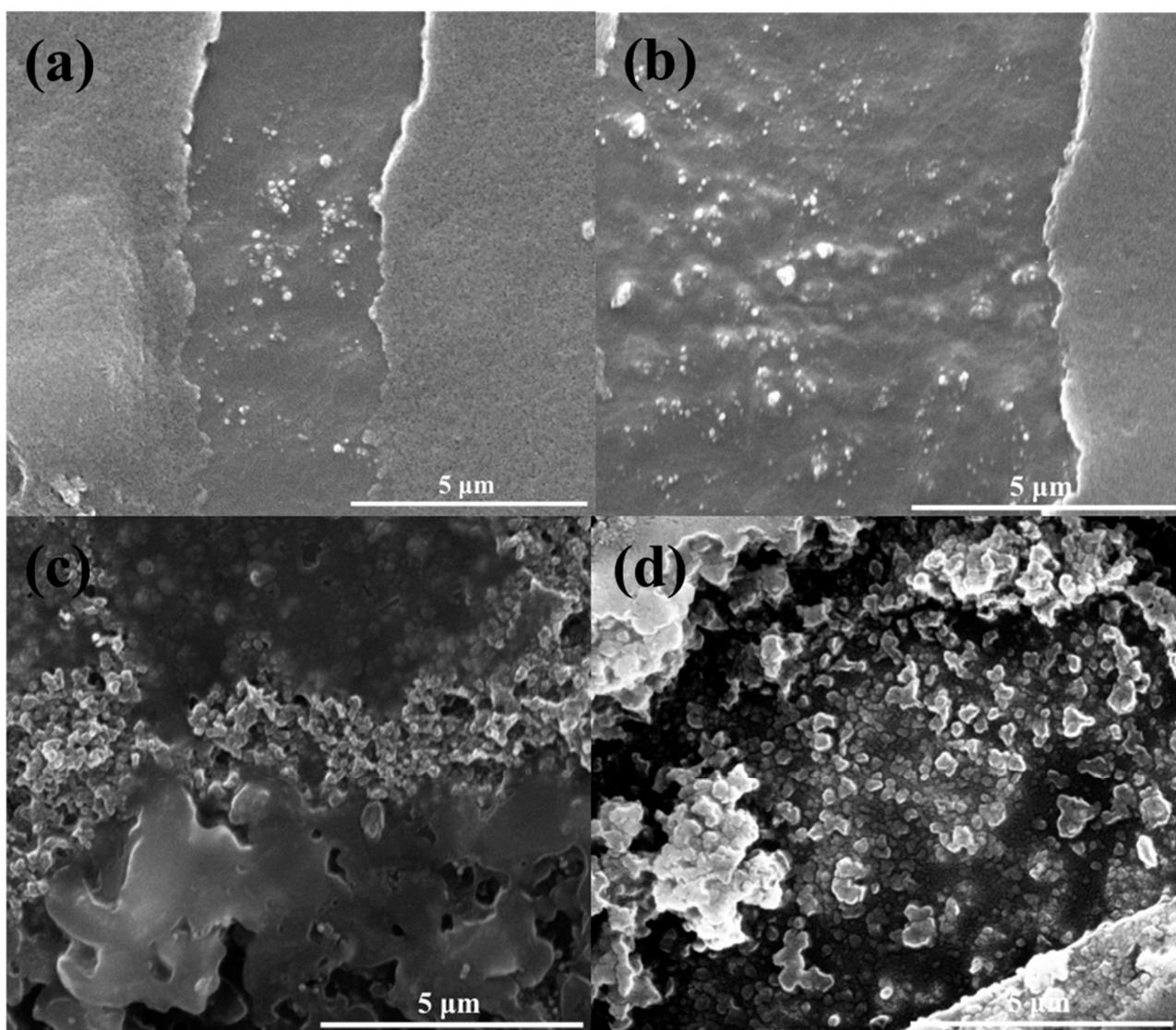
$$\text{DPPH scavenging effect (\%)} = \frac{A_{\text{DPPH}} - A_{\text{Sample}}}{A_{\text{DPPH}}} \times 100, \quad (1)$$

where  $A_{\text{DPPH}}$  and  $A_{\text{Sample}}$  were the absorbance of the methanolic solution of DPPH and the absorbance of the sample, respectively.

