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Properties and chemical modifications of lignin : Towards lignin-based nanomaterials for biomedical applications

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Title: Properties and Chemical Modifications of Lignin: Towards Lignin-Based Nanomaterials for Biomedical Applications

Article Type: Review article

Keywords: Lignin; chemical modifications; lignin-based nanoparticles; lignin-based hydrogels; biomedical applications

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Abstract: Biorenewable polymers have emerged as an attractive alternative to conventional metallic and organic materials for a variety of different applications. This is mainly because of their biocompatibility, biodegradability and low cost of production. Lignocellulosic biomass is the most promising renewable carbon-containing source on Earth. Depending on the origin and species of the biomass, lignin consists of 20-35% of the lignocellulosic biomass. After it has been extracted, lignin can be modified through diverse chemical reactions. There are different categories of chemical modifications, such as lignin depolymerization or fragmentation, modification by synthesizing new chemically active sites, chemical modification of the hydroxyl groups, and the production of lignin graft copolymers. Lignin can be used for different industrial and biomedical applications, including biofuels, chemicals and polymers, and the development of nanomaterials for drug delivery but these uses depend on the source, chemical modifications and physicochemical properties. We provide an overview on the composition and properties, extraction methods and chemical modifications of lignin in this review. Furthermore, we describe different preparation methods for lignin-based nanomaterials with antioxidant UV-absorbing and antimicrobial properties that can be used as reinforcing agents in nanocomposites, in drug delivery and gene delivery vehicles for biomedical applications.

Response to Reviewers: RESPONSE TO THE REVIEWERS' COMMENTS

REVIEWER #1

In this review, the authors summarized the current state of art for the preparation of lignin-based nanomaterials. Recent advances in the application of lignin-based nanomaterials in drug delivery and gene delivery vehicles were also discussed in the manuscript. However, this review is premature for publication in Progress in Materials Science. Some specific comments are listed as follows.

R.: We thank the reviewer for the time spent revising our manuscript. All comments and suggestions were very welcome and helped to improve the quality of our manuscript. We are pleased to clarify the requested points below.

1. In the section of processing methods for lignin extraction, it lacks an insightful analysis of the technologies and pros and cons of different processing methods for lignin extraction. It just involves four kinds of classification, description and simple list of the studies. Hence, the au-thors should add some comprehensive analyses and compare these processing methods.

R.: The advantages and disadvantages of the different processing methods for the lignin extraction were addressed all over the section 3:
3.1. "Similarly to other isolation processes, the native lignin is submitted to some chemical and struc-tural changes [31]. Nevertheless, the production of large amounts of lignin using this process is im-portant for its transformation into high-value lignin-based materials [12]."
3.2. "However, the sulfite process presented some disadvantages, including the formation of new C–C bonds that alter the lignin structure, and the presence of high content of ash and other impurities after isolation [39]."
3.3. "However, comparing to the previous extraction methods, the soda lignin contains no sulfur that makes it a good potential for the preparation of high-value products [6]."
3.4. "Moreover, this process is relatively clean and less aggressive compared to the other processes. The obtained lignin is sulfur-free, with lower ash-content, it has higher purity due to the lower carbo-hydrate content and lower MW (500–5000 Da) [39, 49, 54, 55]. However, despite these advantages, this delignification process is not widely used because of the extensive corrosion of the equipment and the lower quality of the pulp produced compared to the soda and kraft pulping methods [6]. Also, under acidic conditions, random condensation reactions can occur, instead of the depolymerization reactions. For example, the formation of benzylic cations under acidic conditions can take place, leading to the formation of new C–C bonds [39]."

To conclude the section 3, the following paragraph was also added:
"Taking all of this into consideration, the native structure of lignin undergoes a chemical transfor-mation after the extraction process. Besides the limitations abovementioned, the structure of kraft, organosolv, sulfate and soda lignin can also vary according to the plant source. Thus, the introduc-tion of lignin into new markets will strongly depend on its structure, reactivity and physicochemical properties [56]."

2. "4.1 Lignin Depolymerization": There are too many descriptive statements. In order to im-prove the understanding of different lignin depolymerization processes, main reaction equa-tions (similar to fig. 4) should be added in this section.

R.: As each depolymerization process can originate different products, with different chemical structures, we decided to added a figure (Fig. 4) that summarize the different conditions used for each lignin

depolymerization process, with the different products that can obtained from each pro-cess.

3. "4.4 Production of Lignin Graft Copolymers": This section is too long to read. The authors should divide it into several parts and provide some subtitles here. In this way, the readers can easily follow.

R.: In order to facilitate the reading, the following subsections were created in the "4.4. Production of Lignin Graft Copolymers" section, according to the processes described in the Figures 7 and 8:
4.4.1. "Grafting from" Technique
4.4.1.1. Ring Opening Polymerization
4.4.1.2. Radical Polymerization
4.4.1.3. Atom Transfer Radical Polymerization
4.4.2. "Grafting to" Technique

4. In section 6, the authors claimed that lignin has been successfully used to produce different nanomaterials including nanoparticles, nanotubes and hydrogels. The reviewer believes that nanofiber is also one of the important parts of lignin-based nanomaterials. Though the authors introduced PLA-lignin copolymer nanofibers in this section, the reviewer believes they are less extensive than nanoparticles and hydrogels. For example, Gao et al. prepared lignin nanofibers with ionic-responsive shells (Biomacromolecules, 2012, 13, 3602). Salas et al. synthesized soy protein-lignin nanofibers (Reactive and Functional Polymers, 2014, 85, 221). The authors should expand this part and provide more comments/insights on the progress of this field.
5. For a broader impact, the authors should also provide a section discussing the application of lignin-based nanomaterials in other biomedical fields. For example, the authors should provide more insights on how the development of lignin-based nanomaterials can promote their appli-cation in tissue engineering and regenerative medicine.

R.: We recognize that the lignin-based nanofibers are underexplored among the other nanomaterials in this review. Only two studies were lignin nanofibers were referred: Kai et al. (ACS Sustainable Chemistry & Engineering. 2016; 4: 5268-76) about the PLA-lignin copolymer nanofibers, and the study conducted by Gao et al. (Biomacromolecules. 2012; 13: 3602-10) about the lignin nanofibers with ionic-responsive shells, as an example of the atom transfer radical polymerization (section 4.4).
In order to emphasize the potential use of lignin-based materials in other biomedical fields such as tissue engineering, and also include additional studies in which nanofibers were used (namely for tissue engineering), we decided to reorganize the section of the lignin-based nanomaterials accord-ing to the types of application, instead of the division previously established. Thus, the section 6 is now divided by:
6.1. Lignin-based Nanomaterials as Antioxidant
6.2. Lignin-based Nanomaterials for Nanocomposites
6.3. Lignin-based Nanomaterials as Delivery Systems
6.4. Lignin-based Nanomaterials for Tissue Engineering
6.5. Lignin-based Nanomaterials for Other Application

The following studies were added to the section 6:
Quraishi et al., 2015 (https://doi.org/10.1016/j.supflu.2014.12.026)

Kai et al., 2015 (DOI: 10.1021/acssuschemeng.5b00405)
Kai et al., 2017 (DOI: 10.1021/acssuschemeng.7b00850)
Kai et al., 2015 (DOI:10.1039/C5TB00765H)
Salas et al., 2014
(https://doi.org/10.1016/j.reactfunctpolym.2014.09.022)

6. In the conclusion part, the statements about the current challenges in development and usage of lignin are very descriptive and lack in-depth discussion. For example, the authors only said several chemical modifications could be used to increase the potential use of lignins in the de-velopment of advanced lignin-based nanoparticles. The authors should provide more insights and discussions on the challenges.
7. Additionally, in conclusions and perspectives, the authors need to highlight the importance of lignin-based nanomaterials in broader application fields.
8. Finally, the difference and novelty of this review in comparison with previously reported works needs to be discussed.

R.: In order to answer the three comments raised by the reviewer, the conclusion and perspectives section was completely modified:
"Green biomaterials are becoming increasingly in demand due to their low cost of production and eco-friendly properties, which include biocompatibility and biodegradability. For this increased us-age to be achieved, bioengineering and nanotechnology are being developed in order to produce promising vehicles for different applications. Thus, lignin has shown great potential as a component of new green materials, because of its key characteristics, such as antioxidant, antimicrobial and sta-bilizing properties that make this biopolymer promising for end-use applications. The emerging val-orization of lignin by chemistry and pharmaceutical research fields represents an important stimulus towards the development of high-value materials, such as chemicals and biopolymers, from an eco-nomical and renewable aromatic source. Without any chemical modification, lignin is an attractive polymer to reduce the dependence on fossil resources, and it can also be used as a source of carbon for carbon fibers, as well as additive and stabilizer in polymer blends. However, the development and usage of lignin for broader application areas present some challenges, because of its highly variable chemical structure that depends on the extraction method and the source of lignin. To overcome these limitations, different chemical modifications have been proposed to increase the potential use of lignin for the development of advanced lignin-based polymers and nanostructures. For example, esterifica-tion or alkylation of the hydroxyl groups on the lignin structure can be performed to increase the compatibility of the lignin polymer with non-polar polymer matrices. Another challenge that lignin faces is the high polydispersity, which increases the complexity to control its macromolecular struc-ture after chemical modification. The development of nanosized lignin-based materials allows the control of the lignin macromolecular architecture, leading to an improvement on the properties of polymer blends and nanocomposites, as well as their application in the biomedical field.
Most of the existing literature is focused on the extraction processes and chemical modifications of lignin for the development of chemicals and polymers with improved properties. This review covers the topics previously mentioned, and also addresses the main preparation methods of lignin-based nanostructures towards their biomedical application. The use of natural polymers (such as lignin) over the synthetic polymers is

preferable in the biomedical field due to their unique properties such as biocompatibility, biodegradability and abundance in nature. Studies that explored the potential of using lignin in the production of nanosized nanomaterials for drug/gene delivery and other biomedi-cal applications are still limited as yet. Furthermore, in vitro assessment of the LNPs is even more scarcely studied with only few reports on the cytotoxicity evaluation. Apart from nanomaterials, the development of lignin-based hydrogels has promising potential to replace conventional synthetic ma-terials for biomedical and other industrial applications. Given the improvements on the modification chemistry, lignin valorization and its increasing potential at the nanoscale is expected to emerge as an important research field in the near future."

9. Some grammar and typographic errors need to be corrected in the manuscript.

R.: The grammar and typographic errors are now revised.

RESPONSE TO THE REVIEWERS' COMMENTS

REVIEWER #2

The review paper by Patrícia Figueiredo et al. presents different features of lignin materials in terms of chemical modification and application. One of the main credits given to this Work is the right time selected to compile all novel original works performed on lignin materials and prepare a very comprehensive review paper. Currently, plenty of attention towards lignin materials is observed from Scientific communities and despite immensely increasing number of research works in this field, a perceptible lack of complete review paper was recognized in this field. Therefore, I believe, this scholarly presented work has enough potential to fullfill requirements of getting published in Progress in Materials Science because of its broad audience and covering the most recent research efforts on lignin materials, especially in application section.
However, there are still some minor considerations that need to be taken into account by authors before final approval of this manuscript.

R.: We thank the reviewer for the time spent revising our manuscript and for recommending our paper for publication. All comments and suggestions were very welcome and helped to improve the quality of our manuscript. We are pleased to clarify the requested points below.

1. Introduction is very short and mainly provides a general information about the composition of Lignocellulosic materials. I think authors can provide more information about these materials in terms of their potentials, interests of applications and its practical challenges, mainly in one or two paragraphs.

R.: After describing the composition of lignocellulosic materials, we addressed the following text about the potential of lignin as a starting

material for different applications, and the main challenges of using lignin-based materials:
“Thus, lignin is one of the main bio-resource raw materials that can be used for the synthesis of environmentally friendly polymers, and its high content of aromatic structures offers the possibility to replace industrially relevant aromatic polymers and fine chemicals [10]. However, only approximately 2% of the annually extracted lignin by the paper and pulp industry from woody biomass has been mainly used for low-value applications as dispersants, adhesives and fillers [11]. This is mainly due to the complex and variable lignin structure, high polydispersity and immiscibility of lignin with host polymer matrices [6, 11, 12]. The procedures that are applied to separate, to isolate and to chemically transform these three components can lead to a multifunctional array of bio-derived value-added fuels, chemicals and other materials [13, 14]. Another way to overcome these limitations is to prepare lignin nanoparticles, which improves the blending properties with host matrix, and offers a morphological and structural control of the lignin [15-17]. In addition, the preparation of nanostructured lignin also opens the possibility to use lignin-based materials for high-value applications, such as drug/gene delivery and tissue engineering [17].
In this review, we provide an overview of the composition and properties of lignin, the processing methods for lignin extraction, chemical modifications of lignin, and the industrial applications of lignin derivatives. Finally, we discuss the preparation of lignin-based nanomaterials for different applications, highlighting the potential of these nanomaterials for biomedical applications.”

2. Sentences with same meaning are presented in different ways in some parts of the paper. I believe they can be merged to provide better understanding for readers. For example, these two consecutive sentences can be merged:
Lignocellulosic materials are mainly constituted by cellulose, hemicellulose, lignin, and their compositions are dependent on their origin and species (hardwood, softwood or grass). Commonly, they contain 30-50% cellulose, a polymer of glucose; 20-35% hemicellulose, a heteropolymer containing xylose, and 15-30% lignin [3, 8, 9].

R.: The referred sentences were merged into the following sentence:
“Lignocellulosic materials are mainly constituted by 30–50% cellulose, a polymer of glucose, 20–35% hemicellulose, a heteropolymer containing xylose, as well as 15–30% lignin, and their compositions are dependent on their origin and species (hardwood, softwood or grass).”

3. All lignin extraction procedures for different methods are represented very well in detail except for The Organosolv Process in section 3.4. I recommend the authors to use one of the cited examples in this section to include a detailed explanation of this method rather than just mentioning the most appropriate solvents and the cleavage site for the extraction.

R.: In the section 3.4, the extraction of lignin by the organosolv process was approached by adding the following statement:
“The delignification process is carried out by adding mixtures of organic solvent and water that dissolve part of the lignin and hemicellulose [46]. During this process, mainly the lignin-carbohydrate and the α–O–4′

linkages are broken, but β-O-4′ can also be cleaved to a lesser extent when the lignin oligomers are released from the lignocellulose matrix [34, 47]. The recovery and separation of the dissolved lignin and hemicelluloses can be done by precipitation of lignin or evaporation of the organic solvent, after adjusting temperature, pH and concentration of the organic solvent [46, 48].”

4. Provide reference for this sentence in the text:
Although nitrobenzene is a very effective oxidant, it is also a proven carcinogen.

R.: The following reference (Ref. 72) was added to the manuscript to support the abovementioned sentence:
Holder JW. Nitrobenzene carcinogenicity in animals and human hazard evaluation. Toxicology and industrial health. 1999; 15: 445-57.

5. In figure 5, etherification protocol is shown by using propylene oxide or ethylene oxide to form epoxy functionalized lignin. Would the authors check the content of cited papers and provide information in the text how propylene oxide structure (glycidyl ether/epoxy) does not open in the presence of NaOH? This is a very well confirmed chemical reaction that epoxy groups opens in the alkalyne solutions (NaOH) to react with hydroxyl groups. It would be very important to double check the reaction and final product obtained after etherification in the referred papers.

R.: The etherification reaction in mentioned figure was modified to the following reaction:
After checking some papers, we realized that during the oxypropylation reaction in alkaline solutions, the ring on propylene oxide opens. In addition, we rearranged the main text to clarify the reaction protocol:
“The oxypropylation is the most used etherification method to modify lignin using propylene oxide in the presence of an alkaline solution, in order to prepare lignin-based epoxy resins. The resulting solution was treated with epichlorohydrin and cured using m-phenylene diamine for cross-linking [132, 133].”

6. There are few minor typos that need to be revised…

R.: All the typos mentioned by the reviewer were corrected.

RESPONSE TO THE REVIEWERS’ COMMENTS

REVIEWER #3

The authors provide an overview on the composition and properties, extraction methods and chemical modifications of lignin and its potential applications in pharmaceutical nanotechnology and nanomedicine. Furthermore, they report different preparation methods for lignin-based nanomaterials with antioxidant UV-absorbing and antimicrobial properties

that can be used as reinforcing agents in nanocomposites, in drug delivery and gene delivery vehicles for biomedical applications. It is opinion of this reviewer that the review paper can be accepted for publication in the following form without any further revision.

R.: We thank the reviewer for the time spent revising our manuscript and for recommending our paper for publication as such.

*Cover Letter

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Kristi S Anseth
Editor
Progress in Materials Science

Dear Prof. Anseth,

I am submitting the revised manuscript (Ms. Ref. No.: PMS-D-17-00037) entitled "*Properties and Chemical Modifications of Lignin: Towards Lignin-Based Nanomaterials for Biomedical Applications*" by Patrícia Figueiredo, Dr. Kalle Lintinen, Prof. Jouni T. Hirvonen, Prof. Mauri A. Kostiainen, and Prof. Hélder A. Santos for publication as a review paper in **Progress in Materials Science**.

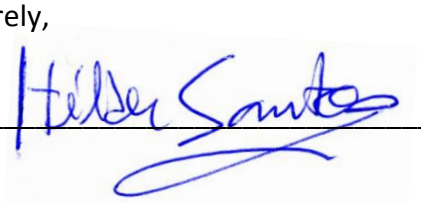
We would like first to thank you and the reviewers for their constructive comments and the given possibility to reply to those comments towards the improvement of this review. All the related comments raised by the reviewers have been addressed. **For this, we have also put additional contents in this review and re-structured few sections, as suggested by the reviewers**.

Please find enclosed the point-by-point response to the reviewers' comments, which also describe the changes made in the main text and supporting information. The marked copy of the revised manuscript shows all the changes made on revised manuscript clearly highlighted in the text.

I strongly believe that the changes introduced in the text have further improved the manuscript and the revised manuscript is now acceptable for publication. If further modifications are needed, we would be pleased to promptly make them.

Thank you in advanced for your consideration!

Yours Sincerely,



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Properties and Chemical Modifications of Lignin: Towards Lignin-Based Nanomaterials for Biomedical Applications

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Keywords: Lignin; chemical modifications; lignin-based nanoparticles; lignin-based hydrogels; biomedical applications.

Abstract (Summary)

Biorenewable polymers have emerged as an attractive alternative to conventional metallic and organic materials for a variety of different applications. This is mainly because of their biocompatibility, biodegradability and low cost of production are beneficial for industry. Lignocellulosic biomass is the most promising renewable carbon-containing source on Earth and it contains mainly cellulose, hemicellulose and lignin. Depending on the origin and species of the biomass (hardwood, softwood or grass), lignin consists of 20–35% of the lignocellulosic biomass, which is the second most abundant biopolymer after cellulose. Currently, there are four main industrial methodologies for extraction, which are sulfite, kraft, organosolv and soda processes. After it has been extracted, lignin can be modified through diverse chemical reactions. There are different categories of chemical modifications, such as lignin depolymerization or fragmentation, modification by synthesizing new chemically active sites, chemical modification of the hydroxyl groups, and the production of lignin graft copolymers. Lignin can be used for different industrial and biomedical applications, including biofuels, chemicals and polymers, and the development of nanomaterials for drug delivery but these uses depend on the source, chemical modifications and physicochemical properties. We provide an overview on the composition and properties, extraction methods and chemical modifications of lignin in this review. Furthermore, we describe different preparation methods for lignin-based nanomaterials with antioxidant UV-absorbing and antimicrobial properties that can be used as reinforcing agents in nanocomposites, in drug delivery and gene delivery vehicles for biomedical applications.

*Manuscript

Properties and Chemical Modifications of Lignin: Towards Lignin-Based Nanomaterials for Biomedical Applications

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Keywords: Lignin; chemical modifications; lignin-based nanoparticles; lignin-based hydrogels; biomedical applications.

Abstract

Biorenewable polymers have emerged as an attractive alternative to conventional metallic and organic materials for a variety of different applications. This is mainly because of their biocompatibility, biodegradability and low cost of production. Lignocellulosic biomass is the most promising renewable carbon-containing source on Earth. Depending on the origin and species of the biomass, lignin consists of 20–35% of the lignocellulosic biomass. After it has been extracted, lignin can be modified through diverse chemical reactions. There are different categories of chemical modifications, such as lignin depolymerization or fragmentation, modification by synthesizing new chemically active sites, chemical modification of the hydroxyl groups, and the production of lignin graft copolymers. Lignin can be used for different industrial and biomedical applications, including biofuels, chemicals and polymers, and the development of nanomaterials for drug delivery but these uses depend on the source, chemical modifications and physicochemical properties. We provide an overview on the composition and properties, extraction methods and chemical modifications of lignin in this review. Furthermore, we describe different preparation methods for lignin-based nanomaterials with antioxidant UV-absorbing and antimicrobial properties that can be used as reinforcing agents in nanocomposites, in drug delivery and gene delivery vehicles for biomedical applications.

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1. Introduction

Biopolymers have shown great potential as alternatives to classic metallic and organic materials for a variety of applications, because of their biocompatibility, biodegradability and low production cost **[1-3]**. The biorenewable polymers *inter alia* that have been extracted from different sources are attracting increasing attention of the research community because of their key advantages, which include: biodegradability, low density and minimization of the environmental effects related with their production and usage **[3-5]**. One example of biorenewable polymers is the lignocellulosic biomass, which are derived from wood and plant sources and is the most promising renewable carbon-containing resource on Earth **[6, 7]**. Lignocellulosic materials are mainly constituted by 30–50% cellulose, a polymer of glucose, 20–35% hemicellulose, a heteropolymer containing xylose, as well as 15–30% lignin, and their compositions are dependent on their origin and species (hardwood, softwood or grass) **[3, 8, 9]**. Thus, lignin is one of the main bio-resource raw materials that can be used for the synthesis of environmentally friendly polymers, and its high content of aromatic structures offers the possibility to replace industrially relevant aromatic polymers and fine chemicals **[10]**. However, only approximately 2% of the annually extracted lignin by the paper and pulp industry from woody biomass has been mainly used for low-value applications as dispersants, adhesives and fillers **[11]**. This is mainly due to the complex and variable lignin structure, high polydispersity and immiscibility of lignin with host polymer matrices **[6, 11, 12]**. The procedures that are applied to separate, to isolate and to chemically transform these three components can lead to a multifunctional array of bio-derived value-added fuels, chemicals and other materials **[13, 14]**. Another way to overcome these limitations is to prepare lignin nanoparticles, which improves the blending properties with host matrix, and offers a morphological and structural control of the lignin **[15-17]**. In addition, the preparation of nanostructured lignin also opens the possibility to use lignin-

based materials for high-value applications, such as drug/gene delivery and tissue engineering **[17]**.

In this review, we provide an overview of the composition and properties of lignin, the processing methods for lignin extraction, chemical modifications of lignin, and the industrial applications of lignin derivatives. Finally, we discuss the preparation of lignin-based nanomaterials for different applications, highlighting the potential of these nanomaterials for biomedical applications.

2. Lignin: Structure, Composition and Properties

Lignin accounts for approximately 30% of the organic carbon in the biosphere, and it is the second most abundant biopolymer of lignocellulosic biomass, after cellulose **[18]**. It is commonly distributed in combination with hemicellulose around the cellulose strands in primary and secondary cell walls, and it is covalently bonded to the carbohydrate/cellulose structure (**Fig. 1a**) **[6, 19]**. Lignin acts primarily as a structural component, by adding strength and rigidity to the cell walls, but it also allows the transport of water and solutes through the vasculature system of the plants and provides physical barriers against invasions by phytopathogens, and other environmental stresses. It can also prevent the degradation of structural polysaccharides by its hydrolytic properties that influence cell wall degradability **[18, 20, 21]**.

Lignin is a highly branched and amorphous biomacromolecule that typically yields molecular masses between 1 000 and 20 000 g mol^{-1}, depending upon the extraction process used **[6, 22]**. It consists of three basic phenylpropanolic monomers known as monolignols: *p*-coumaryl, coniferyl and sinapyl alcohols. When incorporated into the lignin polymer, the units that originated from the monolignols are called *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units, respectively (**Fig. 1b**) **[22-24]**. Lignin biosynthesis starts with the synthesis

of monomers, which is initiated by the deamination of the aromatic amino acid phenylalanine, and its subsequent polymerization by a cascade of oxidases and peroxidases. A sequence of hydroxylation, methylation and reduction reactions by specific enzymes leads to the production of the three basic units for the biosynthesis of lignin **[6, 25, 26]**. Next, the monolignols are exported to the apoplast through the plasma membrane and, finally, the activation of the monolignols leads to the formation of monolignol radicals and their polymerization to produce the lignin polymers, a process that is mediated by enzymes such as peroxidases, laccases and other polyphenol oxidases **[20, 27]**.

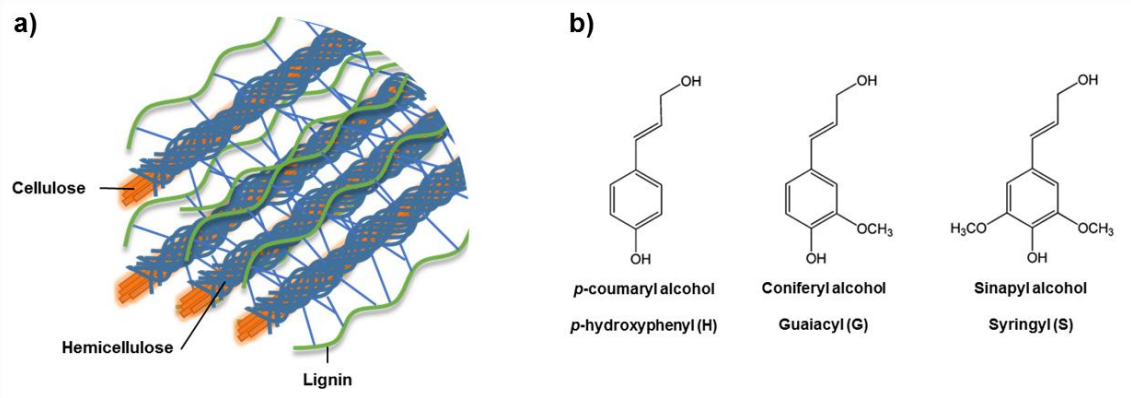

Fig. 1. Lignin distribution in lignocellulosic biomass (a) and monolignol monomers species present in lignin (b).

The main bonds between monomers in lignin are *β*–O–4′ ether linkages that make up more than 50% of the linkage structures of lignin and is a crucial target for most of the degradation mechanisms. Other bonds include *β*–5 phenylcoumaran, *β*–*β*′ resinol, α–O–4′ ether, 4–O–5′ diphenyl ether, 5–5 biphenyl and *β*–1′ diphenyl methane and these account for smaller percentages (**Fig. 2**) **[9, 28-32]**. The amount of lignin varies according to the origin of the lignocellulosic starting material, and the proportion of different monolignols and chemical bonds in lignin structure also depend on the source of the lignocellulosic biomass, because these vary between different hardwood, softwood or grass sources **[3, 8]**. In the case of

softwoods, lignin is mainly composed of guaiacyl units that are linked by ether and carbon-carbon bonds whereas for hardwoods, lignin has equal amounts of guaiacyl and syringyl units. However, the grass lignin is characterized by guaiacyl, syringyl and *p*-hydroxyphenyl units **[19, 24, 33]**. Consequently, the degree of substitution will affect the degree of lignin that crosslinks and also the rigidity of the structure **[6, 33]**.

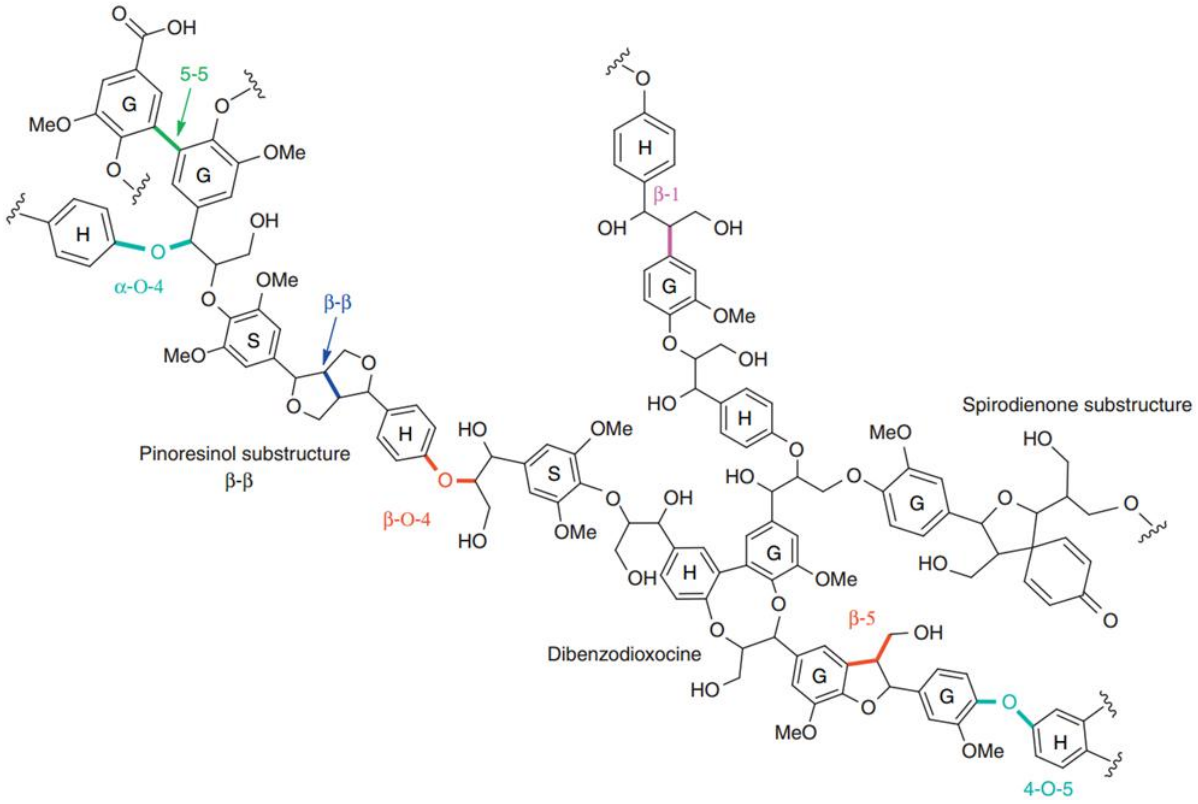

Fig. 2. Example of the lignin structure with the main linkages: *β*–O–4′, *β*–5, *β*–*β*′, *α*–O–4′, 4–O–5′, 5–5 and *β*–1′ bonds. Reproduced from ref. 32 with permission from IFP Energies nouvelles, copyright 2013 **[32]**.

The properties and advantages of lignin are similar to those of other biorenewable polymers and include: antioxidant, antifungal and antimicrobial activities, availability in large amounts as industrial waste byproducts, biodegradability and is CO_2 neutral. Compared to cellulose/hemicellulose, lignin is more resistant to chemical and biological attacks and possesses higher strength that provides structural integrity to the cell wall, protecting other

components from enzymatic degradation **[3, 10, 34, 35]**. Lignin is also able to absorb UV-radiation and has fire-retardant properties **[36, 37]**. Furthermore, lignin has several potentially beneficial physicochemical properties that make it a promising component for the renewable energy industry and other lignin-based products. The presence of the reactive functional groups allows the modification of the molecule by using targeting moieties such as peptides or antibodies and the preparation of graft copolymers. Other beneficial properties include a hydrophilic or hydrophobic feature that depends on the lignin source, good rheological characteristics, good viscoelastic properties, film-forming capacity, and compatibility with a wide variety of industrial chemicals **[6]**.

Natural lignin is almost colorless, but after acid or alkali treatment it takes on a brown or dark brown color **[14, 38]**. Furthermore, the content of phenolic, hydroxyl, carboxyl and sulfonate functional groups are affected by the extraction method used **[22]**. However, the use of lignin in value-added products can be hampered by its complicated molecular structure, which is the most challenging issue in lignin valorization approaches **[7]**. Different methods have been used for the industrial extraction of lignin, which result in intermediate products, which can have several applications.

3. Processing Methods for Lignin Extraction

Lignin extraction from lignocellulosic biomass is performed in such a way that lignin is gradually broken down to lower molecular weight (MW) products **[6, 7]**. Both extraction methodology and source of lignin will influence the physicochemical properties of the isolated product **[6, 9]**. Some key factors that can affect the extraction process are as follows: temperature, pH and pressure of the system, capability of the solvent/solute to take part in the lignin fragmentation process and avoid the lignin recondensation, and also the capacity of the solvent to dissolve lignin molecules **[9, 28]**. Currently, there are four main industrial

processes used to extract pure lignin: sulfite, kraft, organosolv and soda processes **[28, 39]**. The main processes for lignin extraction are discussed in this section and summarized in **Table 1**. The distinctive features of the extraction methods of lignin are described in the following subsections.

Table 1. Methodologies for lignin extraction, treatment conditions, solubility, MW and functional groups of lignin. (ND – No Data; [a]Conventional process; [b]Values referred to softwood).

Extraction Process	Treatment Conditions	Solubility [10]	Lignin MW (Da)	Functional Groups (%) [6]		
				COOH	OH phenolic	Methoxy
Kraft[a]	**1st:** Sodium hydroxide and/or sodium sulfide (pH=13–14 and T≈170 °C) **2nd:** Sulfuric acid (pH=5–7.5)	Alkali; Organic solvents	1000–15000	4.1[b]	2.6[b]	14[b]
Sulfite	Metal sulfite + Sulfur dioxide (Ca^{2+}, Mg^{2+} or Na^{+}) (pH=2–12 and T=120–180 °C, for 1 to 5 h)	Water	1000–50000	ND	ND	ND
Soda	13–16% wt of Sodium hydroxide (T=140–170 °C) + Anthraquinone (catalyzer)	Alkali	1000–3000	7.2–13.6	2.6–5.1	10–16
Organosolv	Organic solvents (ethanol, methanol, acetic and formic acid), usually mixed with water (T=170–190 °C)	Wide range of organic solvents	500– 5000	3.6–7.7	3.4–3.7	15.1–19

3.1. The Kraft Process

Kraft pulping is the principal method used to process lignocellulose, and it accounts for approximately 85% of the produced lignin **[40]**. In the conventional Kraft process, the delignification process is performed at high temperatures and high pH values, during which the lignin is dissolved in sodium hydroxide and sodium sulfide (white liquor) **[28, 39]**. First, lignin is dissolved by the cleavage of the ether linkages, at pH values between 13 and 14 and temperatures around 170 °C, increasing the number of phenolic hydroxyl groups that will

ionize under this pH range and, consequently, allow the solubilization of lignin **[31, 41]**. After that, lignin can be isolated from the remaining alkaline solution by acid (*e.g.*, sulfuric acid) mediated precipitation, which lower the pH to 5–7.5 **[6, 42, 43]**.

Kraft lignin has been produced commercially by the LignoBoost Technology, where lignin is extracted from pulp black liquor **[28]**. In this methodology, lignin is precipitated by acidification by using CO_2 and it is filtered in an initial chamber press filter. After, the filter cake is re-dispersed, acidified using H_2SO_4, filtered and then washed by means of displacement washing in a second chamber press filter **[44]**.

In general, kraft lignin contains small amounts of sulfur groups in its structure (1–3% by wt), and since it is only soluble at pH > 10, most of the commercially available lignin is sulfonated to become soluble in water **[31, 45]**. The mean MW of the kraft lignin usually ranges from 1000 to 3000 Da, but it can go up to as high as 15000 Da **[39, 46]**. Similarly to other isolation processes, the native lignin is submitted to some chemical and structural changes **[31]**. Nevertheless, the production of large amounts of lignin using this process is important for its transformation into high-value lignin-based materials **[12]**.

3.2. The Sulfite Process

The sulfite process is an extraction method that is commonly used in the pulp and paper industry **[28]**. This process involves the reaction between lignin and a metal sulfite and sulfur dioxide, with calcium, magnesium or sodium acting as counter ions **[6, 31]**. By changing the pulping chemicals mix and its dosage, the pH of the system can range between 2 to 12. The process is executed at temperatures that can vary between 120 to 180 °C, with a digestion time of 1 to 5 h **[28, 45]**. Under these conditions, the sulfite delignification process leads to the cleavage of α-ether (α–O–4′) and β-ether (β–O–4′) linkages of lignin, which is an acid catalyzed process. The introduction of polar sulfonic acid groups to the lignin backbone

enables its hydrolysis and dissolution, while the cellulose remains in the solid state **[39, 47]**. The sulfur content of lignosulfonates obtained by this process can range between 4 and 8% and, because of the low pK_a (less than 2) of the sulfonate groups, the isolated lignin is water-soluble under most conditions **[6, 31]**. The lignosulfonates produced by using the sulfite treatment have a higher MW compared to lignin extracted by the kraft process, which is a result of the incorporation of the sulfonate groups into the lignin skeleton. Depending on the source of lignin, the MW can vary between 1000 and 50000 Da, when the lignin comes from hardwood or softwood, respectively **[28, 39, 45]**. However, the sulfite process presented some disadvantages, including the formation of new C–C bonds that alter the lignin's structure, and the presence of high content of ash and other impurities after isolation **[39]**.

3.3. The Soda Process

Soda pulping is typically used for the treatment of non-wood material, including grass, straw and sugarcane bagasse, which accounts for 5% of the total pulp production **[31, 39]**.The solubilization mechanism of lignin is similar to that of the kraft process, as the biomass is digested at temperatures that vary between 140 to 170 °C in the presence of 13–16% by weight of aqueous solution of sodium hydroxide. However, anthraquinone is also used as a catalyzer in order to decrease the degradation of carbohydrates and to dissolve lignin **[6, 31, 39]**. The obtained soda lignin is difficult to recover by filtration or centrifugation due to the high content of carboxylic acid, which results from the oxidation of aliphatic hydroxyl groups **[6]**. The mean MW of the soda lignin is 2400 Da and it can range between 1000 and 3000 Da, depending on its carbon content **[31, 39, 48]**. However, comparing to the previous extraction methods, the soda lignin contains no sulfur, which makes it a good potential for the preparation of high-value products **[6]**.

3.4. The Organosolv Process

The organosolv process is one of the most promising alternatives to the current conventional pulping methodologies, and it is based on the treatment of the biomass using organic solvents as the delignifying agents. The commonly used solvents include: ethanol, methanol, acetic and formic acid that are usually mixed with water at temperatures that range from 170 to 190 °C **[39, 49, 50]**. In addition, basic or acidic catalysts can be added to improve the efficiency of this process **[39]**. The delignification process is carried out by adding mixtures of organic solvent and water that dissolve part of the lignin and hemicellulose **[51]**. During this process, mainly the lignin-carbohydrate and the α–O–4′ linkages are broken, but β–O–4′ can also be cleaved to a lesser extent when the lignin oligomers are released from the lignocellulose matrix **[39, 52]**. The recovery and separation of the dissolved lignin and hemicelluloses can be done by precipitation of lignin or evaporation of the organic solvent, after adjusting temperature, pH and concentration of the organic solvent **[51, 53]**. Organosolv pulping is one of the most efficient options for the further valorization of lignin and it also preserves the native structure of the lignin **[31, 39]**. Moreover, this process is relatively clean and less aggressive compared to the other processes. The obtained lignin is sulfur-free, with lower ash-content, it has higher purity due to the lower carbohydrate content and lower MW (500–5000 Da) **[39, 49, 54, 55]**. However, despite these advantages, this delignification process is not widely used because of the extensive corrosion of the equipment and the lower quality of the pulp produced compared to the soda and kraft pulping methods **[6]**. Also, under acidic conditions, random condensation reactions can occur, instead of the depolymerization reactions. For example, the formation of benzylic cations under acidic conditions can take place, leading to the formation of new C–C bonds **[39]**.

Taking all of this into consideration, the native structure of lignin undergoes a chemical transformation after the extraction process. Besides the limitations abovementioned, the structure of kraft, organosolv, sulfate and soda lignin can also vary according to the plant source. Thus, the introduction of lignin into new markets will strongly depend on its structure, reactivity and physicochemical properties **[56]**.

4. Chemical Modifications of Lignin

The chemical structure of lignin can be easily modified for the development of new materials, especially the phenolic and aliphatic hydroxyl groups. Lignin can be used with or without chemical modification, depending on the target application (**Fig. 3**) **[10]**. Without chemical transformation, lignin can be directly incorporated into a polymeric matrix to reduce production costs and improve properties. For example, unmodified lignin can serve as a UV-light stabilizer, an antioxidant, a flame retardant and an additive to promote plasticity and flow properties of an end product **[57-63]**. Although there is potential for direct industrial applications, unmodified lignin can only be incorporated in small amounts due to its weak mechanical properties and thermal instability. On the other hand, lignin can be chemically modified in order to be used as a starting material for polymer synthesis or conversion into chemicals and fuels **[10, 64]**. There are four different approaches to modify lignin chemically: 1) lignin depolymerization or fragmentation, using the lignin as a carbon source or to cleave it into small fragments that contain aromatic rings; 2) modification of lignin by synthesizing new chemically active sites; 3) chemical modification of the hydroxyl groups present in the lignin structure; and 4) production of graft copolymers **[10, 64]**. These chemical modifications are very dependent on the reactivity of the functional groups and structural features of the lignin that is used. The main features of these processes are discussed in the following sub-sections and are summarized in **Table 2**.

Table 2. Summary of the main chemical modifications of lignin (methodology and final products), including lignin depolymerization, synthesis of new chemical sites and functionalization of the hydroxyl groups.

Chemical Modification	Methodology	Final Products
Lignin Depolymerization		
Pyrolysis ***Conventional (or slow)*** ***Flash (or fast)***	Heating the lignin at T=500 °C, slow heating rate and long residence time from 5–30 min. Heating the lignin at T range 600–1000 °C, high heating rate and short residence time range 0.5–5 seconds. **Catalysts:** Zeolites (ZSM5); Transition metal oxides (Co_3O_4, MoO_3, NiO, Fe_2O_3, MnO_3, CuO); Transition metals (Mo, Cu, Mn, Fe, Ni).	Solid char; Liquid oil (bio-oil); Gases.
Oxidation ***Hydrogen Peroxide*** ***Nitrobenzene*** ***Oxygen***	Alkaline conditions: T range 80–90 °C; Acidic conditions: T range 130–160 °C. **Catalysts:** transition metals, such as Fe^{2+}, Cu^{1+}, Mn^{3+} and Co^{2+}. Alkaline conditions: T range 170–190 °C; residence time of 1–4 h. Alkaline conditions: benzaldehydes; Acidic conditions: benzoic acids; **Catalysts:** Transition metals (Cu^{2+}, Fe^{3+}, Mn^{2+} and Mn^{3+}, Co^{2+} and Zr^{4+}) and metal oxides (CuO, TiO_2 and ZnO).	Mono- and Dicarboxylic acids; Aldehydes: Vanillin, Syringaldehyde, *p*-Hydroxybenzaldehyde, 3,4,5-Trimethoxy benzaldehyde; Acids: Vanillic, Syringic, *p*-Hydroxybenzoic acid; Alcohols: 2-Hydroxy benzyl and Catechol
Hydrogenolysis	Addition of H_2 to break down C–O–C linkages in the lignin structure; T range 300–600 °C; **Catalysts:** noble metals (Pt, Ru, Pd, and Rh), metal oxides (Pd/C, $CoMo/Al_2O_3$).	Phenols, Guaiacols, Syringols, Trimethoxybenzenes,
Hydrolysis	Addition of water to cleave the lignin molecules (under subcritical or supercritical conditions); T range 280–400 °C; Residence time: Some minutes to a few hours; Pressure range 20–25 MPa; **Catalysts:** K_2CO_3, $Ca(OH)_2$ and Na(OH).	Methoxyphenols; Hydroxyphenols; Catechol; Guaiacol.
Gasification	Reactions: hydrolysis, polymerization, hydrogenation, water–gas shift, steam reforming, and methanation. T = 700 °C; Pressure range 15–27.5 MPa	CO_2, CO, H_2 and CH_4, C_2H_4, C_2H_6

	Catalysts: alkali and alkali salts (NaOH, KOH, Na_2CO_3, CaO, $NaHCO_3$, etc.), transition metals (Ni, Pt, Ru and Rh), metal oxides (*e.g.*, $Ni/MgAl_2O_3$).	
Synthesis of New Chemically Active Sites		
Hydroxyalkylation	Reaction processes: demethylation, phenolation and methylolation (reaction of lignin with formaldehyde in alkaline medium).	Lignin-phenol-formaldehyde resins (adhesive properties)
Amination	Mannich reaction: the amination of lignin is done by using different types of amines and formaldehyde (under alkaline, neutral or acidic conditions).	Aminated lignins (asphalt emulsifier, cationic surfactants, epoxy resins and polyurethane synthesis)
Nitration	Using non-aqueous solvents with nitrating agents (*e.g.*, nitric acid with acetic anhydride, acetic acid or sulfuric acid).	Polyurethane-nitrolignin films
Sulfomethylation **Sulfonation**	Adding methylene sulfonate groups to lignin structure, by using methanol and alkali metal sulfite in water at 100 °C, under neutral to basic pH. Adding sulfonate groups to lignin, by using sulfuric acid or sodium sulfite.	Sulfomethylated products (dye dispersant) Sulfonated lignin (cement dispersant)
Functionalization of Hydroxyl Groups		
Alkylation/dealkylation	**a)** Reaction with diazoalkanes; **b)** Reaction with alcohol in the presence of a catalyst (*e.g.*, hydrochloric acid); **c)** Reaction using alkylsulfates and sodium hydroxide.	Demethylated lignin + Polyethylenimine (wood adhesive)
Esterification	**a)** Ring opening reactions using cyclic esters (*e.g.*, ε-caprolactone and lactide); **b)** Condensation polymerization with carboxylic acid chloride (*e.g.*, sebacoyl chloride and terephtaloyl chloride); **c)** Dehydration polymerization with dicarboxylic acids (*e.g.*, dimeric acid and carboxytelechelic polybutadiene).	Lignin-based polyesters (for producing lignin-based epoxy resins, polyurethanes, and unsaturated thermosetting composites)
Etherification	**a)** Polymerization using alkylene oxide (*e.g.*, ethylene oxide and propylene oxide); **b)** Polymerization with epichlorohydrin; **c)** Cross-linking using diglycidyl ethers; **d)** Solvolysis of lignin with ethylene glycol.	Lignin-based polyethers (*e.g.*, lignin-based epoxy resins)
Phenolation	Reaction between lignin and phenol in the presence of organic solvents (*e.g.*, methanol or ethanol).	Lignin phenol formaldehyde resins; Lignin-based polyurethanes films
Urethanization	**a)** One step reaction by adding diisocyanate and another diol to the lignin; **b)** Two-step reaction with the production of an isocyanate-based prepolymer, and then the polymerization of lignin with the prepolymer, acting as a polyol chain extender.	Lignin-based polyurethane

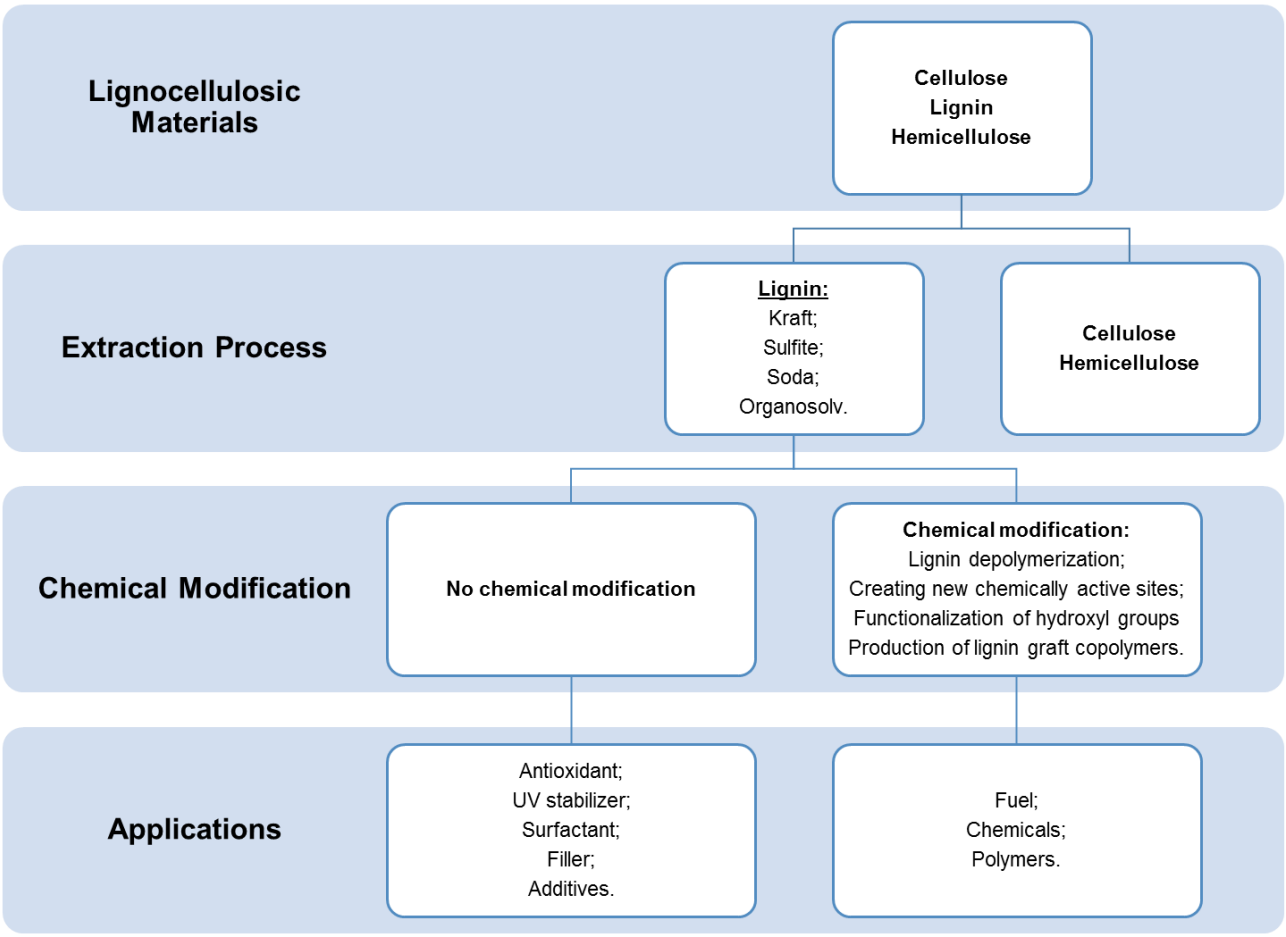

Fig. 3. Schematic representation of the main processes for lignin extraction and possible chemical modifications performed in order to valorize lignin, depending on the applications.

4.1. Lignin Depolymerization

Lignin depolymerization, or fragmentation, is a promising method to transform lignin raw materials and generate valorized lignin-based products. Therefore, the lignin molecules are converted into small compounds for further application, including fuels and basic chemicals or oligomers **[65, 66]**. Several thermochemical methods have been studied for lignin depolymerization, such as pyrolysis, oxidation, hydrogenolysis, hydrolysis and gasification (**Fig. 4**) **[67]**.

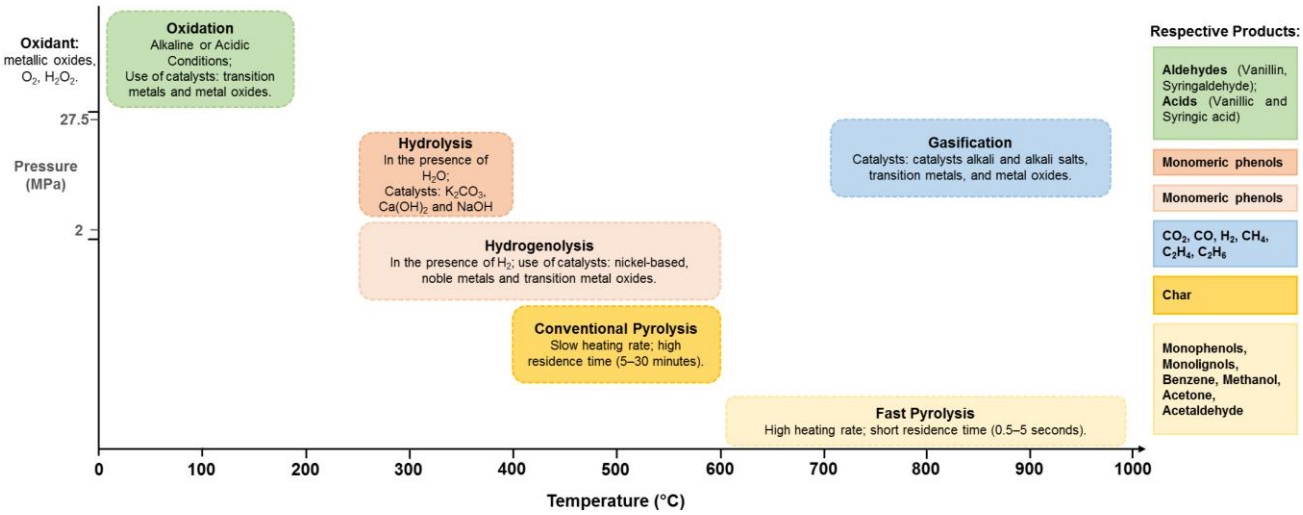

Fig. 4. Overview of the methodologies for lignin depolymerization according to the temperature, presence of oxidants and pressure.

4.1.1. Pyrolysis

Pyrolysis is the thermal treatment that converts lignin into solid char, liquid oil (bio-oil) or gases, in the absence of oxygen with or without any catalyst **[65, 67]**. This is a complex process and the products obtained during the procedure are dependent on several factors, including the feedstock type, temperature of the reaction, heating rate and additives **[67, 68]**. Pyrolysis can be classified into two different categories based on whether it is conventional (slow) pyrolysis and flash (fast) pyrolysis **[10, 69]**. In conventional pyrolysis, lignin is heated to temperatures of around 500 °C, with a slow heating rate and a residence time that ranges between 5 to 30 min. In contrast, the flash pyrolysis heating is carried out at temperatures between 600 to 1000 °C, with a higher heating rate and shorter residence time (0.5–5 seconds) than in conventional pyrolysis **[10, 32, 69, 70]**. By changing all these parameters, it is possible to obtain different products from the same biomass. For example, when the purpose is to obtain a high level of char production, low temperature and low heating rates should be used. On the other hand, low temperature, high heating rate and short residence time should be chosen to maximize the yield of liquid products obtained from the biomass. In order to maximize the yield of gas production, high temperature combined with

extended residence time and low heating rate are required **[69, 70]**. Lignin can also be converted by catalytic fast pyrolysis using different catalysts, such as zeolites (ZSM5), transition metal oxides (Co_3O_4, MoO_3, NiO, Fe_2O_3, MnO_3 and CuO), and supported transition metals (Mo, Cu, Mn, Fe and Ni) **[71, 72]**.

4.1.2. Oxidation

Lignin oxidation is a method that is used to obtain aldehydes, such as vanillin and syringaldehyde, and their corresponding acids, vanillic and syringic acid. Oxidants are used to preserve the aromatic rings present in the lignin structure, with or without catalysts (Cu^{2+} and Co^{2+} salts) **[10, 73, 74]**. The most common oxidants used for lignin depolymerization are nitrobenzene, metallic oxides, oxygen and hydrogen peroxide **[67]**. Lignin is a promising candidate for oxidation reactions due to the presence of hydroxyl (phenolic) groups in its structure. The oxidation rate at a given temperature can be controlled by the amount of phenolic groups, pH (alkaline or acidic oxidation), and concentration of oxygen **[67, 75]**.

The oxidation process with hydrogen peroxide in either alkaline or acidic conditions has been investigated. Under strong alkaline conditions the cracking reaction has been performed at low reaction temperatures that range from 80 to 90 °C. In contrast, under acidic conditions, higher temperatures that range from 130 to 160 °C are required to achieve the same degree of cracking. The main products obtained from the oxidative degradation of lignin were mono- and dicarboxylic acids and the intermediate products were aldehydes **[76]**. Some studies showed that the addition of selected transition metals, such as Fe^{2+}, Cu^{+}, Mn^{3+} and Co^{2+} can accelerate the decomposition of hydrogen peroxide into molecular oxygen **[77, 78]**.

When aldehydes are the desired product, the oxidation of lignin by hydrogen peroxide may not be the optimum method **[67]**. In contrast, the alkaline nitrobenzene oxidation allows the production of the aldehydes (vanillin, syringaldehyde, and *p*-hydroxybenzaldehyde) along with their respective

acids (vanillic acid, syringic acid, and *p*-hydroxybenzoic acid). Moreover, temperatures in the range of 170–190 °C and residence times in the range of 1–4 h are required to obtain these products, though these conditions depend on the source of lignin **[79]**. Although nitrobenzene is a very effective oxidant, it is also a proven carcinogen **[80]**. Consequently, the transition metals and metal oxides have been used in order to replace the use of nitrobenzene in lignin oxidation **[67]**.

The addition of transition metals and metal oxides in combination with oxygen and air have the potential to increase the production of phenolic compounds. For example, transition metal ions such as Cu^{2+}, Fe^{3+}, $Mn^{2+/3+}$, Co^{2+} and Zr^{4+} can improve the production of benzaldehydes and benzoic acids from lignin oxidation with oxygen as the oxidizing agent **[78, 81]**. Furthermore, the pH value of the reaction seems to be an important factor for product selectivity. For example, alkaline conditions are preferred for the formation of benzaldehyde, whereas acidic conditions are favorable for the production of benzoic acids **[78]**. CuO can be utilized in the direct oxidation of lignin and other metal oxides, such as TiO_2 and ZnO. These metal oxides can also be used to facilitate photochemical degradation of lignin into a mixture of aldehydes (vanillin, syringaldehyde and 3,4,5-trimethoxy benzaldehyde), acids (vanillic acid and 4-hydroxybenzoic acid) and alcohols (2-hydroxy benzyl alcohol and catechol) **[78, 82-84]**.

The lignin depolymerization occurs in nature via oxidative enzymes, including laccases and peroxidases (lignin peroxidase and manganese peroxidase), that are secreted by some fungi and bacteria. In addition to the aforementioned, the oxidation methods using these naturally occurring enzymes are undergoing industrial development as new biocatalysts. The redox potential of these enzymes is used to complement other catalyzers, allowing a synergetic action on lignin depolymerization in these developing methodologies **[64, 85, 86]**.

4.1.3. Hydrogenolysis

Hydrogenolysis or hydrogenation represents a process in which the addition of hydrogen will break down the C–O–C bonds into lignin. Compared to pyrolysis, the reaction is generally performed at lower temperatures that range from 300 to 600 °C and allows higher yields of monophenols and less char formation **[65, 67]**. The reaction is performed by adding a hydrogen-donating solvent (tetralin, sodium formate and formic acid) or gaseous hydrogen **[67, 87-91]**. For example, an efficient method for the hydrogenation of lignin in the presence of H_2, isopropanol and using Ni–Mo_2C/C as catalyst has been described, in which under mild conditions (T = 250 °C, a residence time of 2 h and 2.0 MPa initial H_2 pressure), approximately 60% by weight of the lignin was converted into phenols, guaiacols, and trimethoxybenzenes **[92]**. Apart from using nickel-based catalysts, some other studies have reported that lignin is hydrogenated to monomeric phenols (*e.g.*, guaiacols and syringols) by using other type of catalysts, including the noble metals such as Pt, Ru, Pd, and Rh; metal oxides, such as Pd/C, CoMo/Al_2O_3, and activated carbon-, alumina- or silica-supported Ru or Pt **[91, 93-96]**.