### 3. Results and Discussion

#### 3.1. SEM Analysis

The morphology of the synthesized samples was analysed through Scanning Electron Microscopy (SEM), as observed in Figure 1.



**Figure 1.** Micrograph SEM of (a) AS-1LNPs and (b) AS-6LNPs, (c) US-1LNPs and (d) US-6LNPs, with a 30,000× magnification.

Both AS-LNP samples (Figure 1a,b) were composed of isolated pseudo-spherical nanoparticles of about 150 nm in their average diameter resting on a layer of coalescent smaller nanoparticles. The presence of apparently aggregated nanoparticles could be attributed to solvent evaporation during SEM sample preparation and analysis under high

vacuum and temperatures. From this analysis it can be observed that there is no substantial difference between the two samples, implying that the different starting lignin concentration does not significantly affect the lignin nanoparticles' morphology and thus, AS-1LNPs and AS-6LNPs display a similar configuration. On the other hand, SEM Micrographs of US-1LNP and US-2LNP samples (Figure 1c,d) revealed the presence of sheets of lignin nanoparticles with irregular shape as well as larger micrometric lignin fragments. US-LNPs exhibited larger size and size distribution than AS-LNP samples. This finding suggests that the size reduction was most likely caused by cavitation and collisions between aggregates, most likely through a mechanical exfoliation mechanism, resulting in irregularly shaped particles and a more heterogeneous size distribution, in accordance with the data collected by [11,12].

### 3.2. Dynamic Light Scattering

To assess the size of the nanoparticles, DLS measurements were carried out on all samples (Table 1). The analysis revealed no significant differences among samples produced with the same synthesis route.

**Table 1.** Hydrodynamic diameters of all samples.

Sample	Hydrodynamic Diameter $\pm$ 0.5 (nm)
AS-1LNPs	120
AS-6LNPs	158
US-1LNPs	425
US-6LNPs	590

It was possible to observe that both AS-LNPs and US-LNPs behave similarly; indeed, the nanoparticles size was lower in the samples that started with a greater amount of lignin, maybe due to stronger interactions among lignin macromolecules. In accordance with SEM analysis, an anti-solvent approach is characterized by LNPs of about 100–150 nm and an ultrasonication method results in the formation of very large aggregates leading to an inhomogeneous nanoparticle size distribution.

### 3.3. Estimation of Total Phenolic Content

A quantitative investigation of the phenolic group amount within the lignin nanoparticles was carried out by means of a Folin–Ciocálteu assay and the results were reported in Figure 2. All samples displayed a great amount of phenolic content that was around 500 mg of Gallic Acid/g of sample. Comparing nanoparticles with the starting alkali lignin, it can be observed that US-6LNP, AS-1LNP and AS-6LNP samples have a greater value of phenolic content suggesting a different organization of phenolic moieties in the nanoparticles than in the neat lignin, leading to their better exposure; meanwhile, US-1LNPs have a lower value than the alkali lignin which could be explained by the presence of more aggregates that hinder the exposure of phenolic hydroxyl groups to the water phase.

Moreover, both the AS and US sample show a higher content when the starting lignin amount is greater, suggesting an increased surface area and the proportional exposition of these hydroxyl groups. In conclusion, due to the greater amount of time available for better organization and exposure of the phenolic groups during LNP formation, and in addition to the higher initial lignin amount, AS-6LNPs have an increased content than US-1LNPs.