4.1.4. Hydrolysis

Hydrolysis is the process where water is used to cleave the lignin molecules. Aqueous solutions under subcritical or supercritical conditions are used, and commonly at temperatures that vary from 280 to 400 °C. Reaction times range from minutes to a few hours, under 20 to 25 MPa of total pressure **[32, 67]**. Close to its critical point (22 MPa and 374 °C), water manifests several interesting properties, such as low viscosity and high solubility of organic substances, which makes supercritical water an excellent medium for effective and fast reactions. The high concentration of H^+ and OH^- at subcritical conditions can accelerate acid- or base-catalyzed hydrolysis reactions **[32, 97, 98]**. Although the higher conversion to methoxyphenols or hydroxyphenols occurs under supercritical conditions, subcritical conditions are usually preferred. Additionally, various catechols and guaiacols are produced by hydrolysis of the ether bonds. However, with the increase of the

residence time during the hydrothermal treatment, soluble products are converted into insoluble products due to re-condensation reactions. Catalysts, such as K_2CO_3, $Ca(OH)_2$ and NaOH, are required in hydrothermal synthesis in order to overcome this problem, and their use also increase the liquid yield **[32]**.

Mixtures of water with other solvents have been used in order to improve the lignin solubility and prevent cross-linking reactions **[32, 99]**. Mixtures that contain 50/50 (v/v) water/co-solvent, in which the co-solvents are phenol, ethanol, acetone or formic acid, have been demonstrated to be more efficient for the lignin degradation regarding the yield of reaction **[32, 100-103]**.

4.1.5. Gasification

Gasification is the process that converts lignin into gases, where the principal products obtained during this reaction are CO_2, CO, H_2 and CH_4 **[65, 104]**. Additionally, small amounts of C_2H_4 and C_2H_6 can also be produced and these fuel gases have several applications, including use in fuel cells, gas turbines, and synthesis gas **[105]**. The complete gasification of lignin entails a group of successive reactions, such as hydrolysis, polymerization, hydrogenation, water–gas shift, steam reforming, and methanation, all of which are affected by temperature, reaction time, pressure, concentration, and catalysts. Some studies have reported that the temperature required for complete gasification of lignin was at least 700 °C **[105, 106]**. Additionally, higher pressures (15 to 27.5 MPa) improved the gasification efficiency of lignin under hydrothermal conditions and allowed greater yields of H_2 and CH_4. The lignin decomposition rate was also increased by a higher water density caused by using higher pressures **[107-109]**. Furthermore, the addition of catalysts for lignin hydrothermal gasification are necessary to increase the hydrogen yield production and enhance reactions such as water-gas shift, methanation and hydrogenation. These catalysts are mainly alkali and alkali salts (NaOH, KOH, Na_2CO_3, CaO, $NaHCO_3$, etc.), transition metals (Ni, Pt, Ru and Rh), and metal oxides (e.g. $Ni/MgAl_2O_3$) **[105, 106, 110, 111]**.

4.2. Synthesis of New Chemically Active Sites

Lignin possesses several functional groups, which include the following: hydroxyls, methoxyls, carbonyls and carboxyls. These functional groups can be modified for different applications, which will increase the value of the modified lignin. These modifications consist of synthesizing new macromonomers that are more effective and reactive by increasing the reactivity of hydroxyl groups or changing the nature of chemically active sites. Consequently, the chemical reactivity of lignin increases, the fragility of lignin-derived polymers is reduced, the lignin solubility in organic solvents is increased and thus the lignin processing improves. In this way, several chemical modifications have been used to introduce new chemical sites in lignin structure (**Fig. 5**), including hydroxyalkylation, amination, nitration, sulfomethylation and sulfonation, which are briefly discussed in the next subsections **[10, 112]**.

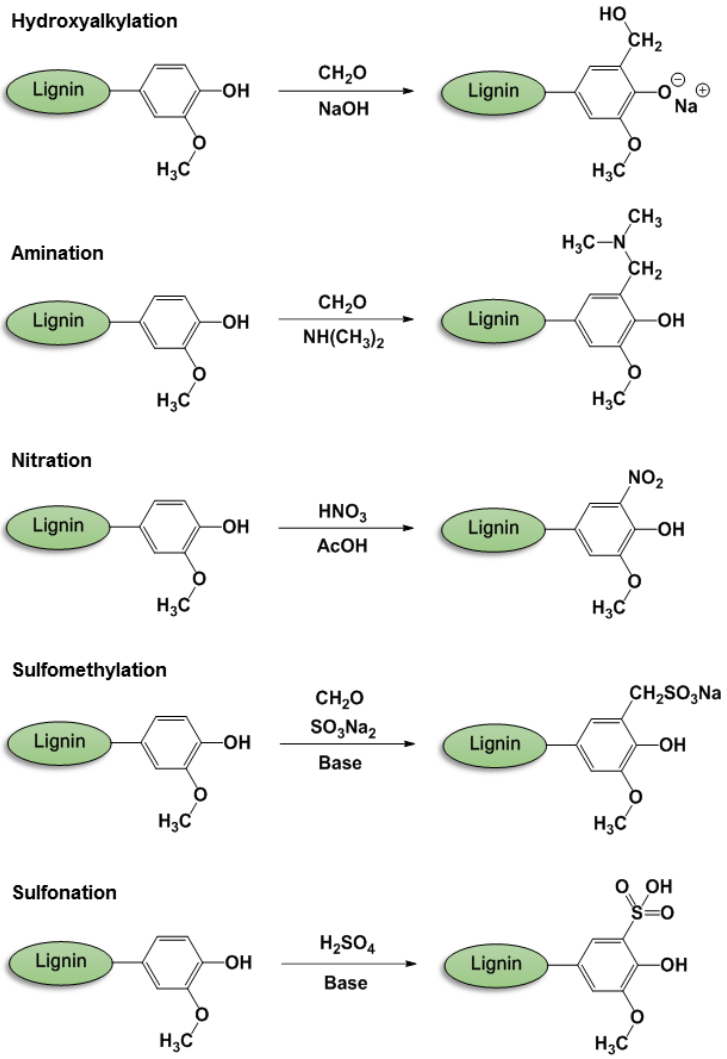

Fig. 5. Overview of the chemical modifications of lignin: synthesis of new chemically active sites.

4.2.1. Hydroxyalkylation

Lignin has been studied as a complement for phenolic wood adhesives. For example, the synthesis of phenol-formaldehyde resins has been replaced by the synthesis of lignin-phenol-formaldehyde resins **[10, 113]**. The reactivity of lignin can be enhanced by demethylation, phenolation and methylolation reaction processes. The reaction of lignin with formaldehyde in an alkaline medium is a prerequisite to the introduction of methylol groups into the lignin structure and is the basis of the lignin methylolation modification process **[10, 114]**. Hydroxyalkylation of lignin has been performed to give adhesive strength with low free formaldehyde content by replacing 40% by weight phenol in phenol-formaldehyde resin synthesis **[10, 115]**. Other aldehydes than

formaldehyde have been used for the integration of lignin into phenolic resins, these include glutaraldehyde and glyoxal, because unlike formaldehyde they are non-toxic and non-volatile **[10, 116-118]**.

4.2.2. Amination

Amination methods introduce the amine functional groups into the lignin structure, which are ionizable and positively charged under acidic conditions **[119, 120]**. There are several chemical methods used for the introduction of amine groups, for example the Mannich reaction is one of the simplest method **[120]**. In Mannich reactions, the amination of lignin structures can be carried out under alkaline, neutral or acidic conditions. Usually, a Mannich reaction is achieved by charging different types of amines and formaldehyde under alkaline conditions **[120]**. For example, the Mannich reaction has been performed for 1-guaiacyl-1-*p*-hydroxyphenylethane, a simple phenolized sulfuric acid lignin model, and it has been reacted with dimethylamine and formaldehyde **[121]**. Its products have been used in the preparation of asphalt emulsifiers, cationic surfactants, epoxy resins and polyurethane **[10, 120-122]**.

4.2.3. Nitration

The nitration of lignin is commonly performed in non-aqueous solvents by using nitrating agents, such as nitric acid with acetic anhydride, acetic acid or sulfuric acid. The resulting product is a yellow to brown amorphous powder, which has a MW that ranges from 600 to 2000 Da and a nitrogen content that varies between 6 to 7% **[10, 119, 123]**. Different graft interpenetrating polymer networks (IPNs), with different NCO/OH molar ratios have been produced from polyurethane (PU) and nitrolignin (NL), by varying the content of 1,4-butanediol as the chain-extender. Increasing the NCO/OH molar ratios also increases the glass transition temperature, the

tensile strength and stiffness of the PU-NL films, whereas the breaking elongation and strain recoverability decreases. Additionally, the introduction of NL into PU limited the motion of the PU molecules, allowing a relatively high cross-link network structure in the PU-NL films. As a consequence, the graft-INPs increased the strength and toughness and also maintained the retractability of the constituent materials **[124, 125]**.

4.2.4. Sulfomethylation and Sulfonation

Sulfomethylation introduces methylene sulfonate groups into the lignin structure in order to increase the number of sulfonate groups in the lignin product and improve its tanning capacity. This reaction is performed with equal moles of methanol, alkali metal sulfite salt and reactive phenolic repeat units of lignin dissolved in water at 100 °C under neutral to basic pH conditions **[119, 126]**. Also, it is possible to synthesize sulfomethyl products of lignin with different degrees of substitution of aromatic rings and side-chains by changing the conditions and ratios of reagents used in this reaction **[127]**. These sulfomethylation products have good dispersibility properties and can be used as a dye dispersant **[128]**.

Sulfonation is a reaction in which sulfonate groups are added to the lignin, by using sulfuric acid or sodium sulfite **[119]**. Among other applications, sulfonated lignin has been used as a dispersant for the cement matrix, due to the high zeta-potential value that contributes to the strong electrostatic repulsion forces between cement particles **[119, 129]**.

4.3. Functionalization of Hydroxyl Groups

As mentioned above, lignin presents in its structure phenolic hydroxyl groups and aliphatic hydroxyl groups at the C-γ and C-α positions on the side chain. Phenolic hydroxyl groups are the most reactive functional groups and can affect the chemical reactivity of the newly formed material. Modifications on hydroxyl groups can lead to the formation of polyol derivatives of lignin. For that

to occur, several reactions have been studied to functionalize the lignin with different functional groups (**Fig. 6**), and these include reactions such as alkylation, esterification, etherification, phenolation and urethanization, which are briefly discussed in the next subsections **[10]**.

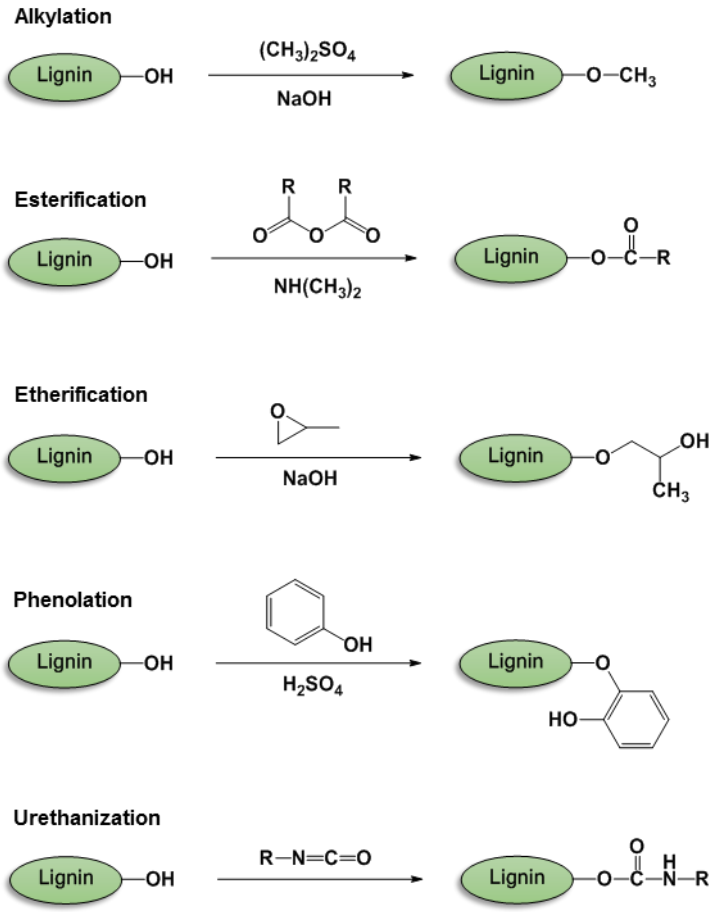

Fig. 6. Overview of the chemical modifications of lignin: functionalization of the hydroxyl groups.

4.3.1. Alkylation/Dealkylation

Lignin presents different sites for alkylation, including the oxygen atoms of the hydroxyl, carbonyl and carboxyl groups. There are three methods for alkylating lignin, which can include a reaction with diazoalkanes, a reaction with alcohol in the presence of a catalyst (*e.g.*, hydrochloric acid), or a reaction using alkylsulfates and sodium hydroxide **[119]**. For example, demethylation is one of the most well-known examples of alkylation/dealkylation reactions that involve lignin in which the demethylated lignin structures are a byproduct in DMSO production. The synthesis starts

with the reaction between lignin and molten sulfur in alkaline media. This demethylated lignin, in combination with polyethylenimine, is used as a formaldehyde-free wood adhesive **[130]**.

4.3.2. Esterification

Esterification is possibly the easiest of the reactions to produce lignin-based polyesters that involve the hydroxyl groups of lignin. Esterification can be performed by using three different procedures, which are: ring opening reactions using cyclic esters, condensation polymerization with carboxylic acid chloride, and dehydration polymerization with dicarboxylic acids **[10, 53]**. For example, the reaction between ε-caprolactone with hydroxypropylated lignin produces lignin-based polyesters with a star-like shape. In these star shapes, hydroxypropylated lignin forms the core, whereas the polycaprolactones form the arm segments **[53, 131]**. Apart from ε-caprolactone, lactide can also be used for copolymerization with lignin to produce lignin-polylactide polyester using triazabicyclodecene as a catalyst in metal and solvent free systems **[53, 132]**. An example of condensation polymerization was obtained when lignin-based polyesters were prepared by reacting lignin with the dicarboxylic acid chlorides, sebacoyl chloride and terephtaloyl chloride in organic solvents **[53, 133]**. Dehydration polymerization procedures that use dicarboxylic acids have been proposed. For example, dimeric acid, a dicarboxylic acid that is synthesized by dimerizing unsaturated fatty acids obtained natural oils, can be applied in the co-esterification with enzymatically hydrolyzed lignin, which makes the co-ester more flexible **[134]**. Additionally, adding carboxytelechelic polybutadiene to lignin can also produce lignin-based polyesters **[135]**.

These lignin esterification reactions that involve the ε-caprolactone and different anhydrides are performed in order to increase the lignin reactivity for the production of lignin-based epoxy resins, polyurethanes and unsaturated thermosetting composites **[53, 136-138]**.

4.3.3. Etherification

The preparation of lignin-based polyethers can be performed by one or any combination of the following procedures: polymerization using the alkylene oxides (*e.g.*, ethylene oxide and propylene oxide), polymerization with epichlorohydrin, cross-linking by using diglycidyl ethers, and solvolysis of lignin with ethylene glycol. The aromatic moieties present in the lignin structure will improve the mechanical and thermal properties of epoxy resins **[53]**. The oxypropylation is the most used etherification method to modify lignin using propylene oxide in the presence of an alkaline solution, in order to prepare lignin-based epoxy resins. The resulting solution was treated with epichlorohydrin and cured using *m*-phenylene diamine for cross-linking **[17, 139]**. Lignosulfonate is first submitted to phenolation using phenol, β-naphtol and bisphenol, and then epoxidized using epichlorohydrin in order to improve the reactivity of the lignin and increase the phenolic group content **[53, 140]**. Lignin from woody biomass can also be grafted with ethylene glycol/ethylene carbonate by solvolysis at 150 °C to introduce ethylene glycol chains into the hydroxyl groups of lignin **[53, 141]**.

4.3.4. Phenolation

Phenolation, also known as phenolysis, is the process by which lignin is modified by reaction with phenol in the presence of organic solvents such as methanol or ethanol in an acidic medium **[10]**. This reaction is commonly used to modify lignosulfonates in order to increase the content of phenol groups and improve the reactivity of the target lignin structure **[10, 142]**. Phenolysis is utilized in the synthesis of phenol–formaldehyde resins before the condensation reaction with formaldehyde. The reaction can be performed at 70 °C for a few hours, after which lignin is added to a phenol-ethanol solution. The curing time and viscosity of the lignin phenol formaldehyde resins are comparable to standard commercial phenol–formaldehyde resins, and this product is used to provide wood adhesive capacity in the construction of particle board **[10, 142, 143]**. Additionally,

lignin that has been modified with cardanol, a natural phenol, can also be used to produce lignin-based polyurethane films with improved properties such as film flexibility **[10, 144]**.

4.3.5. Urethanization

The urethanization process involves the reaction between lignin hydroxyl groups and isocyanate groups to form a urethane link. Traditionally, polyurethanes have been produced from polyols and diisocyanates to provide versatile products. Examples of such products include low temperature elastomers and flexible or rigid adhesives with high tensile strength. Since the lignin structure is rich in hydroxyl groups, it can function as a polyol **[10, 53]**. The improvement of the mechanical properties of lignin-based polyurethanes can be achieved by chemical modifications on lignin such as hydroxyalkylation to introduce soft segments, or by adding other polyols such as polyethylene glycol (PEG) or other diols **[10, 53, 145]**. There are two different approaches for the synthesis of lignin-based polyurethane. The first approach is a one-step reaction that occurs by adding diisocyanate and another by adding diol as the co-monomer. The second approach is a two-step reaction with the first step being the production of a prepolymer using isocyanate together with a polyol, and the second the polymerization of lignin with the prepolymer, acting as a chain extender **[10, 53]**. In the first approach, lignin is directly used in combination with isocyanate and polyols without further chemical modification in order to produce lignin-based polyurethane. For instance, lignin can be mixed with 4,4′-diphenylmethane diisocyanate and PEG to produce lignin-based polyurethane **[10, 146]**. An example of the second approach, is the formation of prepolymers with hydroxyl terminated polybutadiene and 2,4-toluene diisocyanate, which are subsequently reacted with lignin to produce a polyurethane **[53, 147]**.

4.4. Production of Lignin Graft Copolymers

Lignin can be used for the development of lignin graft copolymers in which the polymer chains are attached to the hydroxyl groups on the lignin structure, which produces a star-like branched copolymer with a lignin core. Some examples of polymerization reactions were briefly mentioned above in the chemical modifications section. Two different procedures have been carried out in order to elaborate the lignin graft copolymers: "grafting from" and "grafting to" techniques **[12]**.

4.4.1. "Grafting from" Technique

In the "grafting from" technique, lignin is used as macro-initiator for the polymerization during which a monomer reacts with hydroxyl groups present in lignin and the polymer chain is assembled on the lignin core. The ring opening polymerization of different monomers and radical polymerization of vinylic monomers are two approaches that have been performed to elaborate lignin-graft copolymers by "grafting from" procedure (**Fig. 7**) **[12]**.

4.4.1.1. Ring Opening Polymerization

The reaction of lignin –OH groups with propylene oxide is one of the most common ring opening polymerization reactions that lead to the production of oxypropylated lignin (**Fig. 7**) **[12, 148, 149]**. Using this reaction, the decrease of the *Tg* and viscosity of the copolymer can be achieved by increasing the grafted chain length.**[148, 150]** The resulting copolymer has been commonly used as a "macro"-monomer the synthesis of polyurethane foams as has been described in sub-section *4.3.5***[149]**. Other polymers than propylene oxide have been used for the production of lignin graft copolymers, and these include ε-caprolactone and lactide (**Fig. 7**). The reaction between the hydroxyl groups in lignin and ε-caprolactone leads to the production of lignin-polycaprolactone copolymers in which the ratio of ε-caprolactone/hydroxyl groups determines the length of the grafted polycaprolactone **[12, 131]**.The chain length strongly affects the thermal properties of the resulting copolymers. An amorphous structure can be achieved for ratios of ε-

caprolactone to hydroxyl groups that are below 5, and the grafted polycaprolactone chains crystallize at higher ratios **[131, 138, 151]**. In addition, lignin-poly(lactic acid) copolymers have been synthesized by reacting the hydroxyl groups in lignin and lactide via ring opening polymerization (**Fig. 7**) **[12]**. The thermal properties of the lignin-poly(lactic acid) copolymers depend on the weight fraction of lignin, and also on the chain length of the resulting copolymer. The lignin-poly(lactic acid) copolymers also display a glass transition temperature that ranges from 45 to 85 °C **[132]**.

4.4.1.2. Radical Polymerization

The radical polymerization of vinylic monomers onto lignin has also been used to prepare lignin-graft copolymers by a "grafting from" procedure. This process involves the creation of a radical on the lignin structure that initiates the polymerization of a vinylic monomer, usually by using irradiation or a chemical initiator (peroxide), after which the synthesis proceeds as shown in **Fig. 7** **[12, 152, 153]**. Several copolymers have been produced by this process, including lignin-polystyrene, lignin-poly(acrylic acid) and lignin-poly(vinyl acetate), in order to improve the properties of the resulting copolymers **[154-157]**. However, compared to the ring opening polymerization process, radical polymerization has some disadvantages: 1) there is less control of the grafting reaction; 2) the grafting efficiency can be reduced by the homopolymerization of the vinylic monomers; 3) the radicals are resonance-stabilized on different positions on the aromatic ring of the lignin, thus it is not possible to know exactly where the grafting takes place on the lignin structure; and 4) coupling reactions between the lignin radicals can also occur and this competes with the grafting **[12]**.

4.4.1.3. Atom Transfer Radical Polymerization

In order to overcome the abovementioned limitations, the atom transfer radical polymerization (ATRP) was developed as an efficient method to regulate the radical grafting of vinylic monomers onto the lignin structure. The ATRP method allows the formation of long polymer chains with well-defined structures and low dispersity, provided that the radical polymerization is carried out in a controlled manner **[10, 158, 159]**. Briefly, the method starts with the esterification of lignin by using 2-bromoisobutyryl bromide as the initiator. The monomers can be grafted onto the lignin macroinitiator in the presence of a catalyst such as CuBr with 1,1,4,7,10,10-hexamethyltriethylenetetramine as the ligand, which leads to the formation of lignin-based copolymers (**Fig. 7**) **[17, 158]**. By using this method, the N-isopropylacrylamide (NIPAM) was copolymerized with kraft lignin to produce thermoresponsive lignin-polyNIPAM in which the thermal decomposition temperature of the copolymers increased with increasing of the polymerization degree **[160]**. When heated at temperatures above 32 °C, the copolymer underwent the typical hydrophilic-to-hydrophobic transition that led to the precipitation of the copolymer **[160]**. Later, the same method was used to graft polyNIPAM in lignin nanofiber mats produced by electrospinning, with both thermal and ionic responsive characteristics **[161]**.

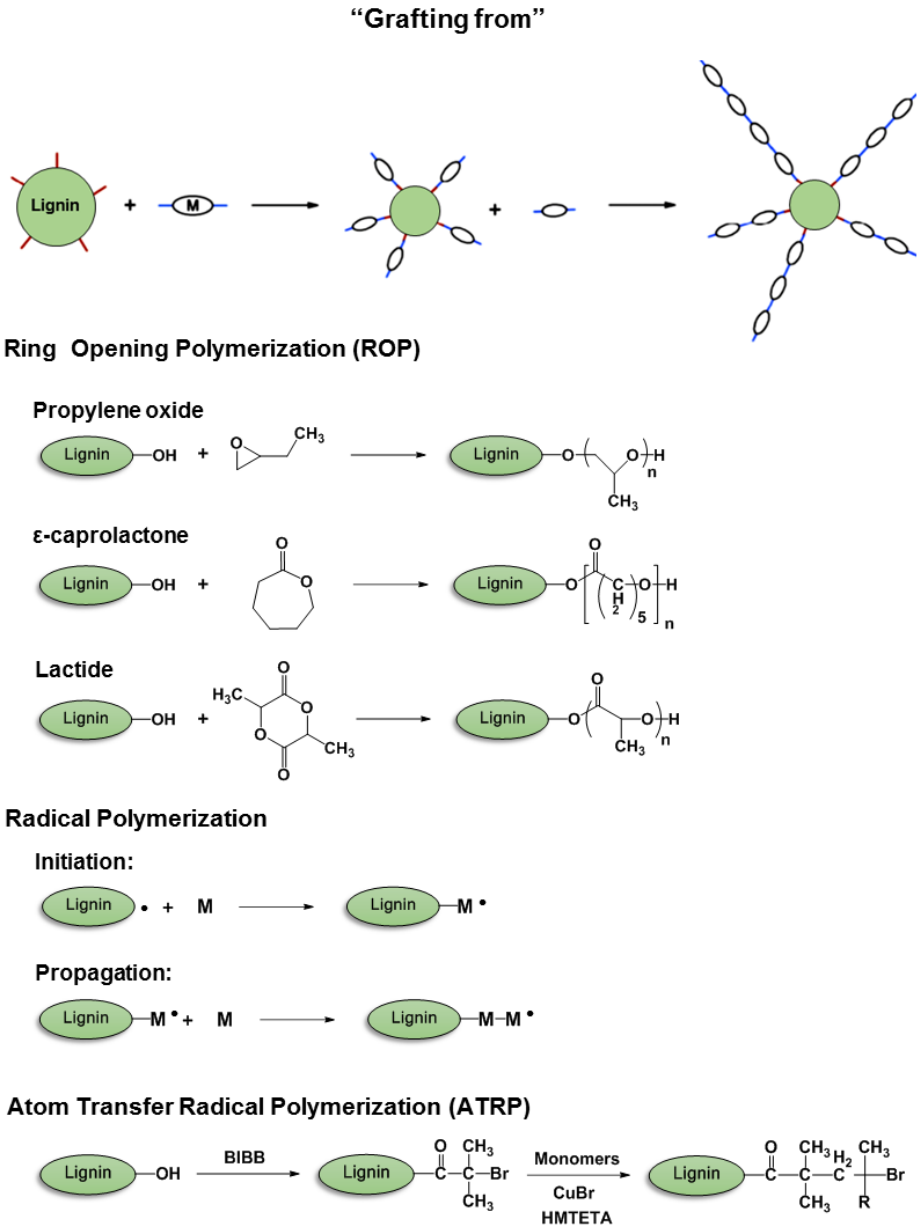

Fig. 7. Schematic representation of the lignin-graft copolymers synthesis by the "grafting from" method (ring opening polymerization and radical polymerization such as ATRP.

4.4.2. "Grafting to" Technique

The "grafting to" method involves the synthesis of the polymer chain that should be functionalized with an end group in order to allow the reaction with the lignin, and then the grafting of the polymer chains to the lignin core by the hydroxyl groups (**Fig. 8**) **[12]**. The properties of the resulting copolymers depend on the number and length of grafted chains. However, in this case, the

number of chains grafted per lignin molecule is the only parameter that can be assessed, since the length of the grafted chains can be tuned and well characterized before the grafting step **[12]**. For example, a boronic acid-terminated polycaprolactone that is covalently linked to the lignin forms a cyclic arylboronate ester bond with the hydroxyl groups in the lignin core (**Fig. 8**) **[162]**.

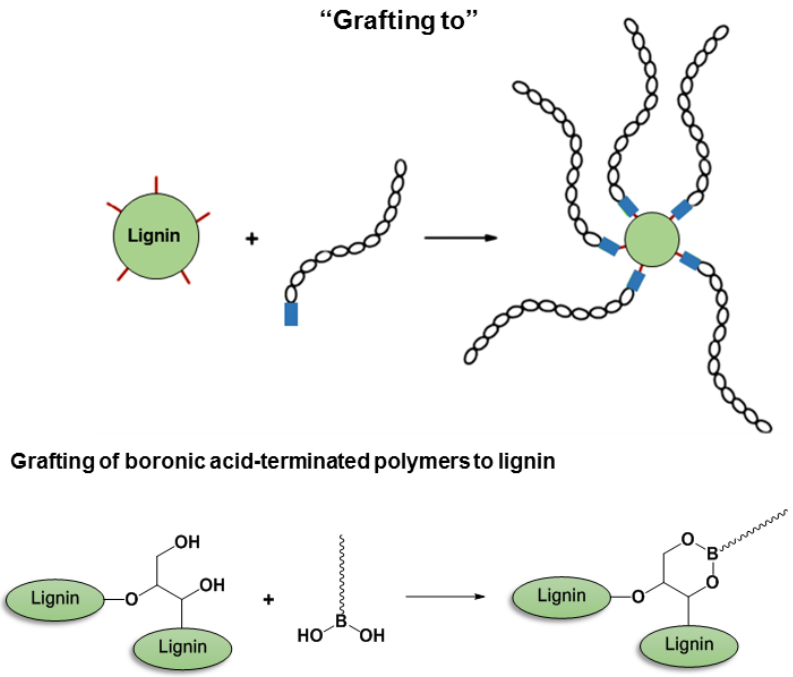

Fig. 8 Schematic representation of the lignin-graft copolymers synthesis by "grafting to" method.

5. Industrial Applications of Lignin

Lignin can be used for the starting point of different industrial applications such as high-value lignin derivatives both before and after any chemical modification. Generally, lignin-based materials can be mainly used as fuels, chemical reagents and polymers (**Fig. 9**). For example, lignin can be used to modify and control the release of fertilizers and herbicides in agriculture, as dispersant agents, as sizing agents in the paper industry and as binders **[14, 22, 31]**. The main industrial applications of lignin derivatives will be discussed in the next subsections.

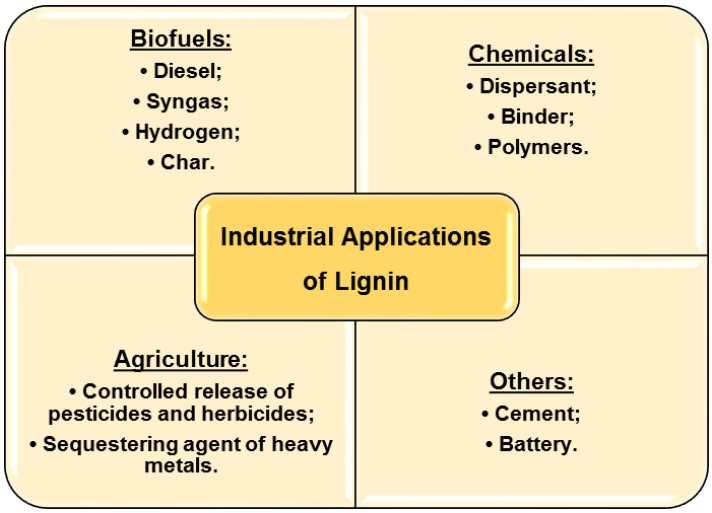

Fig. 9. Main industrial applications of lignin derivatives.

5.1. Biofuels

Lignin and its derivatives have been used as fuel sources in different forms, and these include char, syngas, hydrogen and aromatic hydrocarbons (**Fig. 10**) **[31]**. The main processes to obtain biofuels from biomass begin with pyrolysis, followed by catalytic upgrading of the resulting materials. This pyrolytic breakdown of the biomass produces a large number of chemical substances that can be used as substitutes for conventional fuels **[163]**. Pyrolysis can be fast pyrolysis to obtain oil and slow pyrolysis to obtain char **[164, 165]**. Furthermore, the gasification process can transform lignin into gases that can be used in fuel cells, gas turbines, and as synthesis gas **[105]**.

The char formed by slow pyrolysis can be combusted to produce heat, which is the principal use for the isolated lignin **[31]**. The conditions of pyrolysis will determine the chemical composition and the yield of the solid products, and these conditions include the reactor temperature, heating rate, porosity, initial particle size and initial temperature **[166, 167]**.

The manufacturing of high-value products, such as hydrogen and syngas, is also a promising use of biomass. Hydrogen has been used as an alternative to conventional fuels in order to reduce the dependence on fossil fuels and reduce fossil carbon based emissions, whereas syngas is an important building block for the petrochemical industry **[168]**. The syngas can be combusted to

generate heat and electricity. Pure hydrogen can also be obtained from the upgrading of syngas via the water-gas shift (WGS) reaction followed by gas separation using pressure swing adsorption. The resulting hydrogen can be used as a clean energy carrier for fuel upgrading in petroleum and biorefineries **[31]**.

Lignin has also been used as a source of aromatic monomers, such as guaiacol, phenol, benzene and toluene, and their production depends on the reaction temperature and presence of catalysts **[169]**.This phenolic oil separated from the bio-oil obtained by pyrolysis of lignin is also called pyrolytic lignin **[164]**. Certain properties of the bio-oil, such as its low heating value, incomplete volatility, acidity, instability, and incompatibility with standard petroleum fuels, restrict its application. However, catalytic upgrading of the pyrolytic lignin can be performed in order to increase the stability of the product **[164, 170]**. The pyrolytic products can be blended into transportation fuels, but they can also be utilized in the polymer/chemical and electronic industries **[164]**.

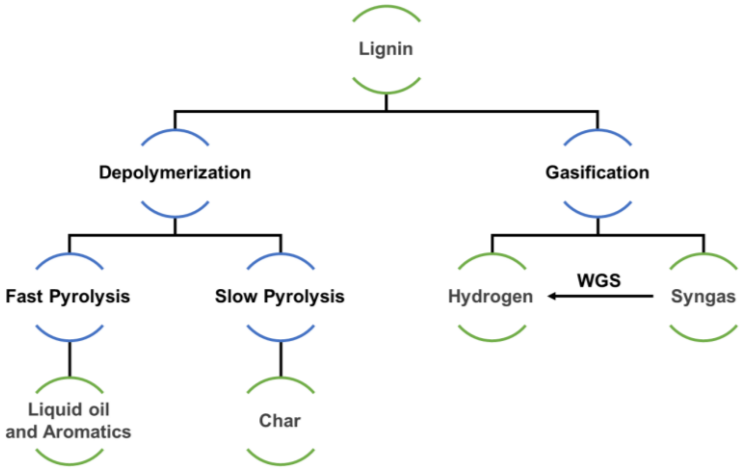

Fig. 10. Overview of the processes to convert lignin into different biofuels.

5.2. Chemicals/Polymers

Lignin has been extensively studied in the field of the material science because of its abundance, low cost, high UV-absorbance, biocompatibility, biodegradability and antioxidant properties **[4, 57]**. In addition, lignin can be used as a phenol substitute in the production of phenol formaldehyde

resins that are to be used as binders because of the presence of phenol groups in its structure **[6]**. As discussed above, different chemical modifications can be applied in order to improve the reactivity of the lignin. However, there is some variability in the properties and thereby in the uses of lignin phenol formaldehyde resins that depend on the source and the extraction method of the original lignin **[6]**. For example, some lignin phenol formaldehyde resins have been effectively used as binders, others as water-barrier coatings for cardboard substrates and yet others can be incorporated into wood adhesives **[171-173]**. Furthermore, the use of phenolic lignin resins as foams reduce thermal conductivity and, consequently, they can act as a thermal isolating foam **[6, 174]**. Conventional phenol formaldehyde resins are toxic, whereas the lignin phenol substitution is environmentally friendly and less expensive than the more conventionally used phenol **[175]**.

The type of lignin used for epoxy resins and its extraction process will influence the properties of the resulting composite **[176]**. For example, epoxy-lignin blends that are derived from the commercial hardwood lignin have enhanced adhesion to epoxy resins when compared to a softwood lignin, which correlates with the density of hydrogen-bonding groups in the material. A commercial soda lignin had the highest adhesive joint shear strength **[6, 177]**. In addition, some reports have suggested that epoxy resins can be used to improve the anti-aging properties of asphalt **[178, 179]**. Additionally, the lignin-based epoxy resins have a good stability when used in electric circuit boards **[176, 180]**.

Polyurethanes are block copolymers constituted by polyesters or polyethers that are covalently bonded by a urethane group. Polyurethanes have unique properties, such as low density, low thermal conductivity and moisture permeability, and high strength and dimensional stability, thus it is used in the production of foams that can be classified as flexible, semi-rigid or rigid foams **[181]**. However, the incorporation of lignin in the synthesis of lignin-based polyurethane has been studied in order to improve the mechanical properties, thermal stability, curing rates, and also the flame

resistance of the lignin-based polyurethane foams **[181, 182]**. In some cases, the resulting lignin-based foams were found to be comparable or even better than the conventional polyurethanes **[181]**.

Carbon fibers are materials that have properties, such as being lightweight, having high strength, great flexibility, dimensional stability, heat resistance and good electric conductivity **[10, 53, 183]**. Having these properties enable the use of carbon fibers in several industries, including spacecraft, aircraft, marine products, sport equipment and automobiles **[10, 183, 184]**. Currently, the main precursor used for the carbon fibers production is polyacrylonitrile, but high production costs has limited its high-end industrial applications **[53]**. Therefore, lignin can be used as a starting material for the production of carbon fibers because it is a low cost precursor, and also because of its high carbon content **[22, 53]**. The production of carbon fibers from hardwood kraft lignin via thermal fiber spinning was first reported by Kadla *et al* **[185]**. This method was used in order to remove impurities from lignin and decrease the hydroxyl content, which enable the lignin-based fibers to keep a characteristic fiber form during the subsequent carbonization. The addition of 3–5% of poly(ethylene oxide) facilitated the fiber spinning. Another study reported the production of carbon fibers from softwood kraft lignin for which the stabilization and carbonization was executed as a one-step process, which indicates great potential to reduce the costs and production time **[186]**. Therefore, the production of lignin-based carbon fibers can provide mechanical properties that are comparable to the conventional carbon fibers that uses polyacrylonitrile as a starting material, and which also show an increased energy efficiency and reduced environmental burden **[10]**.

5.3. Other Applications

Lignosulphonates are strong sequestering agents that trap different metal ions and form complexes with iron, calcium, copper, nickel, aluminum and zinc. Thus, lignosulfonates can be used in the treatment of poor soils by either adding deficient minerals or removing harmful metals

[14]. In addition, the controlled release of herbicide, pesticides and fertilizers can be achieved by using lignin-based biopolymer matrices **[187, 188]**.

Lignin and its derivatives have been utilized for other types of applications in addition the abovementioned applications. For example, amphiphilic lignosulfonates conjugated with poly(ethylene glycol) diglycidyl ether had promising cement dispersant properties without losing any mechanical strength even in the wintertime **[189]**. Moreover, the modification of alkali lignin *via* sulfonation enhanced the dispersion effect to the cement matrix **[129]**.

Lignin can enhance the performance of energy storage devices such as sodium ion batteries, which have been extensively explored as electrical energy storage devices for large-scale and sustainable energy storage applications. In this case, lignin-based materials have been produced in order to be used as precursors in the fabrication of low-cost high-performance carbon electrode materials for sodium ion batteries **[190]**. Another study showed that the mixture of silicon nanoparticles and lignin could be used to replace a conventional polymer binder and carbon black additive. A copper substrate was coated with the mixture and then heat-treated to form a composite electrode with good electrochemical performance. This method produced a high-performance and durable silicon nanoparticle-based negative electrode **[191]**.

6. Preparation of Lignin-Based Nanomaterials and their Applications

Different chemical reactions that can be used to modify lignin in order to produce high-value materials, which were discussed in *section 4*. Lignin is still an underexploited natural source despite its availability and its good structural potential, although the increasing potential of using lignin in the development of nanomaterials is only just emerging. However, its application is limited by the complex macromolecular structure, which is dependent on its source and extraction method. One way to overcome this limitation is to transform raw lignin into nanoparticles of uniform size and

shape **[16]**. Recently, lignin has been successfully used to produce different nanomaterials, such as nanoparticles, nanotubes, nanofibers and hydrogels for different kind of applications **[3, 11, 192]**.

The development of lignin nanoparticles (LNPs) presents key advantages, such as improving the properties of polymer blends and higher anti-oxidant activity due to higher surface-area-to-volume ratios **[15, 193, 194]**. Furthermore, LNPs possess functional groups that can be chemically modified, which significantly increase their application potential. Different approaches such as anti-solvent precipitation, interfacial crosslinking, polymerization, solvent exchange and sonication have been used to produce LNPs all of which have characteristically different shapes (**Fig. 11**) **[11]**. **Table 3** summarizes the preparation methods of LNPs and their main applications. Lignin-based nanoparticles can have potential uses in functional surface coatings (*e.g.*, nanocomposites), and also in biomedical applications, such as drug/gene delivery and tissue engineering.

The development of hydrogels has changed from the adaptation of synthetic polymers to the modification of natural polymers due to the biocompatibility, biodegradability, low toxicity, susceptibility to enzymatic degradation and eco-friendly properties inherent to natural polymers. Lignin, among other biopolymers, is a promising candidate for the development of hydrogels because of its properties, which have already been discussed above **[3]**. Lignin has been used in different types of hydrogels to facilitate their formation and improve their properties. Hydrogels have been used for producing contact lenses, wound dressings, and for drug delivery and tissue engineering **[217]**.

In this section, we will describe the main applications and some methodologies used in the preparation of lignin-based nanomaterials.

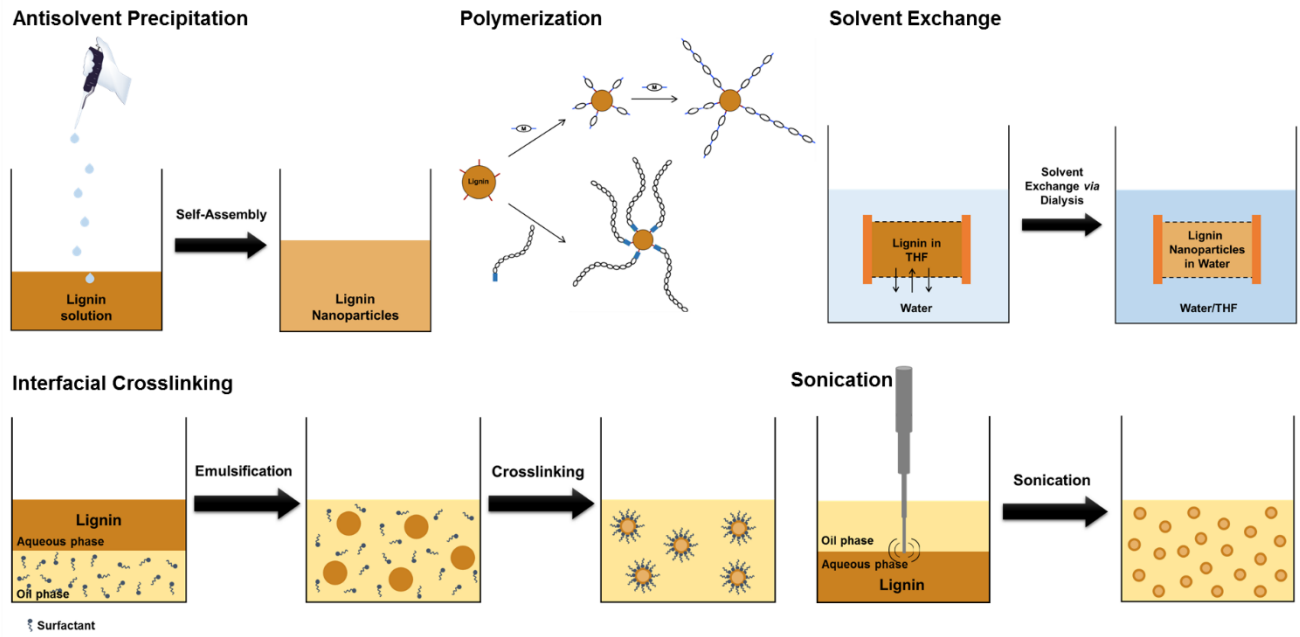

Fig. 11. Schematic representation of the different methodologies to produce LNPs.

Table 3 – Summary of the preparation methods and application of the lignin-based nanoparticles.

Preparation method	Morphology	Description of the preparation method	Potential Applications	References
Anti-solvent Precipitation	Spheres	Organosolv lignin was dissolved in acetone and supercritical CO_2 was used as an anti-solvent.	Possible applications in food processing and pharmaceutical industries.	**[193]**
Anti-solvent Precipitation	Spheres	Lignin was dissolved in acetone/water (9:1 v/v) and added to Milli-Q water.	Possible applications in food, pharmaceutical and cosmetic industries.	**[194]**
Anti-solvent Precipitation	Cluster-forming aggregate	Lignin was dissolved in ethylene glycol and precipitated by HCl.	Hybrid nanocomposites.	**[195]**
Anti-solvent Precipitation	Non-spherical	Lignin was dissolved in ethylene glycol and precipitated by HCl.	Antibacterial effects; drug delivery vehicles and as sorbents for heavy metal ions.	**[196, 197]**
Anti-solvent Precipitation	Quasi-spherical nanoparticles	Lignin was dissolved in DMF, and the solution was sprayed into the precipitator using compressed liquid CO_2 as an anti-solvent.	Potential applications in cosmetics, drug delivery systems, and nanocomposites materials.	**[198]**
Anti-solvent Precipitation	Micelles	Alkali lignin was dissolved in dioxane and cyclohexane was added into the solution.	Lignin/high-density polyethylene blends.	**[199]**
Anti-solvent Precipitation	Colloidal spheres	Acetylated lignin was dissolved in THF and water was gradually added to the solution.	Potential application as drug delivery systems or encapsulation of pesticides for controlling release.	**[200]**
Anti-solvent Precipitation	Nanocapsules	A solution of lignin in ethanol was prepared and the ultra-pure water was dropped into this solution until the water content of the solution reached 90%.	Suitable for different applications.	**[201]**
Interfacial Crosslinking	Hollow nanocapsules	Kraft lignin was dissolved in Milli-Q water (with sodium chloride), mixed with cyclohexane (with a biocompatible surfactant polyglycerol polyricinoleate), and then a solution of toluene diisocyanate in cyclohexane was added.	Encapsulation of hydrophilic compounds for agricultural applications.	**[202]**
Interfacial Crosslinking	Spherical Micro/nanocapsules	The emulsification of an oil in water phase containing lignin using high-intensity ultrasonic technology, creating the crosslinking of lignin at the water/oil interface.	Encapsulation of hydrophobic compounds for biomedical applications.	**[203]**
Interfacial Crosslinking	Micro/nanoparticles	A microemulsion was prepared after adding the lignin solution to the octane containing a mixture of surfactants (Span 80, Tween 80 and 1-pentanol), and subsequent crosslinking of the internal lignin-rich phase by adding epichlorohydrin.	Surfactant for Pickering emulsions; Loading of silver nanoparticles.	**[204]**
Interfacial Crosslinking	Nanocapsules	First, lignin was grafted with allyl groups *via* etherification and dispersed in an oil-in-water miniemulsion by ultrasonication, and then reacted with a thiol-based crosslinking agent at the interface of miniemulsion droplets to form nanocapsules.	Drug delivery and controlled release of hydrophobic compounds (*e.g.*, drugs, essential oils, and antioxidants).	**[205]**
Polymerization	Nanotubes	Lignin reacted nanopores surface of the alumina membranes;	Vehicles for gene delivery into human cells.	**[206]**

		polymerization of the monolignols after deposition of multiple layers of dehydrogenation polymer onto the base layer, and dissolving the templates in phosphoric acid to obtain the lignin nanotubes.		
Polymerization	Polyplexes	PDMAEMA-lignin copolymers synthesized by ATRP.	Gene delivery system.	**[207]**
Polymerization	Not specified	PDEAEMA-grafted lignin nanoparticles prepared *via* ATPR.	Surfactant for Pickering emulsions.	**[208]**
Polymerization	Nanofibers	Grafting of PLA onto the alkylated lignin via ring opening polymerization to form PLA−lignin copolymers. Blending of copolymers with poly(L-lactide) to form nanofibrous composites by electrospinning.	Antioxidant activity; Promising as biomedical materials in tissue engineering scaffolds.	**[209]**
Polymerization	Nanofibers	Preparation of lignin-based copolymers (lignin-poly(ε-caprolactone-co-lactide) via ring-opening polymerization. Blending of lignin-based copolymers with polycaprolactone and poly(ι-lactic acid) via electrospinning.	Antioxidant activity; Promising as biomedical materials in tissue engineering scaffolds.	**[210]**
Polymerization	Nanofibers	Grafting of poly(methyl methacrylate) with lignin by atom transfer radical polymerization. Blending of this copolymer with poly(ε-caprolactone) using electrospinning.	Promote the proliferation, attachment and interactions of human dermal fibroblasts. Promising for tissue engineering.	**[211]**
Sonication	Irregular shape	Sonication of an aqueous suspension of alkali lignin for 60 min.	–	**[212]**
Sonication	Not specified	Lignosulfonate and chitosan solutions were combined in the presence of an organic phase (vegetal oil) and the ultrasonic probe was placed at the aqueous–organic interface.	Antibacterial effect; drug delivery applications.	**[213]**
Solvent Exchange	Spherical nanoparticles	Kraft lignin was dissolved in THF and water was subsequently introduced into the system *via* dialysis.	Potential application in composites, Pickering emulsions and antimicrobial materials. Drug delivery carrier for cancer therapy.	**[214, 215]**
Solvent Exchange	Different nanoparticles morphologies	Condensation reaction of iron isopropoxide with lignin in THF solution and further hydrolysis and dialysis in water.	Potential application to produce materials with enhanced magnetic, antibacterial and catalytic properties.	**[216]**
Solvent Exchange	Spherical nanoparticles	A mixture containing 50:50 w/w of lignin solution and oleic acid-coated Fe_3O_4 NPs in THF was prepared and dialyzed against Milli-Q water.	Promising for cancer therapy and diagnosis, such as magnetic targeting and magnetic resonance imaging.	**[215]**
Aerosol assisted self-assembly	Hollow/solid lignin nanoparticles	Dioxane soluble fraction of alkali lignin at different concentrations was dissolved in DMSO and sprayed upon a liquid nitrogen cooled copper plate using an ultrasonic nebulizer	Sunscreen cosmetics (UV-absorbing ability) and drug delivery applications.	**[217]**
Aerosol Flow Reactor	Spherical nanoparticles	After dissolving alkali, kraft and organosolv lignin with an appropriated solvent, the droplets were generated during the flow-through a collision-type jet atomizer with nitrogen gas, and collected as solid particles.	Surfactant for Pickering emulsions.	**[218]**

6.1. Lignin-Based Nanomaterials as Antioxidant

The complex chemical structure that contains aromatic rings of the methoxy and the hydroxyl groups enables lignin to be incorporated into different materials to produce antioxidant products that can be used in several applications. These functional groups lead to the termination of oxidative propagation reaction *via* hydrogen donation **[17]**. Lu *et al*. prepared LNPs with an average size of ca. 144 nm by using a supercritical anti-solvent precipitation method **[193]**. That study used the nanoscale lignin at 12.4 times higher concentration than the bulk lignin and, consequently, the resulting product had higher antioxidant and free radical scavenging activities and enhanced reducing power. Thus, the resulting nanoscale lignin product could be used in food processing and pharmaceutical industries. Yearla and Padmasree fabricated dioxane LNPs that had an average size of approximately 104 nm by an anti-solvent precipitation method whereby a solution of lignin was dissolved in acetone and water (9:1 v/v) and then the solution was added to water **[194]**. The LNPs manifested greater antioxidant and UV-protection properties than the bulk lignin after monitoring the survival rates of *Escherichia coli* upon UV-irradiation-induced mortality. The resulting LNPs could be further exploited in food, pharmaceutical and cosmetic industries because of these beneficial properties.

6.2. Lignin-based Nanomaterials for Nanocomposites

LNPs have also been exploited as reinforcing agents in polymer matrix and nanocomposites, for which the resulting copolymers have better biocompatibility, mechanical and thermal properties than the original polymers. For example, Gupta *et al*. synthesized LNPs that had an average size of 181 nm by using the anti-solvent precipitation method. Those authors dissolved the lignin in ethylene glycol and then precipitated the LNPs by adding HCl **[195]**. These LNPs were used in the preparation of bio-poly(trimethylene terephthalate) hybrid nanocomposites, that contained 1.5% by weight of LNPs and 7.0% by weight of vapor-grown carbon fibers. Their study reported an

improvement in the mechanical properties of the nanocomposites such as tensile strength, tensile modulus and impact strength in the thermal properties and biodegradation characteristics. The resulting nanocomposites were found to be suitable for several end-use applications. Qian *et al*. used a self-assembly method to prepare lignin reverse micelles after adding cyclohexane into a solution of alkali lignin dissolved in dioxane **[199]**. When further quantities of cyclohexane were added, the lignin micelles tended to flocculate and precipitate. The rendered nanoparticles ranged in size from 120 nm to micrometers, depending on the cyclohexane content, and these had better UV-absorbance properties than the alkali lignin (**Fig. 12**). In addition, the nanoparticles had good miscibility with hydrophobic plastics, such as high-density polyethylene (HDPE), and had improved UV-absorbing and mechanical properties of the resulting lignin/HDPE blends.

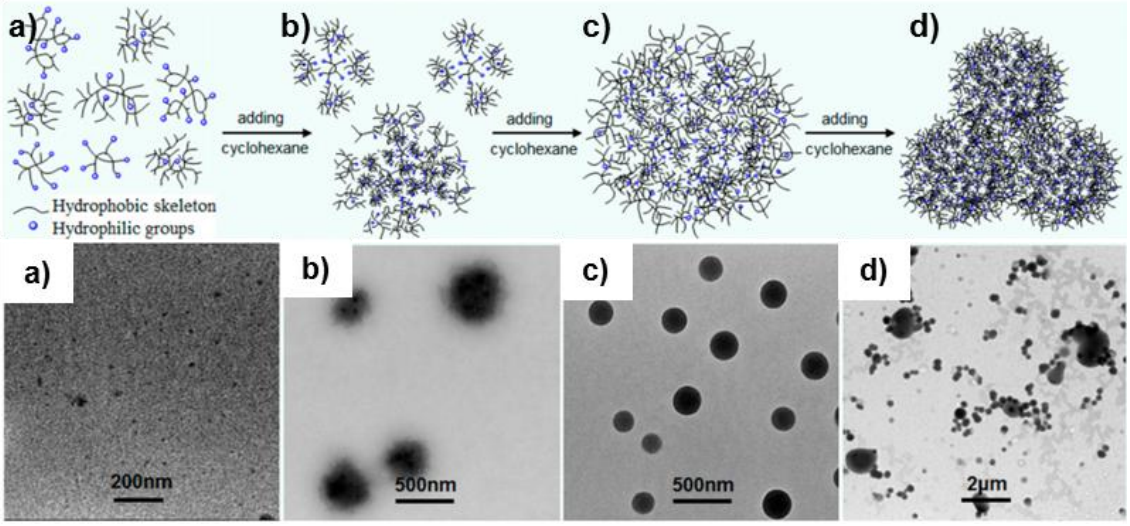

Fig. 12. Schematic representation of the manufacturing process of lignin reverse micelles in dioxane-cyclohexane medium and transmission electron microscopy (TEM) images of the samples from the dispersions with different cyclohexane content: (a) 0−4% vol; (b) 4−7% vol; (c) 7−10% vol, and (d) >10% vol. Adapted from ref. 199 with permission from American Chemical Society, copyright 2015 **[199]**.

Qian *et al*. synthesized *N*,*N*-diethylaminoethyl methacrylate (DEAEMA)-grafted LNPs *via* ATRP, which had sizes that ranged from 237–404 nm **[208]**. These lignin-DEAEMA nanoparticles

were used as surfactant for CO_2/N_2-switchability Pickering emulsions that was correlated with the graft density and chain length of the DEAEMA. A study, by Ago *et al*., described the preparation of spherical LNPs by using an aerosol flow reactor, a high-yield and high-throughput manufacturing approach that rendered particles with sizes that ranged from ca. 30 nm to 2 μm, depending on the collector number (fraction), lignin source, and concentration of the precursor solution **[218]**. The obtained particles were used to stabilize oil-in-water Pickering emulsions with variable droplet size, which depend on the size of the particles, the particle concentration and the origin of the lignin. Nypelö *et al*. fabricated spherical particles of sizes that varied from 90 nm to 1 μm *via* microemulsion and further crosslinking **[204]**. This process required that the water-in-oil microemulsion was prepared by adding a lignin solution to a mixture of surfactants, such as Span 80, Tween 80 and pentanol dissolved in octane, which resulted in a hydrophilic–lipophilic balance of ca. 7. This formulation allowed a spontaneous emulsification, and the solid particles were produced after the crosslinking of the internal lignin-rich phase by the addition of epichlorohydrin and the subsequent separation by removing the continuous oil phase. These particles can be used in the stabilization of oil-in-water Pickering emulsions, in which the larger particle size provided an increased emulsion drop size and stability.

Salas *et al.* produced nanofibers with diameter ranging from 124 to 400 nm by electrospinning aqueous alkaline solutions containing different mass ratios of soy protein and lignin **[219]**. Poly(ethylene glycol) was added as coadjutant in order to facilitate the formation of better-quality nanofibers, and the unfolding of the soy protein improved the interaction with the lignin macromolecules, contributing to the successful electrospinning process. Thus, the mixture of two major renewable resources with exciting chemical features was found to be appropriate for the development of composites.

6.3. Lignin-based Nanomaterials as Delivery Systems

Lignin has the potential to produce nanoparticles for encapsulation of different compounds for different pharmaceutical applications due to its low cost and eco-friendly properties. For example, Frangville *et al*. used an anti-solvent precipitation method, in which the lignin was dissolved in ethylene glycol and precipitated with HCl to form LNPs with sizes that varied from 100 nm to a micrometer scale, depending on the initial concentration of lignin and the concentration of HCl added **[196]**. The LNPs did not have any apparent cytotoxicity for yeast and microalgae, thus, they were considered promising vehicles for drug delivery, stabilizers of cosmetic and pharmaceutical formulations. In addition, these LNPs can also be used as sorbents for heavy metal ions and other environmental pollutants. Later, Richter *et al*. also prepared LNPs by using the same precipitation method referred above, and the LNPs were subsequently infused with silver ions and coated with poly(diallyldimethylammonium chloride) (PDAC) (**Fig. 13**) **[197]**. The coating promoted the adhesion of the biodegradable and environmentally green LNPs infused with silver ions to the bacteria membranes, a process that could efficiently kill a wide-range of bacteria. Thus, by synthetizing such nanoparticles with biodegradable cores and high antimicrobial activity, it is possible to diminish the environmental impact associated with metallic silver nanoparticles.