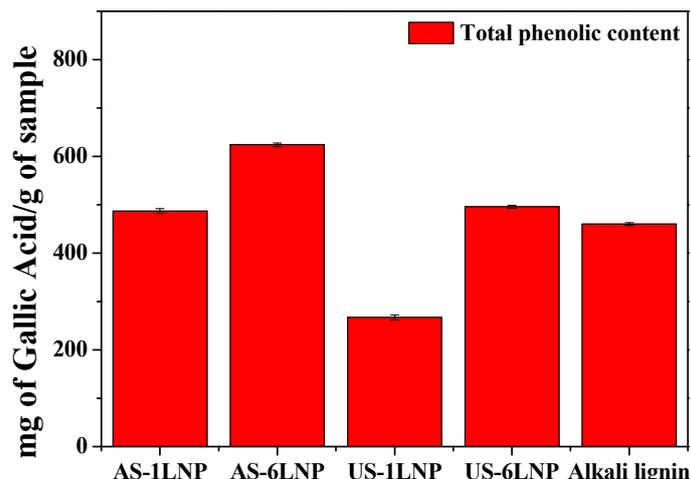


Figure 2. Estimation of phenolic content of all samples.

### 3.4. Estimation of Antioxidant Activity

The presence of functional groups such as quinones and phenolics in the lignin complex chemical structure can play important roles by acting as natural antioxidants. Antioxidant capacity of all the synthesized sample was exploited by means of DPPH Assay and the results were reported in Figure 3.

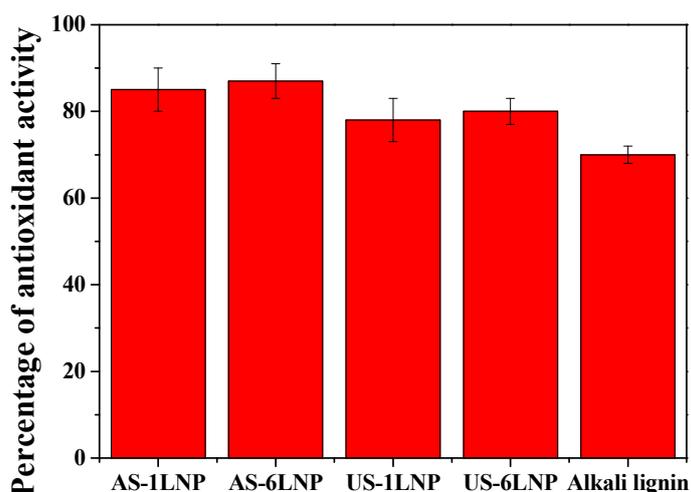


Figure 3. Estimation of antioxidant activity of all samples.

The synthesized samples were studied for their antioxidant activity through the DPPH assay, with a very low concentration of only 0.1 mg/mL. In agreement with the Folin–Ciocâlțeu assay, all of the samples show a high antioxidant capacity with AS-1LNPs and AS-6LNPs giving the greater performance, as expected in view of the larger amount of measured phenolic moieties. This outcome could be attributed to a variety of critical factors, including particle size and shape, as well as the structural changes in the lignin phenolic groups during nanoparticle formation [13,14]. Moreover, in accordance with the number of phenolic moieties present in the samples, both AS-LNPs and US-LNPs display a higher radical scavenging activity when the starting lignin content is greater.

## 4. Conclusions

In this study, two methods for the preparation of LNPs were used and compared, and in terms of morphology, phenolic hydroxyl content, and antioxidant activity of obtained nanoparticles were also compared. Lignin nanoparticles were created using a bottom-up approach by means of antisolvent precipitation and a top-down approach using ultra-

sonication. AS-LNPs have a spherical-like shape with smaller dimensions than US-LNPs, which have an irregular morphology with a larger number of aggregates. Because of the conformation of these LNPs, AS-LNP samples demonstrated improved reorganization of phenolic hydroxyl groups on the surface, resulting in a higher antioxidant activity of around 80% which is even higher than neat lignin. In conclusion, using a straightforward approach, alkali lignin is transformed into nanoparticles with improved functional features than the starting material, thus, providing a significant contribution to the development of a waste-to-wealth strategy and towards addressing environmental and health problems.

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