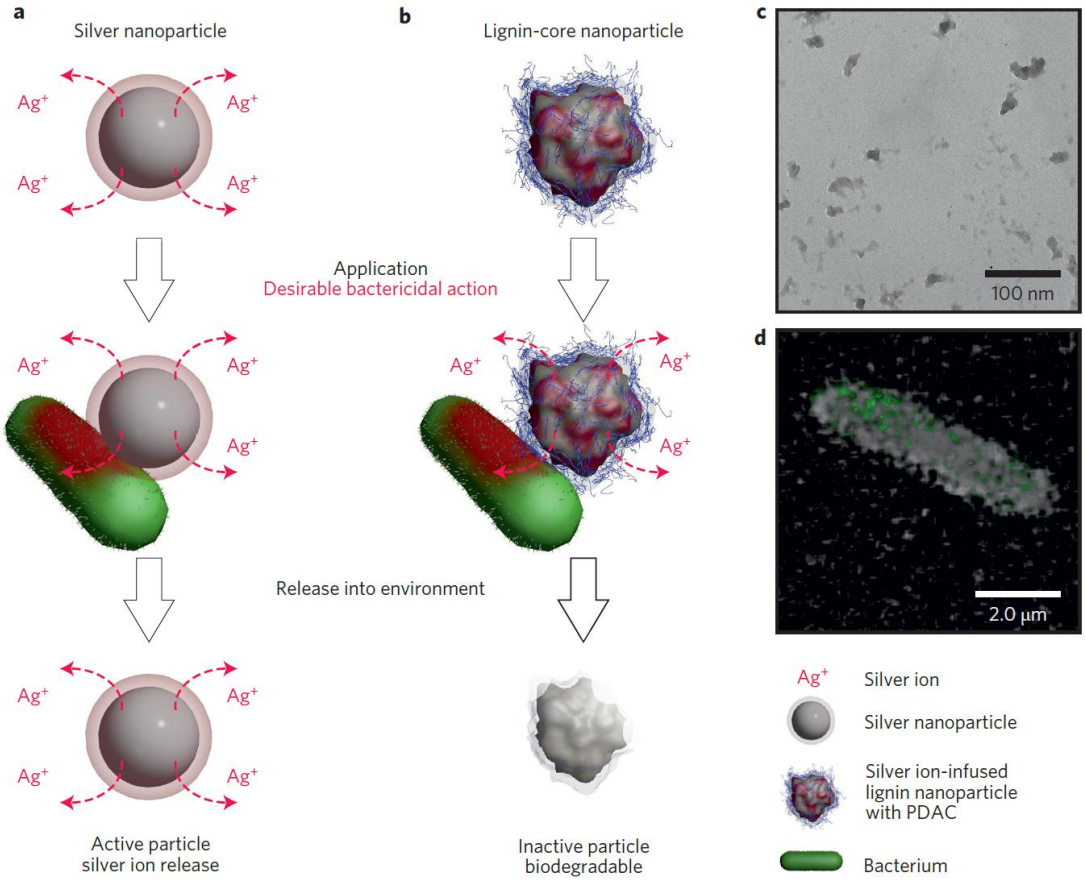

Fig. 133. A schematic representation of (a) general principle of the bactericidal effect of common silver nanoparticles via the release of Ag^+ ions and (b) Ag^+-infused environmentally benign lignin-core nanoparticles with PDAC that promotes electrostatic attraction between the nanoparticles and the negatively charged cell wall. (c) The TEM images of the prepared LNPs. (d) Confocal fluorescence microscopy image of the LNPs with PDAC coating adhering to the cell membrane of *Escherichia coli*. Reproduced from ref. 197 with permission from Nature Publishing Group, copyright 2015 **[197]**.

Qian *et al*. prepared colloidal lignin spheres using the self-assembly method by dissolving acetylated lignin in tetrahydrofuran (THF) and adding water gradually to the solution. This caused the lignin molecules to start associating with each other due to hydrophobic interactions: a process that was completed by adding a water to the extent of 67% by volume of the solution **[200]**. The nanoparticles that formed in water after the evaporation of THF had an average size of around 106

nm and they could be potentially used as drug delivery systems or microencapsulation of pesticides for their controlled release in an environment. In another study, Yiamsawas *et al*. produced hollow nanocapsules *via* interfacial crosslinking, in which lignin was dissolved together with sodium chloride in water and mixed with cyclohexane containing the biocompatible surfactant polyglycerol polyricinoleate **[202]**. Then, after a miniemulsion had been produced by ultrasonication, a polyaddition reaction was started by adding a solution of toluene diisocyanate in cyclohexane solution to the miniemulsion. The obtained nanoparticles had diameters that ranged from 311 to 390 nm in water. In addition, this reaction allowed the encapsulation of hydrophilic compounds such as drugs, fertilizers, and pesticides that can be released by enzymatic degradation of the lignin, which makes these nanocontainers attractive for agricultural applications. In order to form an oil phase in water emulsion with lignin, Tortora *et al*. used a high-intensity ultrasonic technology to synthesize oil-filled microcapsules of kraft lignin of sizes that ranged from 300 nm to 1.1 μm **[203]**. This induced the crosslinking of lignin at the water/oil interface, whose stability was improved by adding a cross-linking agent. This technique also allowed the loading of hydrophobic compounds dissolved in the oil phase and the products had low cytotoxicity and they could be efficiently internalized into Chinese hamster ovary cells.

An ultrasonic spray-freezing method was used by Mishra and Wimmer who prepared hollow/solid lignin colloidal nanoparticles, without chemical modification **[217]**. For this to occur, different concentrations of dioxane soluble fraction of alkali lignin were dissolved in DMSO and sprayed onto a liquid-nitrogen-cooled copper plate using an ultrasonic nebulizer, producing LNPs with sizes in the 96–193 nm range. The morphology of the resulting nanoparticles was dependent on two different processes that can occur in parallel, namely: 1) the time taken by the solvent to freeze, and 2) the time taken by the lignin to diffuse. When the freezing of solvent is enough for the solute to diffuse, a solid particle should be expected, otherwise a hollow particle should be produced (**Fig. 14**). Furthermore, other parameters such as droplet size, nature of polymers and

concentration of solute (higher concentration favors solid particle formation) that can affect the morphology of the nanoparticles. In addition to that, six layers of lignin colloids were deposited on a quartz slide assisted by a negligible UV-absorbing aqueous solution of PDAC, which improved the UV absorbing ability of the lignin product by increasing the number of layers. Thus, the obtained nanoparticles could be used in sunscreen cosmetics due to their UV-absorbing ability, or they could also be used for drug delivery applications.

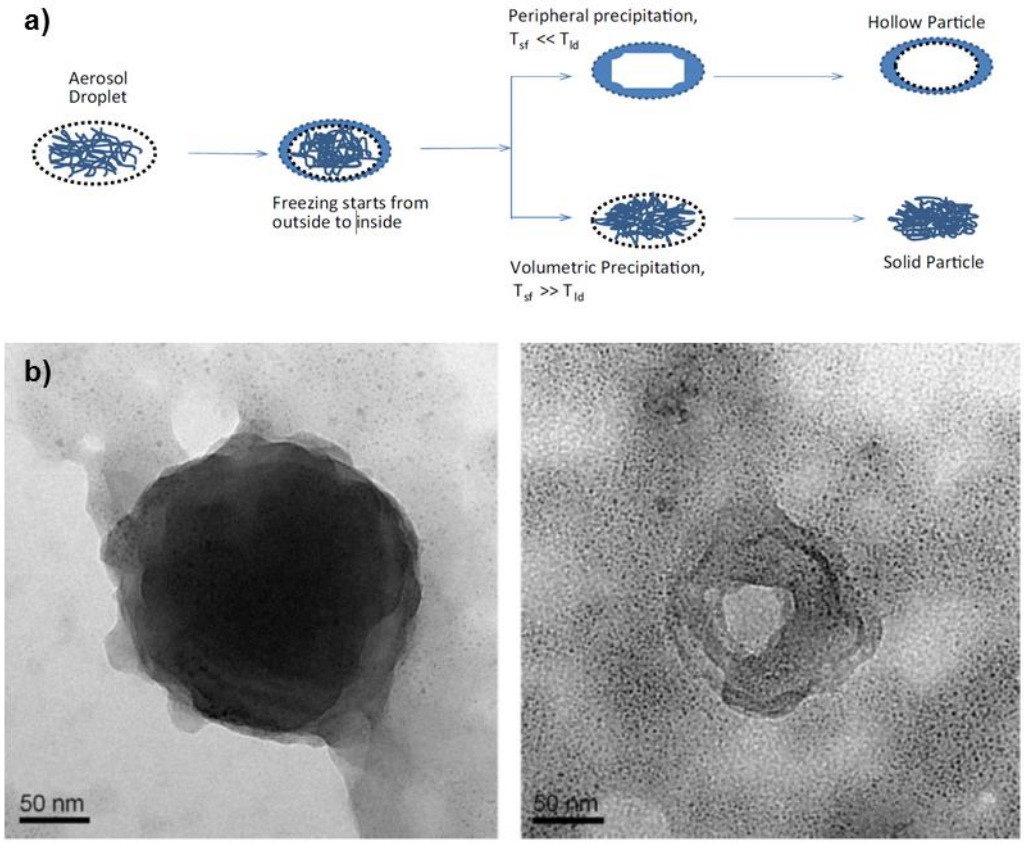

Fig. 14. (a) Schematic representation of the LNPs morphology according to the mechanism followed for their synthesis. T_{sf} – characteristic time for solvent freezing, T_{ld} – typical time for diffusion of lignin. (b) TEM pictures of the solid and hollow LNPs. Adapted from ref. 217 with permission from Elsevier, copyright 2017 **[217]**.

LNPs can also be used for gene delivery applications. Ten *et al*. fabricated lignin nanotubes using nanopore alumina membranes as a template **[206]**. In this method, lignin was first reacted

with the amine groups on the nanoporous surface of the alumina membranes. Then, after the deposition of multiple layers of dehydrogenation polymer onto the base layer, the polymerization of the monolignols occurred with horseradish peroxide as a catalyst. Finally, the templates were dissolved with phosphoric acid in order to obtain lignin nanotubes. The physicochemical properties of the resulting nanotubes depend on the composition, origin and extraction method of the lignin, which affected the size and reactivity of lignin. The lignin nanotubes showed good biocompatibility and a high DNA binding capacity, both of which are good attributes for making these nanotubes a good vehicle for gene delivery into human cells (**Fig. 15**).

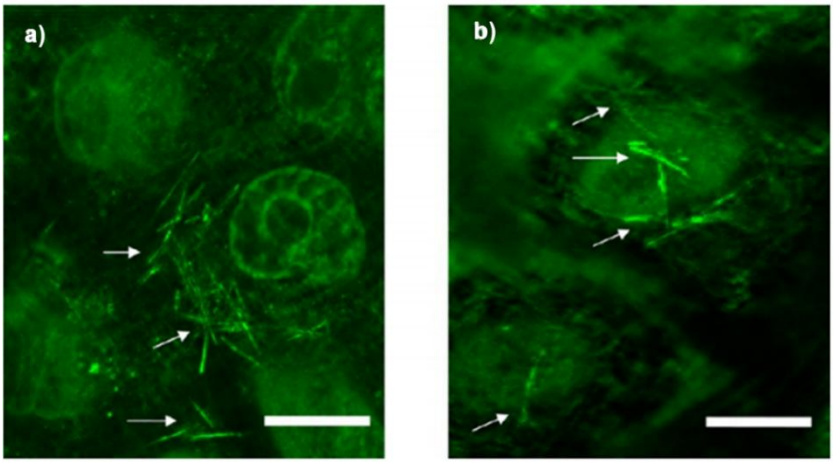

Fig. 15. Confocal microscopy images of lignin nanotubes made of (a) lignin nanotube that had been isolated from PINE with thioglycolic acid and (b) lignin that had been isolated from PINE with NaOH, located inside HeLa cells. The scale bar represents 15 μm. White arrows point to lignin nanotubes. Reproduced from ref. 206 with permission from American Chemical Society, copyright 2014 **[206]**.

Liu *et al*. synthesized poly(2-dimethylaminoethyl methacrylate) (PDMAEMA)-lignin copolymers by ATRP **[207]**. After the synthesis, the copolymers were used to form polyplexes with plasmid DNA by electrostatic interactions between the negatively charged plasmid DNA and the positively charged PDMAEMA-lignin copolymers whose sizes ranged from 100 to 200 nm. The cytotoxicity and gene transfection efficiency of these copolymers were dependent on the chain

length of the grafted PDMAEMA. The copolymers that had very short arm length (i.e. with a mean of 5.5 DMAEMA units) exhibited an excellent *in vitro* transfection efficiency, and is therefore a promising gene delivery system.

An ultrasonication method was used by Kim *et al*. who fabricated chitosan-lignosulfonate nanoparticles after combining chitosan and lignosulfonate solutions in the presence of an organic phase (vegetal oil) and placing the ultrasonic probe at the aqueous–organic interface **[213]**. In this formulation, lignosulfonates act as a counter polyion for the stabilization of chitosan nanoparticles. Different parameters were tested during this process, including the pH of chitosan solution, the sonication time and the presence of surfactant (poloxamer 407) in order to obtain monodisperse and small particles that had a mean size of 230 nm. Furthermore, the obtained nanoparticles were found to be biocompatible with human cells and also had an efficient RNase A loading and enhanced antimicrobial effect compared to the chitosan nanoparticles alone, which makes them promising candidates for cosmetic and drug delivery applications. Gilca *et al*. also reported the preparation of lignin nanoparticles after the sonication of an aqueous suspension of alkali lignin for 60 min, which yielded nanoparticles whose sizes ranged from 10 to 50 nm **[212]**. This method is more attractive for future applications because hazardous solvents were not used in the process.

Uniform and quasi-spherical LNPs were developed by Myint *et al*. *via* a compressed fluid anti-solvent method **[198]**. This method entailed dissolving the kraft lignin in DMF, the solution was then sprayed into the precipitator using compressed liquid CO_2 ($clCO_2$) as an anti-solvent, which produced uniform and quasi-spherical LNPs. The precipitated nanoparticles had a mean particle diameter of around 38 nm, which had higher UV-absorbing, dispersion stability and improved solubility in water when compared with the raw lignin. Furthermore, they could be a good candidate for applications in cosmetics, health and drug delivery systems, and for nanocomposite materials due to the biodegradability and biocompatibility of the LNPs.

Lignin nanocapsules were produced by Li *et al*. *via* self-assembly, which was achieved by taking a solution of kraft lignin that had been dissolved in ethanol and then adding water until the water content of the solution reached 90% **[201]**. The size of the obtained nanocapsules were tunable in the range of tens to hundreds of nanometers, and this together with their biocompatibility, biodegradability, environmentally friendly properties, ease and low cost of preparation make these nanocapsules suitable for multiple applications. In another study, Chen *et al*. synthesized lignin nanocapsules with pH-responsive properties by interfacial miniemulsion polymerization and further crosslinking **[205]**. First, an oil-in-water miniemulsion was obtained *via* ultrasonication, after mixing the oil phase, containing butyl acetate, hexadecane (co-stabilizer), azobisisobutyronitrile (AIBN, oil soluble initiator) and the cross-linker trimethylolpropane tris(3-mercaptopropionate) with the water phase containing lignosulfonate (with and without sodium dodecyl sulfate (SDS)), under vigorous stirring. Then, a cross-linking agent was reacted with lignin at the interface of the miniemulsion droplets to form nanocapsules. Nanocapsules were prepared using the same methodology in the encapsulation process with coumarin-6 dissolved in butyl acetate. The obtained nanocapsules had a mean size range of 50 to 300 nm, and the release of the hydrophobic coumarin-6 was controlled by the variation in pH due to the presence of acid-labile β-thiopropionate cross-linkages in the capsule shell (**Fig. 16**). These lignin nanocapsules are promising vehicles for delivery and controlled release of hydrophobic molecules (*e.g.*, drugs, essential oils and antioxidants).

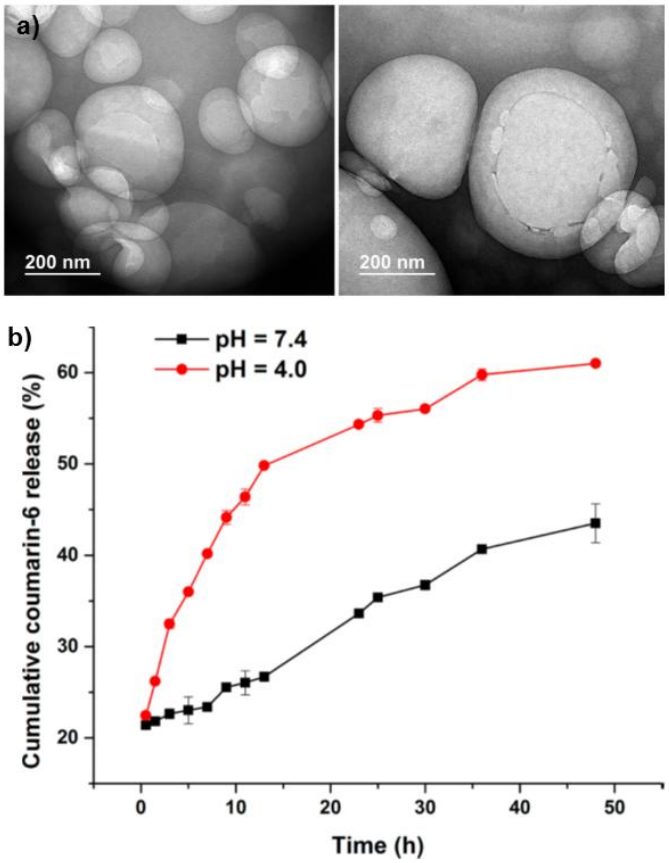

Fig. 16 (a) TEM pictures of lignin nanocapsules in miniemulsion. (b) Coumarin-6 release profile of lignin capsules in a 5% SDS solution at pH 7.4 and at pH 4.0. Reproduced from ref. 205 with permission from American Chemical Society, copyright 2016 **[205]**.

A solvent exchange method was used by Lievonen *et al*. to prepare spherical LNPs, in which kraft lignin was first dissolved in THF and then water was introduced into the system *via* dialysis, without chemical modification of lignin **[214]**. The diameter of the obtained nanoparticles ranged from 200 to 500 nm, depending on the pre-dialysis lignin concentration. In addition, the surface of the LNPs was modified by adsorption of an oppositely charged PDAC by gradually adding a nanoparticle suspension into a solution of PDAC. The obtained LNPs could be used in nanocomposites, Pickering emulsions, antimicrobial materials and drug delivery systems. Using the same solvent exchange process, Lintinen *et al*. also prepared different iron-complexed LNPs by changing the condensation and hydrolysis reaction parameters to yield particles whose sizes ranged

between 50 to 400 nm, with a tunable morphology (**Fig. 17**) **[216]**. This method entailed that lignin was first dissolved in THF and then mixed with an iron isopropoxide [$Fe(OiPr)_3$] solution, which led to a condensation reaction. Subsequently, the hydrolysis reaction was completed by adding water to the previous solutions, and finally, the obtained iron(III)-complexed lignin nanoparticles were solvent exchanged in water. Additionally, nanoparticles synthesized with $Fe(OiPr)_2$ or a mixture of $Fe(OiPr)_2$/$Fe(OiPr)_3$ showed paramagnetic or superparamagnetic behaviors, depending on the size and morphology of the nanoparticles. Thus, depending the obtained nanoparticles, these LNPs could be used to produce biocompatible materials with magnetic, antibacterial and catalytic properties.

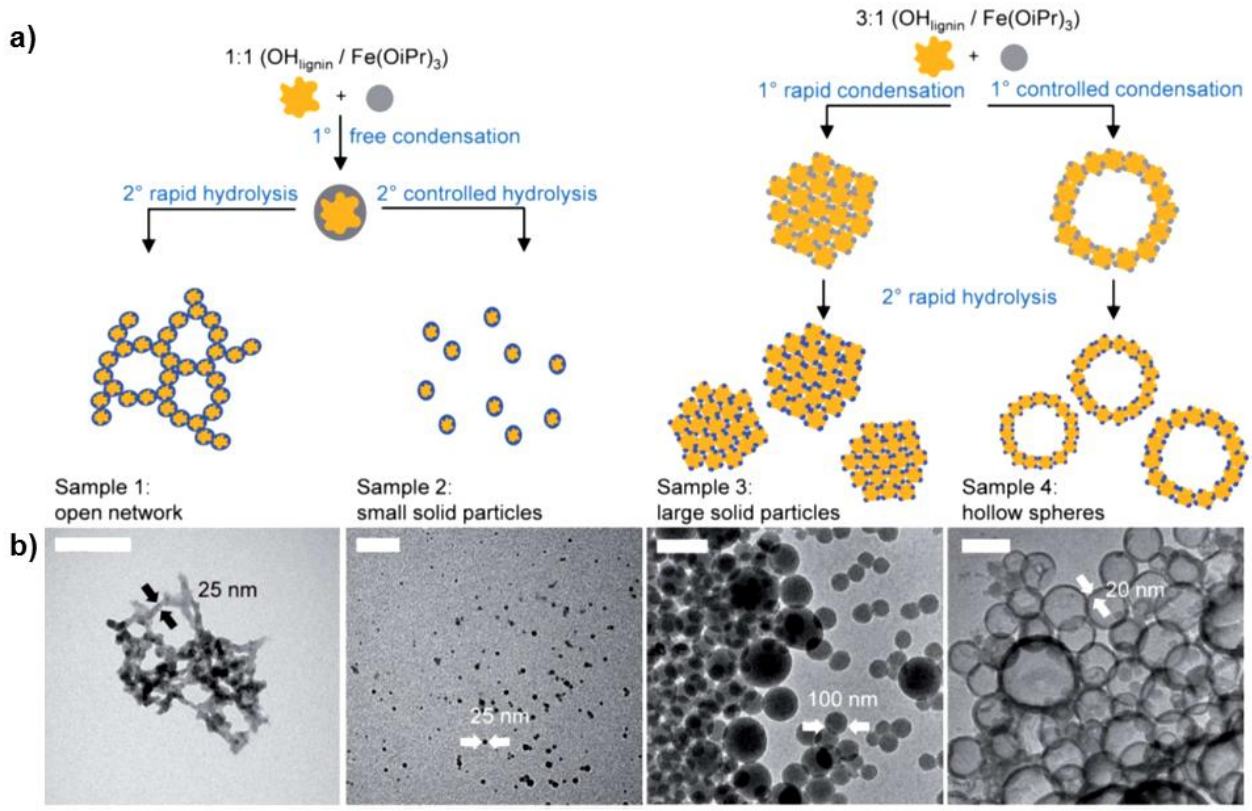

Fig. 177. Morphology of the different nanoparticles obtained by varying the reaction parameters. (a) Schematic representation of the parameters used to obtain nanoparticles with different morphologies. (b) TEM pictures of the obtained LNPs. Scale bars in all TEM images are 200 nm. In Sample 1, a rapid hydrolysis fuses Fe(OiPr) coated Fe:LNPs into fishnet-type open network structures. For Sample 2, a controlled hydrolysis keeps the Fe:LNPs from fusing together and forms

small solid particles. In Sample 3, when less $Fe(OiPr)_3$ is used than the accessible OH groups in lignin, the lignin clusters are partially covered with Fe(OiPr) moieties, which fuse together to form larger (ca. 200 nm) particles. Finally, for Sample 4, when less $Fe(OiPr)_3$ is used than the accessible OH groups and the solutions are slow, the fusing of Fe:LNPs create hollow spheres. Reproduced from ref. 216 with permission from the Royal Society of Chemistry, copyright 2016 **[216]**.

Recently, Figueiredo *et al*. developed and evaluated *in vitro* three types of spherical LNPs that had been prepared by solvent exchange (**Fig. 18) [215]**. These three types were pure lignin nanoparticles (pLNPs) **[214]**, iron(III)-complexed lignin nanoparticles (Fe-LNPs) **[216]**, and iron oxide (Fe_3O_4) nanoparticle infused lignin nanoparticles (Fe_3O_4-LNPs). The Fe_3O_4-LNPs were synthesized by mixing a lignin solution with oleic acid-coated Fe_3O_4 nanoparticles in THF (50:50 w/w) and dialyzed against water. Their superparamagnetic behavior makes them promising for cancer therapy and diagnosis **[215]**. All of the LNPs had low cytotoxicity for different cancer cell lines, and also low hemolytic rates with red blood cells after 12 h incubation. Then, the pLNPs were used to encapsulate hydrophobic drugs, which improved their release profiles and consequently enhanced their anticancer activity. Overall, these LNPs showed important features related to drug delivery and biomedical applications, and their surface chemistry can allow the modification with targeting moieties in order to increase the cellular uptake into specific cells for cancer therapies **[215]**.

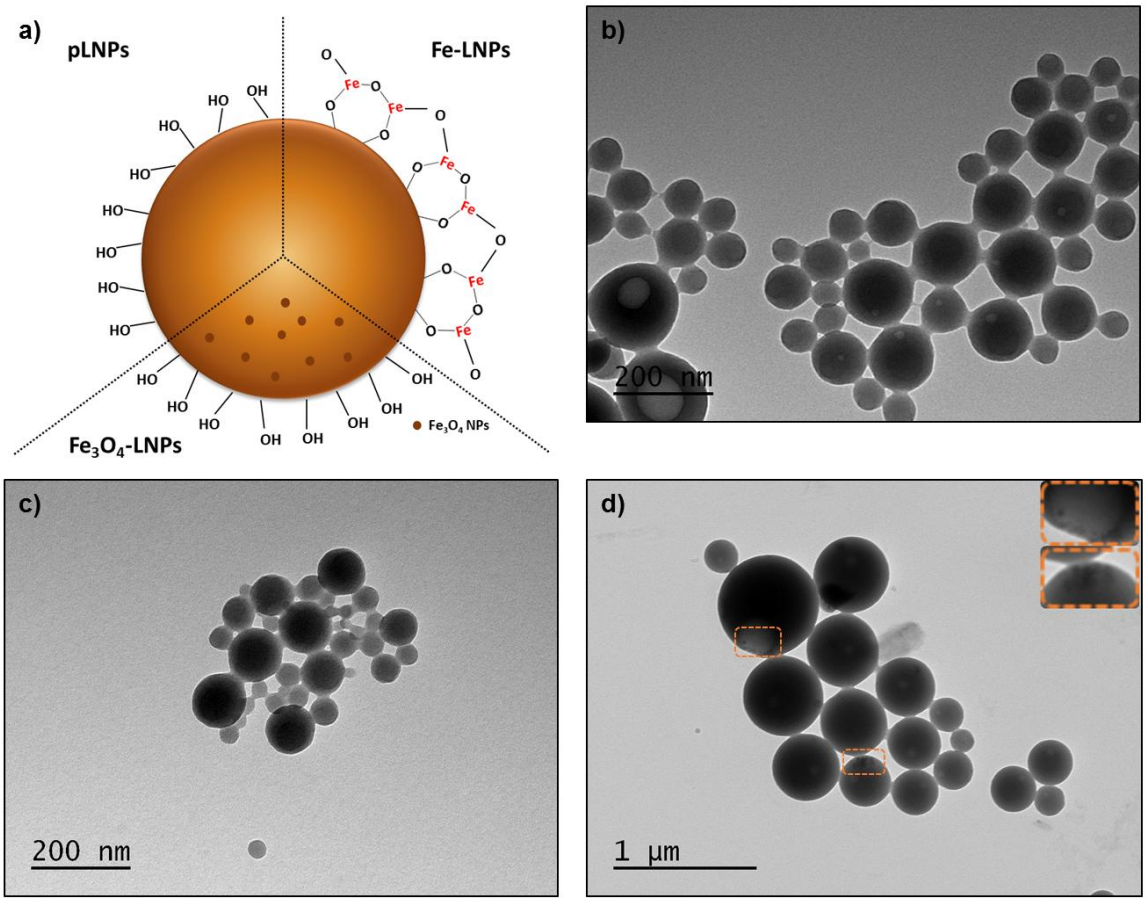

Fig. 18. (a) Schematic representation of LNPs used in the study. TEM images of (b) pLNPs, (c) Fe-LNPs and (d) Fe_3O_4-LNPs and magnification of the Fe_3O_4 NPs inside the LNPs. Reproduced from ref. 215 with permission from Elsevier, copyright 2017 **[215]**.

Besides the lignin-based nanoparticles, hydrogels can also be produced for delivery of different molecules. Superabsorbent cellulose-lignin hydrogels were synthesized by Ciolacu *et al*. by dissolving the cellulose in alkali solution and then mixing with lignin, followed by the chemical crosslinking with epichlorohydrin **[220]**. The hydrogels can incorporate polyphenols and their subsequent release from the hydrogels increased with the lignin content in the matrices. Thus, the swelling process and drug release from hydrogels could be controlled by the composition of the hydrogels, which made the prepared hydrogels potential candidates for biomedical applications as delivery systems.

Wang *et al*. produced sodium lignosulphonate-grafted poly(acrylic acid-*co*-poly(vinyl pyrrolidone)) hydrogels *via* radical polymerization that was assisted by ultrasonic treatment **[221]**. Wang's group also used amoxicillin as a model drug, which exhibited a favorable pH-sensitivity and controllable release behavior *in vitro*. The amoxicillin-loaded hydrogels had better release rates in simulated intestinal fluids than those in simulated gastric fluids.

6.4. Lignin-Based Nanomaterials for Tissue Engineering

Tissue engineering has emerged as a promising tool in the regenerative medicine field for repairing, replacing and enhancing the function of a specific tissue or organ, using biocompatible and biodegradable polymers **[222, 223]**. Several studies explored the potential use of lignin in the development of functional materials for tissue engineering, including hydrogels, aerogels and nanofibers **[210, 211, 224]**.

A study performed by Diao *et al*. reported the synthesis of a temperature sensitive lignin-based thermogelling graft copolymers by ATRP technique **[225]**. They used a lignin core and multiple graft polymer chains composed by a block of poly(*N*-isopropylacrylamide) (PNIPAAm) and block of brush-like random copolymer of PEG and poly(propylene glycol) (PPG) in that method. In aqueous solutions, the copolymers displayed thermogelling behaviors, going from a solution at low temperatures to a hydrogel at temperatures between 32 and 34 °C, and further to a dehydrated gel at higher temperatures (**Fig. 19**). The block architecture of the copolymers grafted onto the lignin is crucial to hydrogel formation in such cases, because the hydrogel is produced when the lignin-based PNIPAAm-*block*-PEG/PPG copolymer is used. However, when a lignin-based PNIPAAm-*random*-PEG/PPG copolymer was used, with the same monomer ratio, the formation of hydrogels was not observed at any temperature. Furthermore, the thermogelling copolymers presented very low critical gelation concentrations that ranged from 1.3 to 2.5% by weight, in which the PNIPAAm block is important for gelling at the critical gelation temperature, and the PPG segments can assist

in water retention by balancing the hydrophilicity/hydrophobicity of the hydrogel network at the temperatures up to 52 °C. Thus, the resulting copolymer solutions experienced a thermogelling transition at a temperature higher than room temperature and lower than human body temperature, suggesting that the hydrogels can be useful for biomedical applications, such as drug delivery and tissue engineering (*e.g.*, stem cells culture and differentiation).

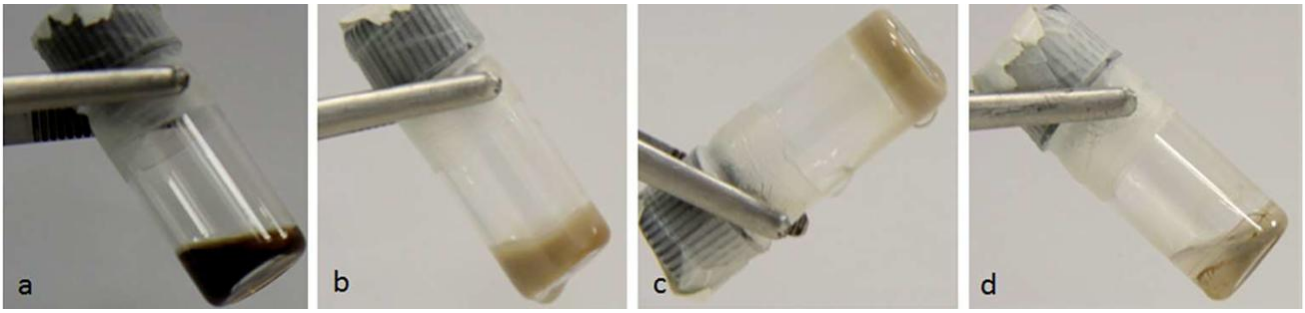

Fig. 1919. A sequence of pictures showing the lignin-based PNIPAAm-*block*-PEG/PPG copolymer behavior at different temperatures: (a) at 25 °C, (b) at 33 °C, (c) at 35 °C, and (d) at 52 °C. Reproduced from ref. 225 with permission from Royal Society of Chemistry, copyright 2014 **[225]**.

Xanthan/lignin hydrogels were prepared by Raschip *et al*., using epichlorohydrin as a crosslinking agent in an alkaline medium and different types of lignin **[226]**. Compared to xanthan gums, the hydrogels had higher stability due to the incorporation of lignin. The morphology and thermal decomposition of the hydrogels were influenced by the type of lignin used. In another study by Raschip *et al*., they prepared biodegradable and superabsorbant xanthan/lignin hydrogels by crosslinking between xanthan gum and different types of lignin, and they also used epichlorhydrin as a crosslinking agent **[227]**. The hydrogels that were produced had combined antimicrobial and antioxidative properties, with an increased thermal stability, hydrophilicity and biocompatibility of hydrogel-films by incorporating lignin. Owing to these properties, the resulting hydrogels can be used in pharmaceutical and biomedical field.

Quraishi *et al.* developed alginate-lignin aerogels using CO_2, as an acidifier to release Ca^{2+} ions for the crosslinking of alginate–lignin mixture and promote the gelation, followed by solvent exchange and supercritical drying **[228]**. Foaming by rapid expansion of CO_2 showed to be an effective way to introduce pores of few hundred microns into the aerogels. The obtained alginate-lignin aerogels presented textural and morphological properties (**Fig. 20**), cytocompatibility and good cell adhesion that fulfill the requisites for tissue engineering and regenerative medicine applications.

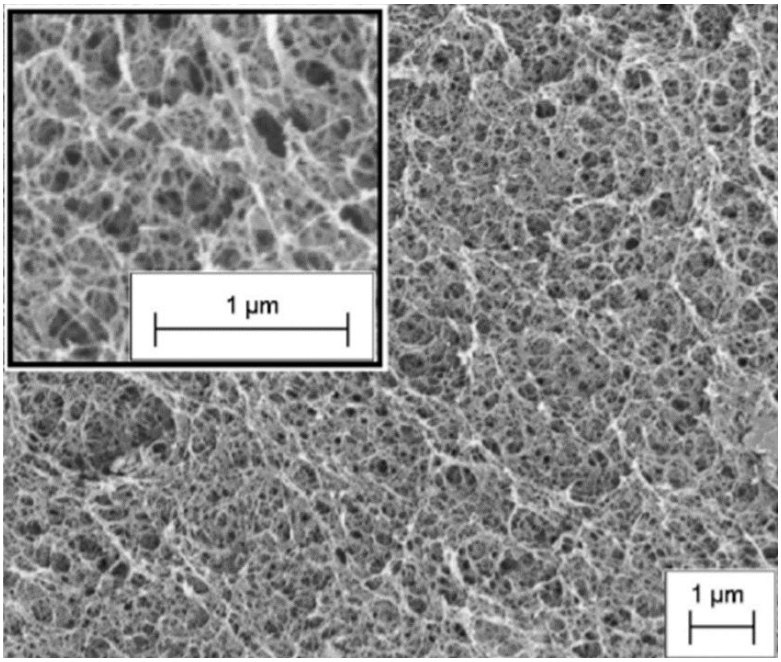

Fig. 20. SEM structure of alginate–lignin aerogel (alginate/lignin; mass ratio 4:1). Reproduced from ref. 228 with permission from Elsevier, copyright 2017 **[228]**.

Xanthan/lignin hydrogels were prepared by Raschip *et al*., using epichlorohydrin as a crosslinking agent in an alkaline medium and different types of lignin **[226]**. Compared to xanthan gums, the hydrogels had higher stability due to the incorporation of lignin. The morphology and thermal decomposition of the hydrogels were influenced by the type of lignin used. In another study by Raschip *et al*., they prepared biodegradable and superabsorbant xanthan/lignin hydrogels by crosslinking between xanthan gum and different types of lignin, and they also used epichlorhydrin

as a crosslinking agent **[227]**. The hydrogels that were produced had combined antimicrobial and antioxidative properties, with an increased thermal stability, hydrophilicity and biocompatibility of hydrogel-films by incorporating lignin. Owing to these properties, the resulting hydrogels can be used in pharmaceutical and biomedical field.

Using ATRP, Kai *et al.* synthesized poly(ethylene glycol) methyl ether methacrylate (PEGMA)-grafted lignin hyperbranched copolymers with hyperbranched architecture **[229]**. In the presence of α-cyclodextrin, aqueous solutions of the copolymers formed supramolecular hydrogels with a very low critical gelation concentration. The lignin biodegradable core was found to be particularly important for inducing the gelation, in which 1 wt-% copolymers were sufficient to form stable hydrogels at body temperature. Combining the rheological properties and excellent self-healing ability with the good biocompatibility, the resulting lignin–PEGMA/cyclodextrin hydrogels were proposed as promising smart biomaterial for biomedical applications.

Scaffolds are widely used for tissue engineering purposes due to their similarity to the native extracellular matrix in terms of both chemical composition and physical structure. Polymeric nanofiber matrices constituted by lignin are also candidates to mimic the extracellular matrix, because of its nanoscaled size, being a potential candidate for the development of future tissue engineering scaffolds **[209, 230]**. In this way, Kai *et al*. prepared poly(lactic acid) (PLA)−lignin copolymers by grafting the PLA onto the alkylated lignin *via* ring opening polymerization, using different proportions of lignin (10–50%) **[209]**. They found that the PLA chain length of the resulting copolymers varied from 5 to 38. These copolymers were then blended with poly(L-lactide) (PLLA) to form nanofibrous composites by electrospinning with fiber diameters of 350–500 nm (**Fig. 21**). The resulting nanofibers showed a good antioxidant activity and biocompatibility when they were evaluated by incubation with three different cell lines (PC12, human dermal fibroblasts, and human mesenchymal stem cells).

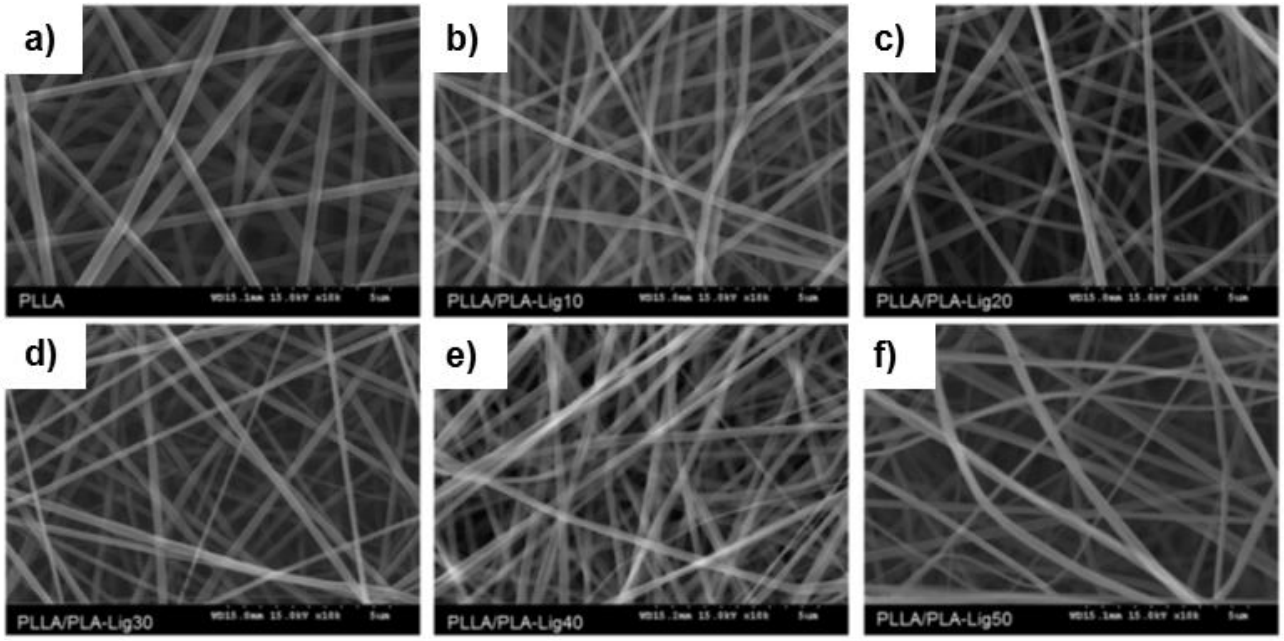

Fig. 21. Scanning electron microscopy (SEM) images of electrospun nanofibers: (a) PLLA; (b) PLLA/PLA–10% Lignin; (c) PLLA/PLA–20% Lignin; (d) PLLA/PLA–30% Lignin; (e) PLLA/PLA–40% Lignin; and (f) PLLA/PLA–50% Lignin. Scale bars = 5 μm. Reproduced from ref. 209 with permission from American Chemical Society, copyright 2016 **[209]**.

The same group also fabricated poly(methyl methacrylate) (PMMA) grafted lignin copolymers by atom transfer radical polymerization, and further blending with poly(ε-caprolactone) to produce nanofiber structures by electrospinning, with reinforced mechanical properties **[211]**. These biocompatible electrospun nanofibers were found promote the proliferation, attachment and interactions of human dermal fibroblasts. More recently, Kai *et al.* synthetized new lignin-based copolymers (lignin-poly(ε-caprolactone-co-lactide), lignin-PCLLA) via ring-opening polymerization **[210]**. Then, the lignin-PCLLA copolymers were blended with polycaprolactone and poly(ι-lactic acid) via electrospinning, originating uniform nanofibers with diameter ranging from 300 to 500 nm. The mechanical properties of the nanofibers were significantly improved after adding the lignin copolymers. As in the previous study, these nanofibers also showed antioxidant

activity and *in vitro* cytocompatibility, suggesting that these nanofibers could be used as biomedical material in tissue engineering scaffolds for protecting cells from oxidative stress.

6.5. Lignin-based Nanomaterials for Other Applications

Lignin-based hydrogels have also been developed for other kind of applications, such as water-storing soil conditioner for agriculture, forestry, and soil rehabilitation, as well as for controlled release of fertilizers.

Lignin-modified alginate hydrogels were synthesized by Flores-Céspedes *et al*., with high entrapment efficiency (99.5%) of azadirachtin **[231]**. The addition of lignin to the alginate formulation allowed a slow release and improved stability against photodegradation of the botanical insecticide azadirachtin, which enhanced the storage period of the insecticide. Peng *et al*., prepared pH-responsive hydrogels by the chemical crosslinking of acetic acid lignin with isocyanate group-terminated polyurethane ionomers, in which the swelling ratios increased with the rising of the pH values **[232]**. In addition, the thermal stabilities of the resulting hydrogels were improved and the release profiles of ammonium sulfate indicated that the hydrogels could be used as coating materials to control the release of fertilizer for different agricultural and horticultural applications. Gao *et al*. also prepared pH-responsive lignin-based hydrogels by blending lignin with PDMAEMA and poly(ethylene oxide). The components formed hydrogels at neutral pH and turned to liquid solutions under both acidic and alkaline conditions **[233]**.

Green polymer hydrogels were synthesized by El-Zawawy, by graft polymerization of acrylamide and poly(vinyl alcohol) with alkali or kraft lignin, and then mixing with acrylamide monomer **[234]**. The alkali lignin hydrogel has high swelling ratios and slower water uptake and deswelling rates than the kraft lignin hydrogel. The slower deswelling rate is due to the hydrogel's compatible network structure, whereas the high swelling ratio was attributed to the cooperative interactions between the sponge particles and the bulk matrix. Later, El-Zawawy and co-workers

prepared lignin-based hydrogels using black liquor, a mixture of lignin/carbohydrates that had been obtained from alkaline pulping of rice straw as the backbone material **[235]**. The hydrogels were prepared by two different approaches: 1) by graft copolymerization of poly(vinyl alcohol) and polyacrylamide involving a radical polymerization in the presence of an initiator and mixed with black liquor, and 2) by an addition reaction in which the same reagents were mixed with black liquor in the absence of an initiator. The hydrogels prepared by radical polymerization exhibited higher swelling capacity (60%) when compared to the hydrogels that had been prepared by an addition reaction (27%). Thus, the black liquor containing lignin can also be used to prepare hydrogels, with potential to solve the environmental water pollution.

Lignosulfonate-*g*-acrylic acid hydrogels with superabsorbent capacity were synthesized by Yu *et al*., by copolymerization of acrylic acid with lignosulfonates using *N,N′*-methylene-*bis*-acrylamide as a cross-linker and laccase/tert-butyl hydroperoxide as an initiator **[236]**. The absorption capacities of the obtained hydrogels were evaluated using methylene blue, a cationic organic dye that can be found in the wastewater of textile mills. The absorbing capacity and removal efficiency of methylene blue were improved by introducing carboxylic acid groups from the acrylic acid, enhancing the interaction between the lignin-based hydrogel and cationic dyes. These hydrogels can be successfully used for absorbing contaminants. In another study, Xue *et al*. prepared acrylamide-based hydrogels with different amounts of ethanol organosolv lignin as a reactive filler in the presence of *N,N′*-methylene-*bis*-acrylamide as a cross-linker and ammonium persulfate (APS) as an initiator, and they used the crosslinking copolymer network (**Fig. 22**) **[237]**. The water swelling capacity increased with the increase of the lignin content. In addition, the obtained hydrogels showed excellent properties, including high tensile modulus and elongation at break, which make them promising materials for water retention applications.

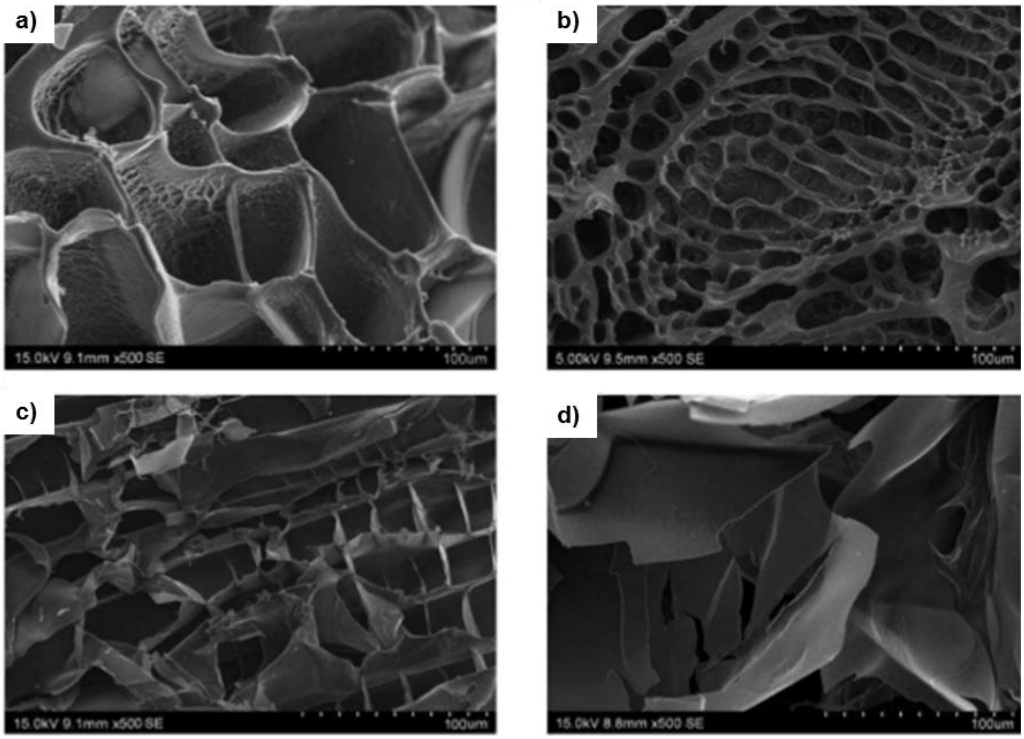

Fig. 202. SEM images of the morphology of acrylamide-based hydrogels that contain an organosolv lignin content of: (a) 0 mg, (b) 10 mg, (c) 20 mg, and (d) 30 mg. Reproduced from ref. 237 with permission from Wiley, copyright 2015 **[237]**.

Passauer *et al*. prepared highly swellable lignin-based hydrogels by crosslinking organosolv lignin with PEG diglycidyl ether (PEGDGE). The swelling properties of the hydrogels and the moisture sorption of the resulting xerogel were strongly dependent on the degree of chemical modification with PEGDGE **[238]**. The total and free water content of the hydrogels decreased by increasing the crosslinking density. The water bound in hydrogels and moisture sorption of xerogels also increased, because of the hydration/dissolution and plasticization that was mediated by the hydrophobic oligo(oxyethylene) and oligo(oxyethylene) glycol substituents, which resulted in moisture diffusion into the xerogel matrix, a plasticization, and swelling of the gels. The hydrogels that were obtained can be used for various applications, particularly as water-storing soil conditioner for agriculture, forestry, and soil rehabilitation.

7. Conclusions and Future Perspectives

Green biomaterials are becoming increasingly in demand due to their low cost of production and eco-friendly properties, which include biocompatibility and biodegradability. For this increased usage to be achieved, bioengineering and nanotechnology are being developed in order to produce promising vehicles for different applications. Thus, lignin shown great potential as a component of new green materials, because of its key characteristics, such as antioxidant, antimicrobial and stabilizing properties that make this biopolymer promising for end-use applications. The emerging valorization of lignin by the chemical and pharmaceutical research fields represents an important stimulus towards the development of high-value materials, such as chemicals and biopolymers, from an economical and renewable aromatic source. Without any chemical modification, lignin is an attractive polymer to reduce the dependence on fossil resources, and it can also be used as a source of carbon for carbon fibers, as well as additive and stabilizer in polymer blends. However, the development and usage of lignin for broader application areas present some challenges, because of its highly variable chemical structure that depends on the extraction method and the source of lignin. To overcome these limitations, different chemical modifications have been proposed to increase the potential use of lignin for the development of advanced lignin-based polymers and nanostructures. For example, esterification or alkylation of the hydroxyl groups on the lignin structure can be performed to increase the compatibility of the lignin polymer with non-polar polymer matrices. Another challenge that lignin faces is the high polydispersity, which increases the complexity to control its macromolecular structure after chemical modification. The development of nanosized lignin-based materials allows the control of the lignin macromolecular architecture, leading to an improvement on the properties of polymer blends and nanocomposites, as well as their application in the biomedical field.

Most of the existing literature is focused on the extraction processes and chemical modifications of lignin for the development of chemicals and polymers with improved properties. This review

covers the topics previously mentioned, and also addresses the main preparation methods of lignin-based nanostructures towards their biomedical applications. The use of natural polymers (such as lignin) over the synthetic polymers is preferable in the biomedical field due to their unique properties (*e.g.*, biocompatibility, biodegradability and abundance in nature). Studies that explored the potential of using lignin in the production of nanosized nanomaterials for drug/gene delivery and other biomedical applications are still limited. Furthermore, *in vitro* and *in vivo* assessments of the LNPs is even more scarcely studied with only few reports on the cytotoxicity evaluation. Apart from nanomaterials, the development of lignin-based hydrogels has promising potential to replace conventional synthetic materials for biomedical and other industrial applications. Given the improvements on the modification chemistry, lignin valorization and its increasing potential at the nanoscale is expected to emerge as an important research field in the near future.

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Properties and Chemical Modifications of Lignin: Towards Lignin-Based Nanomaterials for Biomedical Applications

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Abstract

Biorenewable polymers have emerged as an attractive alternative to conventional metallic and organic materials for a variety of different applications. This is mainly because of their biocompatibility, biodegradability and low cost of production. Lignocellulosic biomass is the most promising renewable carbon-containing source on Earth. Depending on the origin and species of the biomass, lignin consists of 20–35% of the lignocellulosic biomass. After it has been extracted, lignin can be modified through diverse chemical reactions. There are different categories of chemical modifications, such as lignin depolymerization or fragmentation, modification by synthesizing new chemically active sites, chemical modification of the hydroxyl groups, and the production of lignin graft copolymers. Lignin can be used for different industrial and biomedical applications, including biofuels, chemicals and polymers, and the development of nanomaterials for drug delivery but these uses depend on the source, chemical modifications and physicochemical properties. We provide an overview on the composition and properties, extraction methods and chemical modifications of lignin in this review. Furthermore, we describe different preparation methods for lignin-based nanomaterials with antioxidant UV-absorbing and antimicrobial properties that can be used as reinforcing agents in nanocomposites, in drug delivery and gene delivery vehicles for biomedical applications.

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1. Introduction

Biopolymers have shown great potential as alternatives to classic metallic and organic materials for a variety of applications, because of their biocompatibility, biodegradability and low production cost **[1-3]**. The biorenewable polymers *inter alia* that have been extracted from different sources are attracting increasing attention of the research community because of their key advantages, which include: biodegradability, low density and minimization of the environmental effects related with their production and usage **[3-5]**. One example of biorenewable polymers is the lignocellulosic biomass, which are derived from wood and plant sources and is the most promising renewable carbon-containing resource on Earth **[6, 7]**. Lignocellulosic materials are mainly constituted by 30–50% cellulose, a polymer of glucose, 20–35% hemicellulose, a heteropolymer containing xylose, as well as 15–30% lignin, and their compositions are dependent on their origin and species (hardwood, softwood or grass) **[3, 8, 9]**. Thus, lignin is one of the main bio-resource raw materials that can be used for the synthesis of environmentally friendly polymers, and its high content of aromatic structures offers the possibility to replace industrially relevant aromatic polymers and fine chemicals **[10]**. However, only approximately 2% of the annually extracted lignin by the paper and pulp industry from woody biomass has been mainly used for low-value applications as dispersants, adhesives and fillers **[11]**. This is mainly due to the complex and variable lignin structure, high polydispersity and immiscibility of lignin with host polymer matrices **[6, 11, 12]**. The procedures that are applied to separate, to isolate and to chemically transform these three components can lead to a multifunctional array of bio-derived value-added fuels, chemicals and other materials **[13, 14]**. Another way to overcome these limitations is to prepare lignin nanoparticles, which improves the blending properties with host matrix, and offers a morphological and structural control of the lignin **[15-17]**. In addition, the preparation of nanostructured lignin also opens the possibility to use lignin-

based materials for high-value applications, such as drug/gene delivery and tissue engineering **[17]**.

In this review, we provide an overview of the composition and properties of lignin, the processing methods for lignin extraction, chemical modifications of lignin, and the industrial applications of lignin derivatives. Finally, we discuss the preparation of lignin-based nanomaterials for different applications, highlighting the potential of these nanomaterials for biomedical applications.

2. Lignin: Structure, Composition and Properties

Lignin accounts for approximately 30% of the organic carbon in the biosphere, and it is the second most abundant biopolymer of lignocellulosic biomass, after cellulose **[18]**. It is commonly distributed in combination with hemicellulose around the cellulose strands in primary and secondary cell walls, and it is covalently bonded to the carbohydrate/cellulose structure (**Fig. 1a**) **[6, 19]**. Lignin acts primarily as a structural component, by adding strength and rigidity to the cell walls, but it also allows the transport of water and solutes through the vasculature system of the plants and provides physical barriers against invasions by phytopathogens, and other environmental stresses. It can also prevent the degradation of structural polysaccharides by its hydrolytic properties that influence cell wall degradability **[18, 20, 21]**.

Lignin is a highly branched and amorphous biomacromolecule that typically yields molecular masses between 1 000 and 20 000 g mol^{-1}, depending upon the extraction process used **[6, 22]**. It consists of three basic phenylpropanolic monomers known as monolignols: *p*-coumaryl, coniferyl and sinapyl alcohols. When incorporated into the lignin polymer, the units that originated from the monolignols are called *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units, respectively (**Fig. 1b**) **[22-24]**. Lignin biosynthesis starts with the synthesis

of monomers, which is initiated by the deamination of the aromatic amino acid phenylalanine, and its subsequent polymerization by a cascade of oxidases and peroxidases. A sequence of hydroxylation, methylation and reduction reactions by specific enzymes leads to the production of the three basic units for the biosynthesis of lignin **[6, 25, 26]**. Next, the monolignols are exported to the apoplast through the plasma membrane and, finally, the activation of the monolignols leads to the formation of monolignol radicals and their polymerization to produce the lignin polymers, a process that is mediated by enzymes such as peroxidases, laccases and other polyphenol oxidases **[20, 27]**.

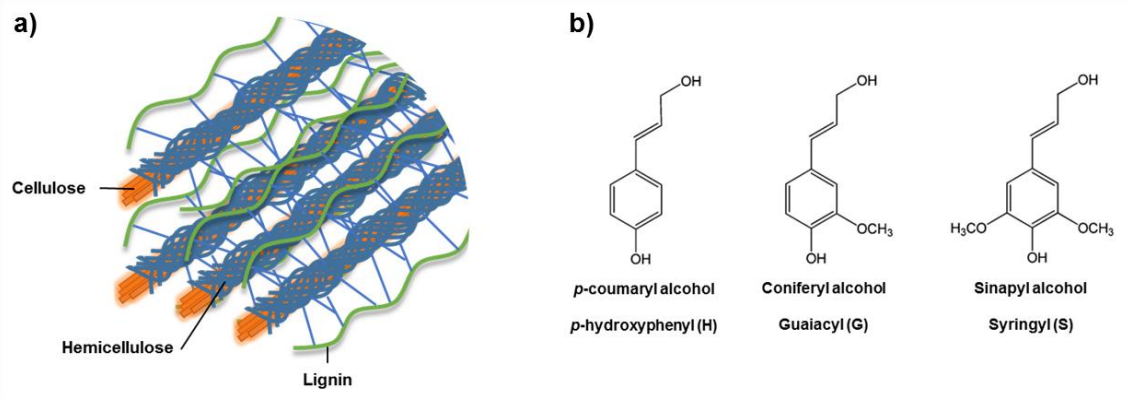

Fig. 1. Lignin distribution in lignocellulosic biomass (a) and monolignol monomers species present in lignin (b).

The main bonds between monomers in lignin are *β*–O–4′ ether linkages that make up more than 50% of the linkage structures of lignin and is a crucial target for most of the degradation mechanisms. Other bonds include *β*–5 phenylcoumaran, *β*–*β*′ resinol, α–O–4′ ether, 4–O–5′ diphenyl ether, 5–5 biphenyl and *β*–1′ diphenyl methane and these account for smaller percentages (**Fig. 2**) **[9, 28-32]**. The amount of lignin varies according to the origin of the lignocellulosic starting material, and the proportion of different monolignols and chemical bonds in lignin structure also depend on the source of the lignocellulosic biomass, because these vary between different hardwood, softwood or grass sources **[3, 8]**. In the case of

softwoods, lignin is mainly composed of guaiacyl units that are linked by ether and carbon-carbon bonds whereas for hardwoods, lignin has equal amounts of guaiacyl and syringyl units. However, the grass lignin is characterized by guaiacyl, syringyl and *p*-hydroxyphenyl units **[19, 24, 33]**. Consequently, the degree of substitution will affect the degree of lignin that crosslinks and also the rigidity of the structure **[6, 33]**.

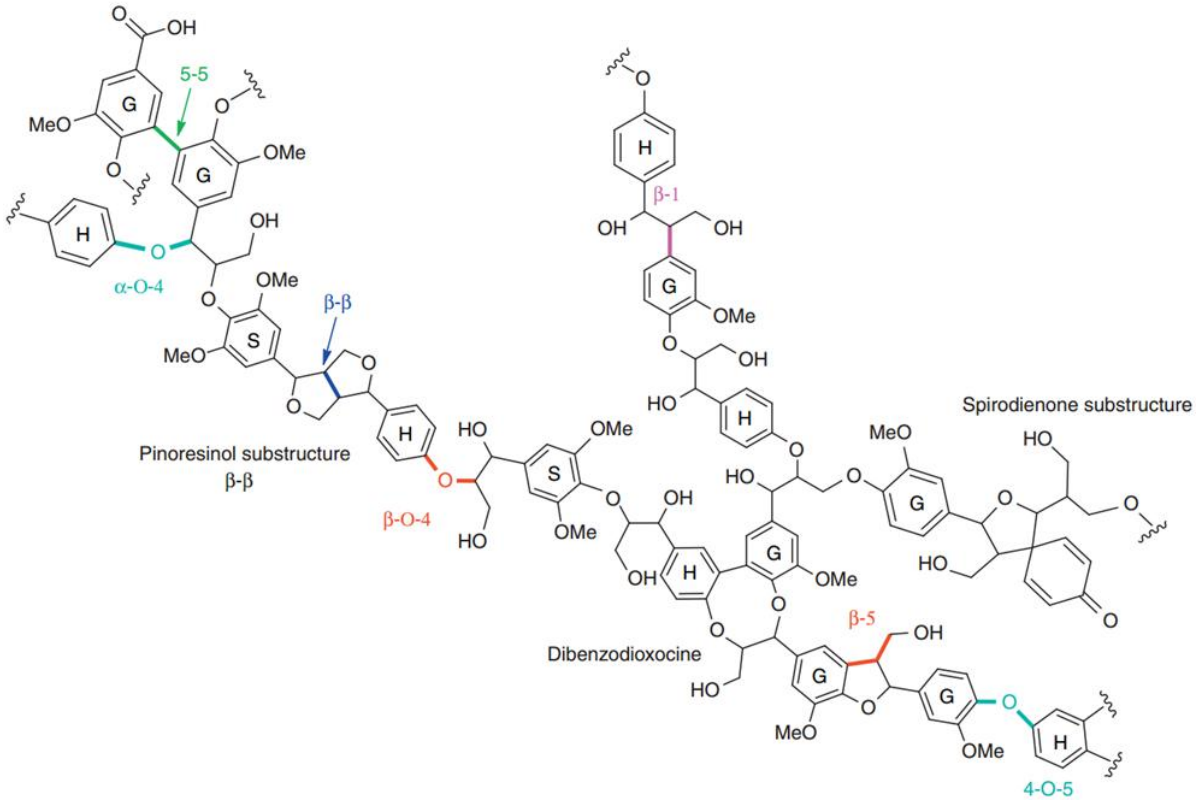

Fig. 2. Example of the lignin structure with the main linkages: *β*–O–4′, *β*–5, *β*–*β*′, *α*–O–4′, 4–O–5′, 5–5 and *β*–1′ bonds. Reproduced from ref. 32 with permission from IFP Energies nouvelles, copyright 2013 **[32]**.

The properties and advantages of lignin are similar to those of other biorenewable polymers and include: antioxidant, antifungal and antimicrobial activities, availability in large amounts as industrial waste byproducts, biodegradability and is CO_2 neutral. Compared to cellulose/hemicellulose, lignin is more resistant to chemical and biological attacks and possesses higher strength that provides structural integrity to the cell wall, protecting other

components from enzymatic degradation **[3, 10, 34, 35]**. Lignin is also able to absorb UV-radiation and has fire-retardant properties **[36, 37]**. Furthermore, lignin has several potentially beneficial physicochemical properties that make it a promising component for the renewable energy industry and other lignin-based products. The presence of the reactive functional groups allows the modification of the molecule by using targeting moieties such as peptides or antibodies and the preparation of graft copolymers. Other beneficial properties include a hydrophilic or hydrophobic feature that depends on the lignin source, good rheological characteristics, good viscoelastic properties, film-forming capacity, and compatibility with a wide variety of industrial chemicals **[6]**.

Natural lignin is almost colorless, but after acid or alkali treatment it takes on a brown or dark brown color **[14, 38]**. Furthermore, the content of phenolic, hydroxyl, carboxyl and sulfonate functional groups are affected by the extraction method used **[22]**. However, the use of lignin in value-added products can be hampered by its complicated molecular structure, which is the most challenging issue in lignin valorization approaches **[7]**. Different methods have been used for the industrial extraction of lignin, which result in intermediate products, which can have several applications.

3. Processing Methods for Lignin Extraction

Lignin extraction from lignocellulosic biomass is performed in such a way that lignin is gradually broken down to lower molecular weight (MW) products **[6, 7]**. Both extraction methodology and source of lignin will influence the physicochemical properties of the isolated product **[6, 9]**. Some key factors that can affect the extraction process are as follows: temperature, pH and pressure of the system, capability of the solvent/solute to take part in the lignin fragmentation process and avoid the lignin recondensation, and also the capacity of the solvent to dissolve lignin molecules **[9, 28]**. Currently, there are four main industrial

processes used to extract pure lignin: sulfite, kraft, organosolv and soda processes **[28, 39]**. The main processes for lignin extraction are discussed in this section and summarized in **Table 1**. The distinctive features of the extraction methods of lignin are described in the following subsections.

Table 1. Methodologies for lignin extraction, treatment conditions, solubility, MW and functional groups of lignin. (ND – No Data; [a]Conventional process; [b]Values referred to softwood).

Extraction Process	Treatment Conditions	Solubility [10]	Lignin MW (Da)	Functional Groups (%) [6]		
				COOH	OH phenolic	Methoxy
Kraft[a]	**1st:** Sodium hydroxide and/or sodium sulfide (pH=13–14 and T≈170 °C) **2nd:** Sulfuric acid (pH=5–7.5)	Alkali; Organic solvents	1000–15000	4.1[b]	2.6[b]	14[b]
Sulfite	Metal sulfite + Sulfur dioxide (Ca^{2+}, Mg^{2+} or Na^{+}) (pH=2–12 and T=120–180 °C, for 1 to 5 h)	Water	1000–50000	ND	ND	ND
Soda	13–16% wt of Sodium hydroxide (T=140–170 °C) + Anthraquinone (catalyzer)	Alkali	1000–3000	7.2–13.6	2.6–5.1	10–16
Organosolv	Organic solvents (ethanol, methanol, acetic and formic acid), usually mixed with water (T=170–190 °C)	Wide range of organic solvents	500– 5000	3.6–7.7	3.4–3.7	15.1–19

3.1. The Kraft Process

Kraft pulping is the principal method used to process lignocellulose, and it accounts for approximately 85% of the produced lignin **[40]**. In the conventional Kraft process, the delignification process is performed at high temperatures and high pH values, during which the lignin is dissolved in sodium hydroxide and sodium sulfide (white liquor) **[28, 39]**. First, lignin is dissolved by the cleavage of the ether linkages, at pH values between 13 and 14 and temperatures around 170 °C, increasing the number of phenolic hydroxyl groups that will

ionize under this pH range and, consequently, allow the solubilization of lignin **[31, 41]**. After that, lignin can be isolated from the remaining alkaline solution by acid (*e.g.*, sulfuric acid) mediated precipitation, which lower the pH to 5–7.5 **[6, 42, 43]**.

Kraft lignin has been produced commercially by the LignoBoost Technology, where lignin is extracted from pulp black liquor **[28]**. In this methodology, lignin is precipitated by acidification by using CO_2 and it is filtered in an initial chamber press filter. After, the filter cake is re-dispersed, acidified using H_2SO_4, filtered and then washed by means of displacement washing in a second chamber press filter **[44]**.

In general, kraft lignin contains small amounts of sulfur groups in its structure (1–3% by wt), and since it is only soluble at pH > 10, most of the commercially available lignin is sulfonated to become soluble in water **[31, 45]**. The mean MW of the kraft lignin usually ranges from 1000 to 3000 Da, but it can go up to as high as 15000 Da **[39, 46]**. Similarly to other isolation processes, the native lignin is submitted to some chemical and structural changes **[31]**. Nevertheless, the production of large amounts of lignin using this process is important for its transformation into high-value lignin-based materials **[12]**.

3.2. The Sulfite Process

The sulfite process is an extraction method that is commonly used in the pulp and paper industry **[28]**. This process involves the reaction between lignin and a metal sulfite and sulfur dioxide, with calcium, magnesium or sodium acting as counter ions **[6, 31]**. By changing the pulping chemicals mix and its dosage, the pH of the system can range between 2 to 12. The process is executed at temperatures that can vary between 120 to 180 °C, with a digestion time of 1 to 5 h **[28, 45]**. Under these conditions, the sulfite delignification process leads to the cleavage of α-ether (α–O–4′) and β-ether (β–O–4′) linkages of lignin, which is an acid catalyzed process. The introduction of polar sulfonic acid groups to the lignin backbone

enables its hydrolysis and dissolution, while the cellulose remains in the solid state **[39, 47]**. The sulfur content of lignosulfonates obtained by this process can range between 4 and 8% and, because of the low pK_a (less than 2) of the sulfonate groups, the isolated lignin is water-soluble under most conditions **[6, 31]**. The lignosulfonates produced by using the sulfite treatment have a higher MW compared to lignin extracted by the kraft process, which is a result of the incorporation of the sulfonate groups into the lignin skeleton. Depending on the source of lignin, the MW can vary between 1000 and 50000 Da, when the lignin comes from hardwood or softwood, respectively **[28, 39, 45]**. However, the sulfite process presented some disadvantages, including the formation of new C–C bonds that alter the lignin's structure, and the presence of high content of ash and other impurities after isolation **[39]**.

3.3. The Soda Process

Soda pulping is typically used for the treatment of non-wood material, including grass, straw and sugarcane bagasse, which accounts for 5% of the total pulp production **[31, 39]**.The solubilization mechanism of lignin is similar to that of the kraft process, as the biomass is digested at temperatures that vary between 140 to 170 °C in the presence of 13–16% by weight of aqueous solution of sodium hydroxide. However, anthraquinone is also used as a catalyzer in order to decrease the degradation of carbohydrates and to dissolve lignin **[6, 31, 39]**. The obtained soda lignin is difficult to recover by filtration or centrifugation due to the high content of carboxylic acid, which results from the oxidation of aliphatic hydroxyl groups **[6]**. The mean MW of the soda lignin is 2400 Da and it can range between 1000 and 3000 Da, depending on its carbon content **[31, 39, 48]**. However, comparing to the previous extraction methods, the soda lignin contains no sulfur, which makes it a good potential for the preparation of high-value products **[6]**.

3.4. The Organosolv Process

The organosolv process is one of the most promising alternatives to the current conventional pulping methodologies, and it is based on the treatment of the biomass using organic solvents as the delignifying agents. The commonly used solvents include: ethanol, methanol, acetic and formic acid that are usually mixed with water at temperatures that range from 170 to 190 °C **[39, 49, 50]**. In addition, basic or acidic catalysts can be added to improve the efficiency of this process **[39]**. The delignification process is carried out by adding mixtures of organic solvent and water that dissolve part of the lignin and hemicellulose **[51]**. During this process, mainly the lignin-carbohydrate and the α–O–4′ linkages are broken, but β–O–4′ can also be cleaved to a lesser extent when the lignin oligomers are released from the lignocellulose matrix **[39, 52]**. The recovery and separation of the dissolved lignin and hemicelluloses can be done by precipitation of lignin or evaporation of the organic solvent, after adjusting temperature, pH and concentration of the organic solvent **[51, 53]**. Organosolv pulping is one of the most efficient options for the further valorization of lignin and it also preserves the native structure of the lignin **[31, 39]**. Moreover, this process is relatively clean and less aggressive compared to the other processes. The obtained lignin is sulfur-free, with lower ash-content, it has higher purity due to the lower carbohydrate content and lower MW (500–5000 Da) **[39, 49, 54, 55]**. However, despite these advantages, this delignification process is not widely used because of the extensive corrosion of the equipment and the lower quality of the pulp produced compared to the soda and kraft pulping methods **[6]**. Also, under acidic conditions, random condensation reactions can occur, instead of the depolymerization reactions. For example, the formation of benzylic cations under acidic conditions can take place, leading to the formation of new C–C bonds **[39]**.

Taking all of this into consideration, the native structure of lignin undergoes a chemical transformation after the extraction process. Besides the limitations abovementioned, the structure of kraft, organosolv, sulfate and soda lignin can also vary according to the plant source. Thus, the introduction of lignin into new markets will strongly depend on its structure, reactivity and physicochemical properties **[56]**.

4. Chemical Modifications of Lignin

The chemical structure of lignin can be easily modified for the development of new materials, especially the phenolic and aliphatic hydroxyl groups. Lignin can be used with or without chemical modification, depending on the target application (**Fig. 3**) **[10]**. Without chemical transformation, lignin can be directly incorporated into a polymeric matrix to reduce production costs and improve properties. For example, unmodified lignin can serve as a UV-light stabilizer, an antioxidant, a flame retardant and an additive to promote plasticity and flow properties of an end product **[57-63]**. Although there is potential for direct industrial applications, unmodified lignin can only be incorporated in small amounts due to its weak mechanical properties and thermal instability. On the other hand, lignin can be chemically modified in order to be used as a starting material for polymer synthesis or conversion into chemicals and fuels **[10, 64]**. There are four different approaches to modify lignin chemically: 1) lignin depolymerization or fragmentation, using the lignin as a carbon source or to cleave it into small fragments that contain aromatic rings; 2) modification of lignin by synthesizing new chemically active sites; 3) chemical modification of the hydroxyl groups present in the lignin structure; and 4) production of graft copolymers **[10, 64]**. These chemical modifications are very dependent on the reactivity of the functional groups and structural features of the lignin that is used. The main features of these processes are discussed in the following sub-sections and are summarized in **Table 2**.

Table 2. Summary of the main chemical modifications of lignin (methodology and final products), including lignin depolymerization, synthesis of new chemical sites and functionalization of the hydroxyl groups.

Chemical Modification	Methodology	Final Products
Lignin Depolymerization		
Pyrolysis ***Conventional (or slow)*** ***Flash (or fast)***	Heating the lignin at T=500 °C, slow heating rate and long residence time from 5–30 min. Heating the lignin at T range 600–1000 °C, high heating rate and short residence time range 0.5–5 seconds. **Catalysts:** Zeolites (ZSM5); Transition metal oxides (Co_3O_4, MoO_3, NiO, Fe_2O_3, MnO_3, CuO); Transition metals (Mo, Cu, Mn, Fe, Ni).	Solid char; Liquid oil (bio-oil); Gases.
Oxidation ***Hydrogen Peroxide*** ***Nitrobenzene*** ***Oxygen***	Alkaline conditions: T range 80–90 °C; Acidic conditions: T range 130–160 °C. **Catalysts:** transition metals, such as Fe^{2+}, Cu^{1+}, Mn^{3+} and Co^{2+}. Alkaline conditions: T range 170–190 °C; residence time of 1–4 h. Alkaline conditions: benzaldehydes; Acidic conditions: benzoic acids; **Catalysts:** Transition metals (Cu^{2+}, Fe^{3+}, Mn^{2+} and Mn^{3+}, Co^{2+} and Zr^{4+}) and metal oxides (CuO, TiO_2 and ZnO).	Mono- and Dicarboxylic acids; Aldehydes: Vanillin, Syringaldehyde, *p*-Hydroxybenzaldehyde, 3,4,5-Trimethoxy benzaldehyde; Acids: Vanillic, Syringic, *p*-Hydroxybenzoic acid; Alcohols: 2-Hydroxy benzyl and Catechol
Hydrogenolysis	Addition of H_2 to break down C–O–C linkages in the lignin structure; T range 300–600 °C; **Catalysts:** noble metals (Pt, Ru, Pd, and Rh), metal oxides (Pd/C, $CoMo/Al_2O_3$).	Phenols, Guaiacols, Syringols, Trimethoxybenzenes,
Hydrolysis	Addition of water to cleave the lignin molecules (under subcritical or supercritical conditions); T range 280–400 °C; Residence time: Some minutes to a few hours; Pressure range 20–25 MPa; **Catalysts:** K_2CO_3, $Ca(OH)_2$ and Na(OH).	Methoxyphenols; Hydroxyphenols; Catechol; Guaiacol.
Gasification	Reactions: hydrolysis, polymerization, hydrogenation, water–gas shift, steam reforming, and methanation. T = 700 °C; Pressure range 15–27.5 MPa	CO_2, CO, H_2 and CH_4, C_2H_4, C_2H_6

	Catalysts: alkali and alkali salts (NaOH, KOH, Na_2CO_3, CaO, $NaHCO_3$, etc.), transition metals (Ni, Pt, Ru and Rh), metal oxides (*e.g.*, $Ni/MgAl_2O_3$).	
Synthesis of New Chemically Active Sites		
Hydroxyalkylation	Reaction processes: demethylation, phenolation and methylolation (reaction of lignin with formaldehyde in alkaline medium).	Lignin-phenol-formaldehyde resins (adhesive properties)
Amination	Mannich reaction: the amination of lignin is done by using different types of amines and formaldehyde (under alkaline, neutral or acidic conditions).	Aminated lignins (asphalt emulsifier, cationic surfactants, epoxy resins and polyurethane synthesis)
Nitration	Using non-aqueous solvents with nitrating agents (*e.g.*, nitric acid with acetic anhydride, acetic acid or sulfuric acid).	Polyurethane-nitrolignin films
Sulfomethylation **Sulfonation**	Adding methylene sulfonate groups to lignin structure, by using methanol and alkali metal sulfite in water at 100 °C, under neutral to basic pH. Adding sulfonate groups to lignin, by using sulfuric acid or sodium sulfite.	Sulfomethylated products (dye dispersant) Sulfonated lignin (cement dispersant)
Functionalization of Hydroxyl Groups		
Alkylation/dealkylation	**a)** Reaction with diazoalkanes; **b)** Reaction with alcohol in the presence of a catalyst (*e.g.*, hydrochloric acid); **c)** Reaction using alkylsulfates and sodium hydroxide.	Demethylated lignin + Polyethylenimine (wood adhesive)
Esterification	**a)** Ring opening reactions using cyclic esters (*e.g.*, ε-caprolactone and lactide); **b)** Condensation polymerization with carboxylic acid chloride (*e.g.*, sebacoyl chloride and terephtaloyl chloride); **c)** Dehydration polymerization with dicarboxylic acids (*e.g.*, dimeric acid and carboxytelechelic polybutadiene).	Lignin-based polyesters (for producing lignin-based epoxy resins, polyurethanes, and unsaturated thermosetting composites)
Etherification	**a)** Polymerization using alkylene oxide (*e.g.*, ethylene oxide and propylene oxide); **b)** Polymerization with epichlorohydrin; **c)** Cross-linking using diglycidyl ethers; **d)** Solvolysis of lignin with ethylene glycol.	Lignin-based polyethers (*e.g.*, lignin-based epoxy resins)
Phenolation	Reaction between lignin and phenol in the presence of organic solvents (*e.g.*, methanol or ethanol).	Lignin phenol formaldehyde resins; Lignin-based polyurethanes films
Urethanization	**a)** One step reaction by adding diisocyanate and another diol to the lignin; **b)** Two-step reaction with the production of an isocyanate-based prepolymer, and then the polymerization of lignin with the prepolymer, acting as a polyol chain extender.	Lignin-based polyurethane

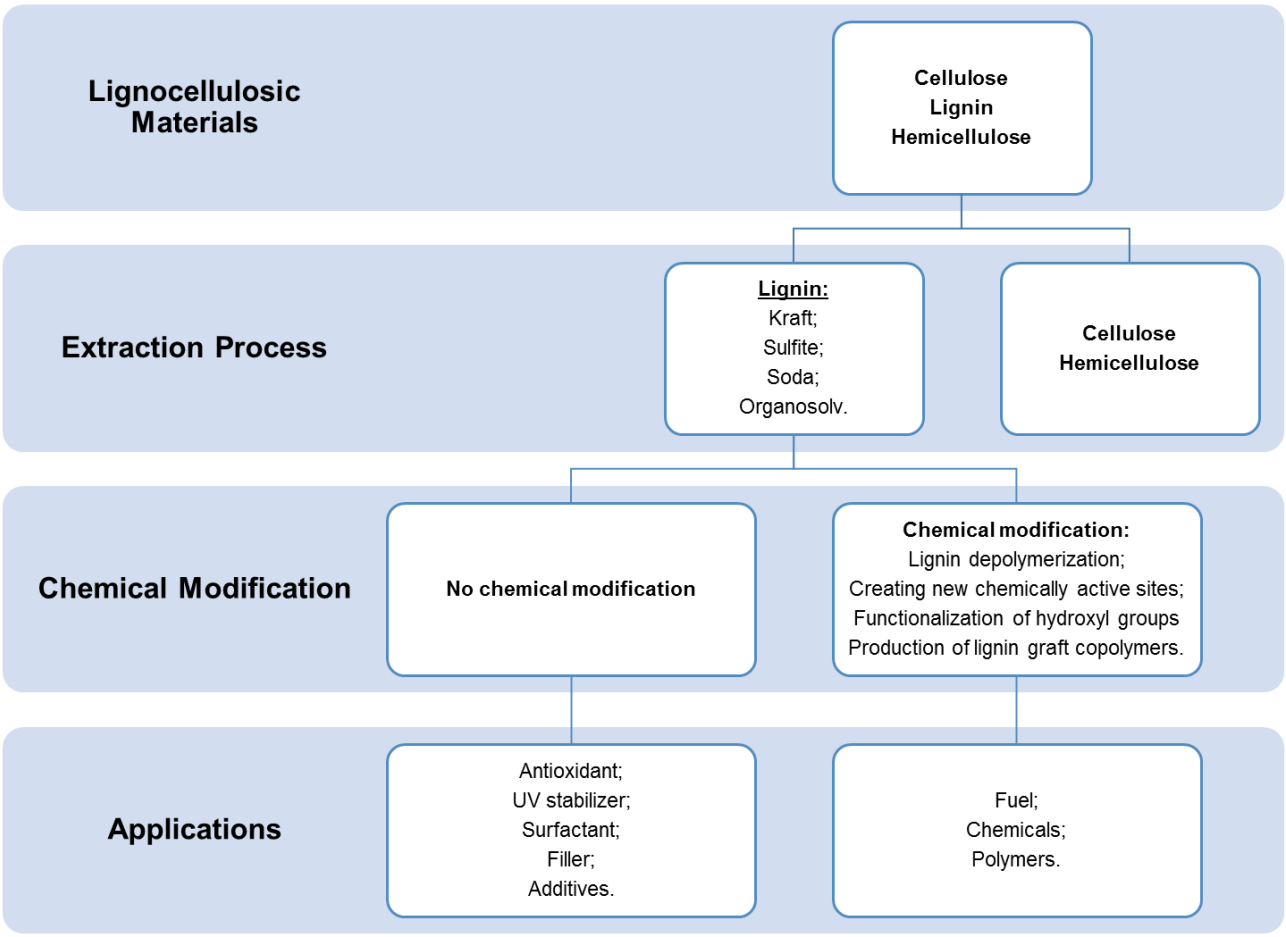

Fig. 3. Schematic representation of the main processes for lignin extraction and possible chemical modifications performed in order to valorize lignin, depending on the applications.

4.1. Lignin Depolymerization

Lignin depolymerization, or fragmentation, is a promising method to transform lignin raw materials and generate valorized lignin-based products. Therefore, the lignin molecules are converted into small compounds for further application, including fuels and basic chemicals or oligomers **[65, 66]**. Several thermochemical methods have been studied for lignin depolymerization, such as pyrolysis, oxidation, hydrogenolysis, hydrolysis and gasification (**Fig. 4**) **[67]**.

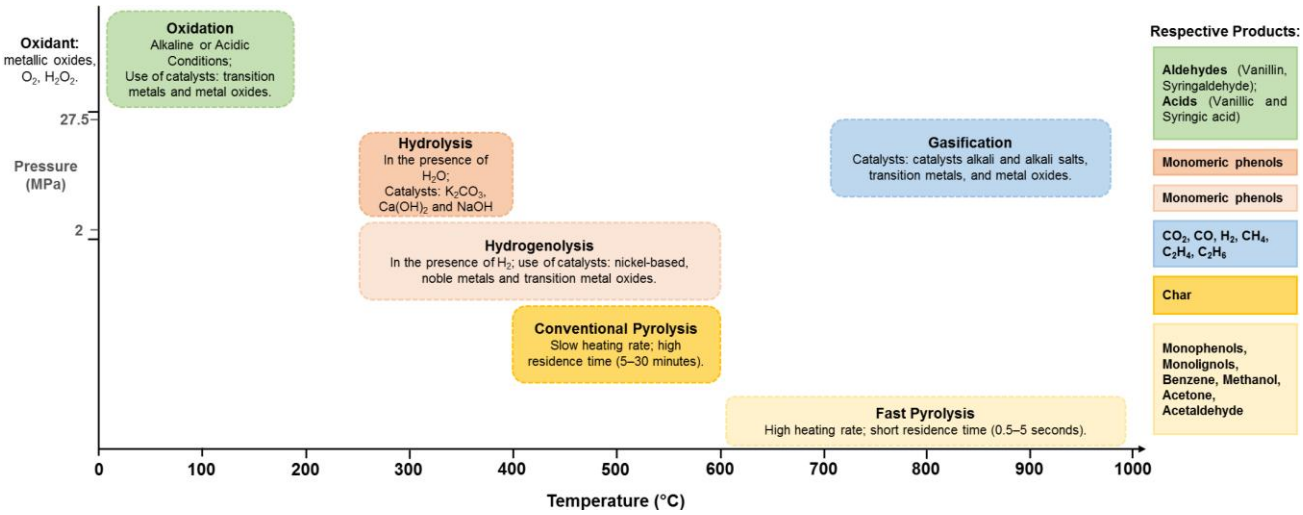

Fig. 4. Overview of the methodologies for lignin depolymerization according to the temperature, presence of oxidants and pressure.

4.1.1. Pyrolysis

Pyrolysis is the thermal treatment that converts lignin into solid char, liquid oil (bio-oil) or gases, in the absence of oxygen with or without any catalyst **[65, 67]**. This is a complex process and the products obtained during the procedure are dependent on several factors, including the feedstock type, temperature of the reaction, heating rate and additives **[67, 68]**. Pyrolysis can be classified into two different categories based on whether it is conventional (slow) pyrolysis and flash (fast) pyrolysis **[10, 69]**. In conventional pyrolysis, lignin is heated to temperatures of around 500 °C, with a slow heating rate and a residence time that ranges between 5 to 30 min. In contrast, the flash pyrolysis heating is carried out at temperatures between 600 to 1000 °C, with a higher heating rate and shorter residence time (0.5–5 seconds) than in conventional pyrolysis **[10, 32, 69, 70]**. By changing all these parameters, it is possible to obtain different products from the same biomass. For example, when the purpose is to obtain a high level of char production, low temperature and low heating rates should be used. On the other hand, low temperature, high heating rate and short residence time should be chosen to maximize the yield of liquid products obtained from the biomass. In order to maximize the yield of gas production, high temperature combined with

extended residence time and low heating rate are required **[69, 70]**. Lignin can also be converted by catalytic fast pyrolysis using different catalysts, such as zeolites (ZSM5), transition metal oxides (Co_3O_4, MoO_3, NiO, Fe_2O_3, MnO_3 and CuO), and supported transition metals (Mo, Cu, Mn, Fe and Ni) **[71, 72]**.

4.1.2. Oxidation

Lignin oxidation is a method that is used to obtain aldehydes, such as vanillin and syringaldehyde, and their corresponding acids, vanillic and syringic acid. Oxidants are used to preserve the aromatic rings present in the lignin structure, with or without catalysts (Cu^{2+} and Co^{2+} salts) **[10, 73, 74]**. The most common oxidants used for lignin depolymerization are nitrobenzene, metallic oxides, oxygen and hydrogen peroxide **[67]**. Lignin is a promising candidate for oxidation reactions due to the presence of hydroxyl (phenolic) groups in its structure. The oxidation rate at a given temperature can be controlled by the amount of phenolic groups, pH (alkaline or acidic oxidation), and concentration of oxygen **[67, 75]**.

The oxidation process with hydrogen peroxide in either alkaline or acidic conditions has been investigated. Under strong alkaline conditions the cracking reaction has been performed at low reaction temperatures that range from 80 to 90 °C. In contrast, under acidic conditions, higher temperatures that range from 130 to 160 °C are required to achieve the same degree of cracking. The main products obtained from the oxidative degradation of lignin were mono- and dicarboxylic acids and the intermediate products were aldehydes **[76]**. Some studies showed that the addition of selected transition metals, such as Fe^{2+}, Cu^{+}, Mn^{3+} and Co^{2+} can accelerate the decomposition of hydrogen peroxide into molecular oxygen **[77, 78]**.

When aldehydes are the desired product, the oxidation of lignin by hydrogen peroxide may not be the optimum method **[67]**. In contrast, the alkaline nitrobenzene oxidation allows the production of the aldehydes (vanillin, syringaldehyde, and *p*-hydroxybenzaldehyde) along with their respective

acids (vanillic acid, syringic acid, and *p*-hydroxybenzoic acid). Moreover, temperatures in the range of 170–190 °C and residence times in the range of 1–4 h are required to obtain these products, though these conditions depend on the source of lignin **[79]**. Although nitrobenzene is a very effective oxidant, it is also a proven carcinogen **[80]**. Consequently, the transition metals and metal oxides have been used in order to replace the use of nitrobenzene in lignin oxidation **[67]**.

The addition of transition metals and metal oxides in combination with oxygen and air have the potential to increase the production of phenolic compounds. For example, transition metal ions such as Cu^{2+}, Fe^{3+}, $Mn^{2+/3+}$, Co^{2+} and Zr^{4+} can improve the production of benzaldehydes and benzoic acids from lignin oxidation with oxygen as the oxidizing agent **[78, 81]**. Furthermore, the pH value of the reaction seems to be an important factor for product selectivity. For example, alkaline conditions are preferred for the formation of benzaldehyde, whereas acidic conditions are favorable for the production of benzoic acids **[78]**. CuO can be utilized in the direct oxidation of lignin and other metal oxides, such as TiO_2 and ZnO. These metal oxides can also be used to facilitate photochemical degradation of lignin into a mixture of aldehydes (vanillin, syringaldehyde and 3,4,5-trimethoxy benzaldehyde), acids (vanillic acid and 4-hydroxybenzoic acid) and alcohols (2-hydroxy benzyl alcohol and catechol) **[78, 82-84]**.

The lignin depolymerization occurs in nature via oxidative enzymes, including laccases and peroxidases (lignin peroxidase and manganese peroxidase), that are secreted by some fungi and bacteria. In addition to the aforementioned, the oxidation methods using these naturally occurring enzymes are undergoing industrial development as new biocatalysts. The redox potential of these enzymes is used to complement other catalyzers, allowing a synergetic action on lignin depolymerization in these developing methodologies **[64, 85, 86]**.

4.1.3. Hydrogenolysis

Hydrogenolysis or hydrogenation represents a process in which the addition of hydrogen will break down the C–O–C bonds into lignin. Compared to pyrolysis, the reaction is generally performed at lower temperatures that range from 300 to 600 °C and allows higher yields of monophenols and less char formation **[65, 67]**. The reaction is performed by adding a hydrogen-donating solvent (tetralin, sodium formate and formic acid) or gaseous hydrogen **[67, 87-91]**. For example, an efficient method for the hydrogenation of lignin in the presence of H_2, isopropanol and using Ni–Mo_2C/C as catalyst has been described, in which under mild conditions (T = 250 °C, a residence time of 2 h and 2.0 MPa initial H_2 pressure), approximately 60% by weight of the lignin was converted into phenols, guaiacols, and trimethoxybenzenes **[92]**. Apart from using nickel-based catalysts, some other studies have reported that lignin is hydrogenated to monomeric phenols (*e.g.*, guaiacols and syringols) by using other type of catalysts, including the noble metals such as Pt, Ru, Pd, and Rh; metal oxides, such as Pd/C, CoMo/Al_2O_3, and activated carbon-, alumina- or silica-supported Ru or Pt **[91, 93-96]**.

4.1.4. Hydrolysis

Hydrolysis is the process where water is used to cleave the lignin molecules. Aqueous solutions under subcritical or supercritical conditions are used, and commonly at temperatures that vary from 280 to 400 °C. Reaction times range from minutes to a few hours, under 20 to 25 MPa of total pressure **[32, 67]**. Close to its critical point (22 MPa and 374 °C), water manifests several interesting properties, such as low viscosity and high solubility of organic substances, which makes supercritical water an excellent medium for effective and fast reactions. The high concentration of H^+ and OH^- at subcritical conditions can accelerate acid- or base-catalyzed hydrolysis reactions **[32, 97, 98]**. Although the higher conversion to methoxyphenols or hydroxyphenols occurs under supercritical conditions, subcritical conditions are usually preferred. Additionally, various catechols and guaiacols are produced by hydrolysis of the ether bonds. However, with the increase of the

residence time during the hydrothermal treatment, soluble products are converted into insoluble products due to re-condensation reactions. Catalysts, such as K_2CO_3, $Ca(OH)_2$ and NaOH, are required in hydrothermal synthesis in order to overcome this problem, and their use also increase the liquid yield **[32]**.

Mixtures of water with other solvents have been used in order to improve the lignin solubility and prevent cross-linking reactions **[32, 99]**. Mixtures that contain 50/50 (v/v) water/co-solvent, in which the co-solvents are phenol, ethanol, acetone or formic acid, have been demonstrated to be more efficient for the lignin degradation regarding the yield of reaction **[32, 100-103]**.

4.1.5. Gasification

Gasification is the process that converts lignin into gases, where the principal products obtained during this reaction are CO_2, CO, H_2 and CH_4 **[65, 104]**. Additionally, small amounts of C_2H_4 and C_2H_6 can also be produced and these fuel gases have several applications, including use in fuel cells, gas turbines, and synthesis gas **[105]**. The complete gasification of lignin entails a group of successive reactions, such as hydrolysis, polymerization, hydrogenation, water–gas shift, steam reforming, and methanation, all of which are affected by temperature, reaction time, pressure, concentration, and catalysts. Some studies have reported that the temperature required for complete gasification of lignin was at least 700 °C **[105, 106]**. Additionally, higher pressures (15 to 27.5 MPa) improved the gasification efficiency of lignin under hydrothermal conditions and allowed greater yields of H_2 and CH_4. The lignin decomposition rate was also increased by a higher water density caused by using higher pressures **[107-109]**. Furthermore, the addition of catalysts for lignin hydrothermal gasification are necessary to increase the hydrogen yield production and enhance reactions such as water-gas shift, methanation and hydrogenation. These catalysts are mainly alkali and alkali salts (NaOH, KOH, Na_2CO_3, CaO, $NaHCO_3$, etc.), transition metals (Ni, Pt, Ru and Rh), and metal oxides (e.g. $Ni/MgAl_2O_3$) **[105, 106, 110, 111]**.

4.2. Synthesis of New Chemically Active Sites

Lignin possesses several functional groups, which include the following: hydroxyls, methoxyls, carbonyls and carboxyls. These functional groups can be modified for different applications, which will increase the value of the modified lignin. These modifications consist of synthesizing new macromonomers that are more effective and reactive by increasing the reactivity of hydroxyl groups or changing the nature of chemically active sites. Consequently, the chemical reactivity of lignin increases, the fragility of lignin-derived polymers is reduced, the lignin solubility in organic solvents is increased and thus the lignin processing improves. In this way, several chemical modifications have been used to introduce new chemical sites in lignin structure (**Fig. 5**), including hydroxyalkylation, amination, nitration, sulfomethylation and sulfonation, which are briefly discussed in the next subsections **[10, 112]**.

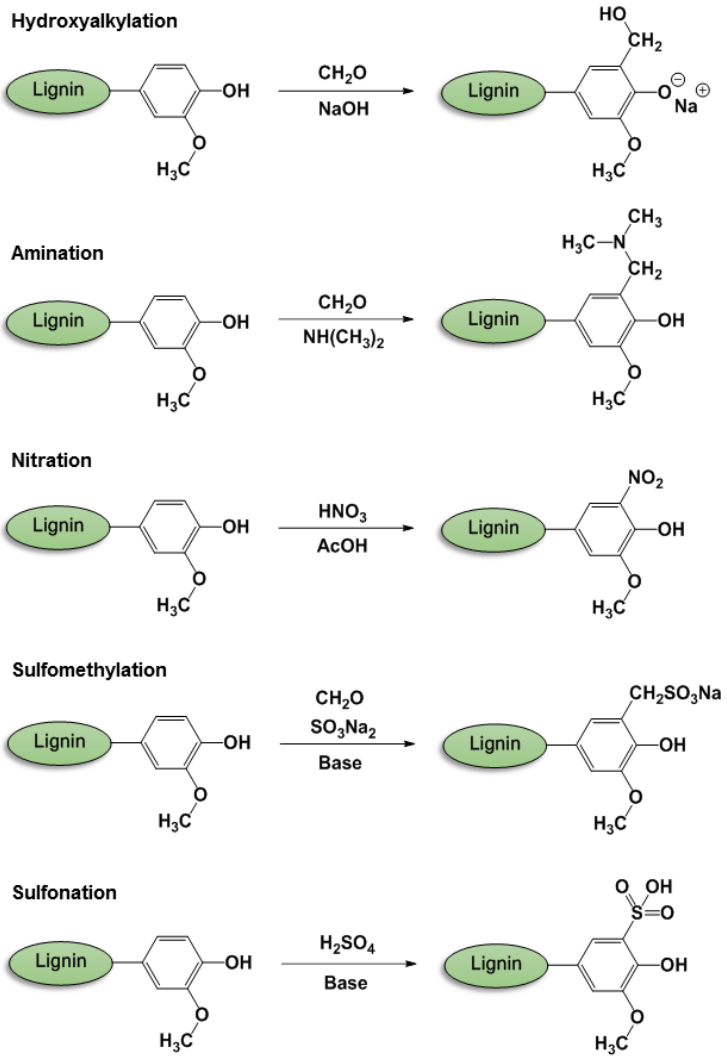

Fig. 5. Overview of the chemical modifications of lignin: synthesis of new chemically active sites.

4.2.1. Hydroxyalkylation

Lignin has been studied as a complement for phenolic wood adhesives. For example, the synthesis of phenol-formaldehyde resins has been replaced by the synthesis of lignin-phenol-formaldehyde resins **[10, 113]**. The reactivity of lignin can be enhanced by demethylation, phenolation and methylolation reaction processes. The reaction of lignin with formaldehyde in an alkaline medium is a prerequisite to the introduction of methylol groups into the lignin structure and is the basis of the lignin methylolation modification process **[10, 114]**. Hydroxyalkylation of lignin has been performed to give adhesive strength with low free formaldehyde content by replacing 40% by weight phenol in phenol-formaldehyde resin synthesis **[10, 115]**. Other aldehydes than

formaldehyde have been used for the integration of lignin into phenolic resins, these include glutaraldehyde and glyoxal, because unlike formaldehyde they are non-toxic and non-volatile **[10, 116-118]**.

4.2.2. Amination

Amination methods introduce the amine functional groups into the lignin structure, which are ionizable and positively charged under acidic conditions **[119, 120]**. There are several chemical methods used for the introduction of amine groups, for example the Mannich reaction is one of the simplest method **[120]**. In Mannich reactions, the amination of lignin structures can be carried out under alkaline, neutral or acidic conditions. Usually, a Mannich reaction is achieved by charging different types of amines and formaldehyde under alkaline conditions **[120]**. For example, the Mannich reaction has been performed for 1-guaiacyl-1-*p*-hydroxyphenylethane, a simple phenolized sulfuric acid lignin model, and it has been reacted with dimethylamine and formaldehyde **[121]**. Its products have been used in the preparation of asphalt emulsifiers, cationic surfactants, epoxy resins and polyurethane **[10, 120-122]**.

4.2.3. Nitration

The nitration of lignin is commonly performed in non-aqueous solvents by using nitrating agents, such as nitric acid with acetic anhydride, acetic acid or sulfuric acid. The resulting product is a yellow to brown amorphous powder, which has a MW that ranges from 600 to 2000 Da and a nitrogen content that varies between 6 to 7% **[10, 119, 123]**. Different graft interpenetrating polymer networks (IPNs), with different NCO/OH molar ratios have been produced from polyurethane (PU) and nitrolignin (NL), by varying the content of 1,4-butanediol as the chain-extender. Increasing the NCO/OH molar ratios also increases the glass transition temperature, the

tensile strength and stiffness of the PU-NL films, whereas the breaking elongation and strain recoverability decreases. Additionally, the introduction of NL into PU limited the motion of the PU molecules, allowing a relatively high cross-link network structure in the PU-NL films. As a consequence, the graft-INPs increased the strength and toughness and also maintained the retractability of the constituent materials **[124, 125]**.

4.2.4. Sulfomethylation and Sulfonation

Sulfomethylation introduces methylene sulfonate groups into the lignin structure in order to increase the number of sulfonate groups in the lignin product and improve its tanning capacity. This reaction is performed with equal moles of methanol, alkali metal sulfite salt and reactive phenolic repeat units of lignin dissolved in water at 100 °C under neutral to basic pH conditions **[119, 126]**. Also, it is possible to synthesize sulfomethyl products of lignin with different degrees of substitution of aromatic rings and side-chains by changing the conditions and ratios of reagents used in this reaction **[127]**. These sulfomethylation products have good dispersibility properties and can be used as a dye dispersant **[128]**.

Sulfonation is a reaction in which sulfonate groups are added to the lignin, by using sulfuric acid or sodium sulfite **[119]**. Among other applications, sulfonated lignin has been used as a dispersant for the cement matrix, due to the high zeta-potential value that contributes to the strong electrostatic repulsion forces between cement particles **[119, 129]**.

4.3. Functionalization of Hydroxyl Groups

As mentioned above, lignin presents in its structure phenolic hydroxyl groups and aliphatic hydroxyl groups at the C-γ and C-α positions on the side chain. Phenolic hydroxyl groups are the most reactive functional groups and can affect the chemical reactivity of the newly formed material. Modifications on hydroxyl groups can lead to the formation of polyol derivatives of lignin. For that

to occur, several reactions have been studied to functionalize the lignin with different functional groups (**Fig. 6**), and these include reactions such as alkylation, esterification, etherification, phenolation and urethanization, which are briefly discussed in the next subsections **[10]**.

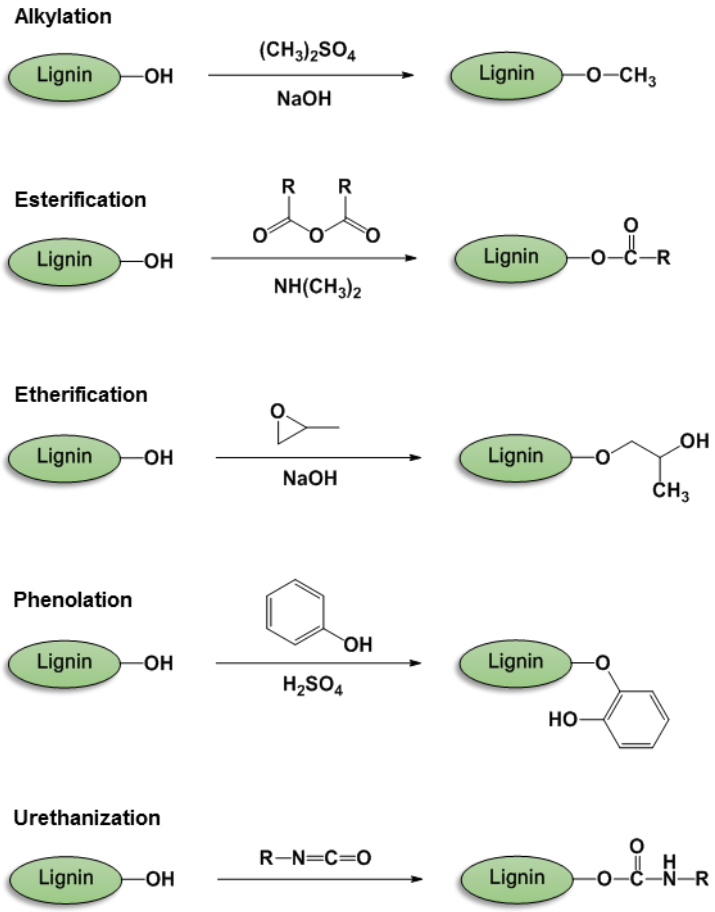

Fig. 6. Overview of the chemical modifications of lignin: functionalization of the hydroxyl groups.

4.3.1. Alkylation/Dealkylation

Lignin presents different sites for alkylation, including the oxygen atoms of the hydroxyl, carbonyl and carboxyl groups. There are three methods for alkylating lignin, which can include a reaction with diazoalkanes, a reaction with alcohol in the presence of a catalyst (*e.g.*, hydrochloric acid), or a reaction using alkylsulfates and sodium hydroxide **[119]**. For example, demethylation is one of the most well-known examples of alkylation/dealkylation reactions that involve lignin in which the demethylated lignin structures are a byproduct in DMSO production. The synthesis starts

with the reaction between lignin and molten sulfur in alkaline media. This demethylated lignin, in combination with polyethylenimine, is used as a formaldehyde-free wood adhesive **[130]**.

4.3.2. Esterification

Esterification is possibly the easiest of the reactions to produce lignin-based polyesters that involve the hydroxyl groups of lignin. Esterification can be performed by using three different procedures, which are: ring opening reactions using cyclic esters, condensation polymerization with carboxylic acid chloride, and dehydration polymerization with dicarboxylic acids **[10, 53]**. For example, the reaction between ε-caprolactone with hydroxypropylated lignin produces lignin-based polyesters with a star-like shape. In these star shapes, hydroxypropylated lignin forms the core, whereas the polycaprolactones form the arm segments **[53, 131]**. Apart from ε-caprolactone, lactide can also be used for copolymerization with lignin to produce lignin-polylactide polyester using triazabicyclodecene as a catalyst in metal and solvent free systems **[53, 132]**. An example of condensation polymerization was obtained when lignin-based polyesters were prepared by reacting lignin with the dicarboxylic acid chlorides, sebacoyl chloride and terephtaloyl chloride in organic solvents **[53, 133]**. Dehydration polymerization procedures that use dicarboxylic acids have been proposed. For example, dimeric acid, a dicarboxylic acid that is synthesized by dimerizing unsaturated fatty acids obtained natural oils, can be applied in the co-esterification with enzymatically hydrolyzed lignin, which makes the co-ester more flexible **[134]**. Additionally, adding carboxytelechelic polybutadiene to lignin can also produce lignin-based polyesters **[135]**.

These lignin esterification reactions that involve the ε-caprolactone and different anhydrides are performed in order to increase the lignin reactivity for the production of lignin-based epoxy resins, polyurethanes and unsaturated thermosetting composites **[53, 136-138]**.

4.3.3. Etherification

The preparation of lignin-based polyethers can be performed by one or any combination of the following procedures: polymerization using the alkylene oxides (*e.g.*, ethylene oxide and propylene oxide), polymerization with epichlorohydrin, cross-linking by using diglycidyl ethers, and solvolysis of lignin with ethylene glycol. The aromatic moieties present in the lignin structure will improve the mechanical and thermal properties of epoxy resins **[53]**. The oxypropylation is the most used etherification method to modify lignin using propylene oxide in the presence of an alkaline solution, in order to prepare lignin-based epoxy resins. The resulting solution was treated with epichlorohydrin and cured using *m*-phenylene diamine for cross-linking **[17, 139]**. Lignosulfonate is first submitted to phenolation using phenol, β-naphtol and bisphenol, and then epoxidized using epichlorohydrin in order to improve the reactivity of the lignin and increase the phenolic group content **[53, 140]**. Lignin from woody biomass can also be grafted with ethylene glycol/ethylene carbonate by solvolysis at 150 °C to introduce ethylene glycol chains into the hydroxyl groups of lignin **[53, 141]**.

4.3.4. Phenolation

Phenolation, also known as phenolysis, is the process by which lignin is modified by reaction with phenol in the presence of organic solvents such as methanol or ethanol in an acidic medium **[10]**. This reaction is commonly used to modify lignosulfonates in order to increase the content of phenol groups and improve the reactivity of the target lignin structure **[10, 142]**. Phenolysis is utilized in the synthesis of phenol–formaldehyde resins before the condensation reaction with formaldehyde. The reaction can be performed at 70 °C for a few hours, after which lignin is added to a phenol-ethanol solution. The curing time and viscosity of the lignin phenol formaldehyde resins are comparable to standard commercial phenol–formaldehyde resins, and this product is used to provide wood adhesive capacity in the construction of particle board **[10, 142, 143]**. Additionally,

lignin that has been modified with cardanol, a natural phenol, can also be used to produce lignin-based polyurethane films with improved properties such as film flexibility **[10, 144]**.

4.3.5. Urethanization

The urethanization process involves the reaction between lignin hydroxyl groups and isocyanate groups to form a urethane link. Traditionally, polyurethanes have been produced from polyols and diisocyanates to provide versatile products. Examples of such products include low temperature elastomers and flexible or rigid adhesives with high tensile strength. Since the lignin structure is rich in hydroxyl groups, it can function as a polyol **[10, 53]**. The improvement of the mechanical properties of lignin-based polyurethanes can be achieved by chemical modifications on lignin such as hydroxyalkylation to introduce soft segments, or by adding other polyols such as polyethylene glycol (PEG) or other diols **[10, 53, 145]**. There are two different approaches for the synthesis of lignin-based polyurethane. The first approach is a one-step reaction that occurs by adding diisocyanate and another by adding diol as the co-monomer. The second approach is a two-step reaction with the first step being the production of a prepolymer using isocyanate together with a polyol, and the second the polymerization of lignin with the prepolymer, acting as a chain extender **[10, 53]**. In the first approach, lignin is directly used in combination with isocyanate and polyols without further chemical modification in order to produce lignin-based polyurethane. For instance, lignin can be mixed with 4,4′-diphenylmethane diisocyanate and PEG to produce lignin-based polyurethane **[10, 146]**. An example of the second approach, is the formation of prepolymers with hydroxyl terminated polybutadiene and 2,4-toluene diisocyanate, which are subsequently reacted with lignin to produce a polyurethane **[53, 147]**.

4.4. Production of Lignin Graft Copolymers

Lignin can be used for the development of lignin graft copolymers in which the polymer chains are attached to the hydroxyl groups on the lignin structure, which produces a star-like branched copolymer with a lignin core. Some examples of polymerization reactions were briefly mentioned above in the chemical modifications section. Two different procedures have been carried out in order to elaborate the lignin graft copolymers: "grafting from" and "grafting to" techniques **[12]**.

4.4.1. "Grafting from" Technique

In the "grafting from" technique, lignin is used as macro-initiator for the polymerization during which a monomer reacts with hydroxyl groups present in lignin and the polymer chain is assembled on the lignin core. The ring opening polymerization of different monomers and radical polymerization of vinylic monomers are two approaches that have been performed to elaborate lignin-graft copolymers by "grafting from" procedure (**Fig. 7**) **[12]**.

4.4.1.1. Ring Opening Polymerization

The reaction of lignin –OH groups with propylene oxide is one of the most common ring opening polymerization reactions that lead to the production of oxypropylated lignin (**Fig. 7**) **[12, 148, 149]**. Using this reaction, the decrease of the *Tg* and viscosity of the copolymer can be achieved by increasing the grafted chain length.**[148, 150]** The resulting copolymer has been commonly used as a "macro"-monomer the synthesis of polyurethane foams as has been described in sub-section *4.3.5***[149]**. Other polymers than propylene oxide have been used for the production of lignin graft copolymers, and these include ε-caprolactone and lactide (**Fig. 7**). The reaction between the hydroxyl groups in lignin and ε-caprolactone leads to the production of lignin-polycaprolactone copolymers in which the ratio of ε-caprolactone/hydroxyl groups determines the length of the grafted polycaprolactone **[12, 131]**.The chain length strongly affects the thermal properties of the resulting copolymers. An amorphous structure can be achieved for ratios of ε-

caprolactone to hydroxyl groups that are below 5, and the grafted polycaprolactone chains crystallize at higher ratios **[131, 138, 151]**. In addition, lignin-poly(lactic acid) copolymers have been synthesized by reacting the hydroxyl groups in lignin and lactide via ring opening polymerization (**Fig. 7**) **[12]**. The thermal properties of the lignin-poly(lactic acid) copolymers depend on the weight fraction of lignin, and also on the chain length of the resulting copolymer. The lignin-poly(lactic acid) copolymers also display a glass transition temperature that ranges from 45 to 85 °C **[132]**.

4.4.1.2. Radical Polymerization

The radical polymerization of vinylic monomers onto lignin has also been used to prepare lignin-graft copolymers by a "grafting from" procedure. This process involves the creation of a radical on the lignin structure that initiates the polymerization of a vinylic monomer, usually by using irradiation or a chemical initiator (peroxide), after which the synthesis proceeds as shown in **Fig. 7** **[12, 152, 153]**. Several copolymers have been produced by this process, including lignin-polystyrene, lignin-poly(acrylic acid) and lignin-poly(vinyl acetate), in order to improve the properties of the resulting copolymers **[154-157]**. However, compared to the ring opening polymerization process, radical polymerization has some disadvantages: 1) there is less control of the grafting reaction; 2) the grafting efficiency can be reduced by the homopolymerization of the vinylic monomers; 3) the radicals are resonance-stabilized on different positions on the aromatic ring of the lignin, thus it is not possible to know exactly where the grafting takes place on the lignin structure; and 4) coupling reactions between the lignin radicals can also occur and this competes with the grafting **[12]**.

4.4.1.3. Atom Transfer Radical Polymerization

In order to overcome the abovementioned limitations, the atom transfer radical polymerization (ATRP) was developed as an efficient method to regulate the radical grafting of vinylic monomers onto the lignin structure. The ATRP method allows the formation of long polymer chains with well-defined structures and low dispersity, provided that the radical polymerization is carried out in a controlled manner **[10, 158, 159]**. Briefly, the method starts with the esterification of lignin by using 2-bromoisobutyryl bromide as the initiator. The monomers can be grafted onto the lignin macroinitiator in the presence of a catalyst such as CuBr with 1,1,4,7,10,10-hexamethyltriethylenetetramine as the ligand, which leads to the formation of lignin-based copolymers (**Fig. 7**) **[17, 158]**. By using this method, the N-isopropylacrylamide (NIPAM) was copolymerized with kraft lignin to produce thermoresponsive lignin-polyNIPAM in which the thermal decomposition temperature of the copolymers increased with increasing of the polymerization degree **[160]**. When heated at temperatures above 32 °C, the copolymer underwent the typical hydrophilic-to-hydrophobic transition that led to the precipitation of the copolymer **[160]**. Later, the same method was used to graft polyNIPAM in lignin nanofiber mats produced by electrospinning, with both thermal and ionic responsive characteristics **[161]**.

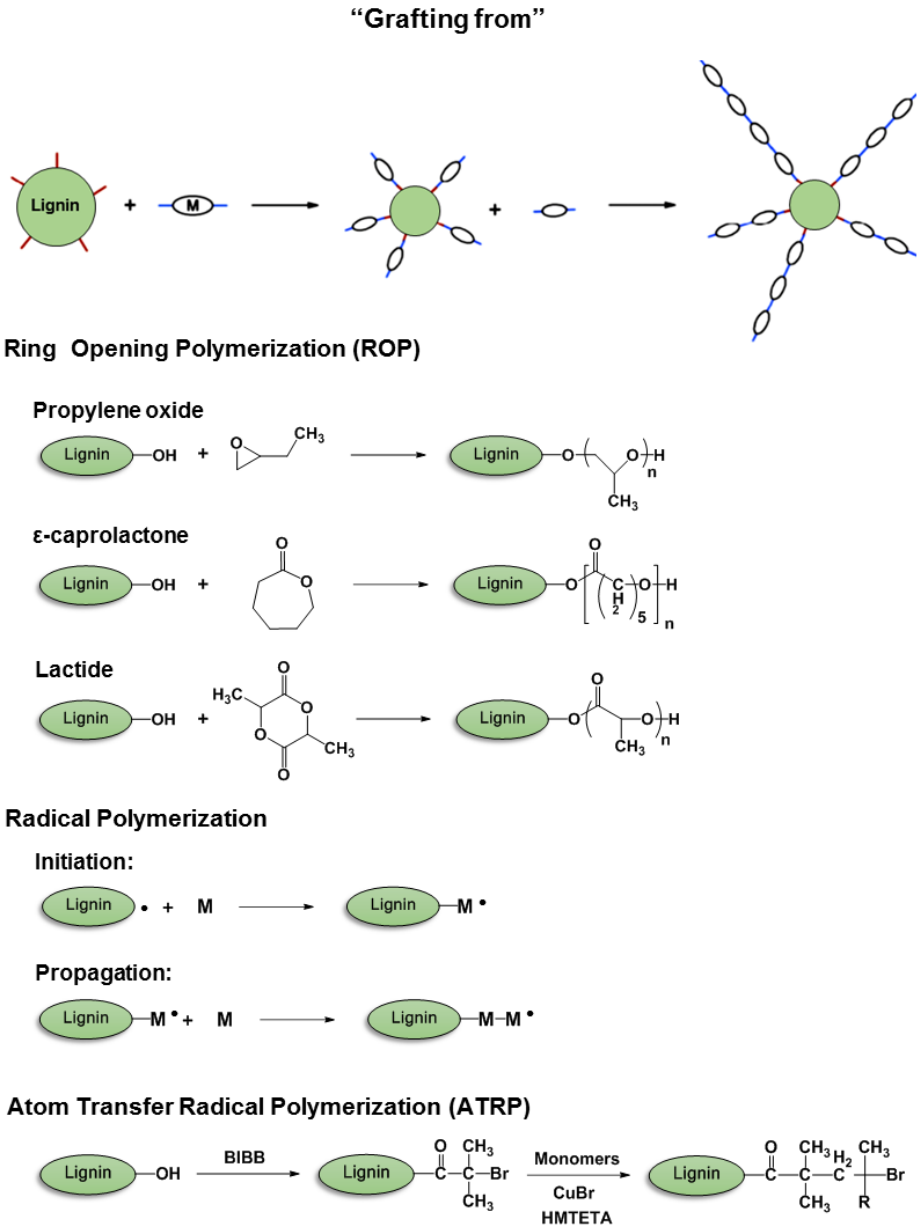

Fig. 7. Schematic representation of the lignin-graft copolymers synthesis by the "grafting from" method (ring opening polymerization and radical polymerization such as ATRP.

4.4.2. "Grafting to" Technique

The "grafting to" method involves the synthesis of the polymer chain that should be functionalized with an end group in order to allow the reaction with the lignin, and then the grafting of the polymer chains to the lignin core by the hydroxyl groups (**Fig. 8**) **[12]**. The properties of the resulting copolymers depend on the number and length of grafted chains. However, in this case, the

number of chains grafted per lignin molecule is the only parameter that can be assessed, since the length of the grafted chains can be tuned and well characterized before the grafting step **[12]**. For example, a boronic acid-terminated polycaprolactone that is covalently linked to the lignin forms a cyclic arylboronate ester bond with the hydroxyl groups in the lignin core (**Fig. 8**) **[162]**.

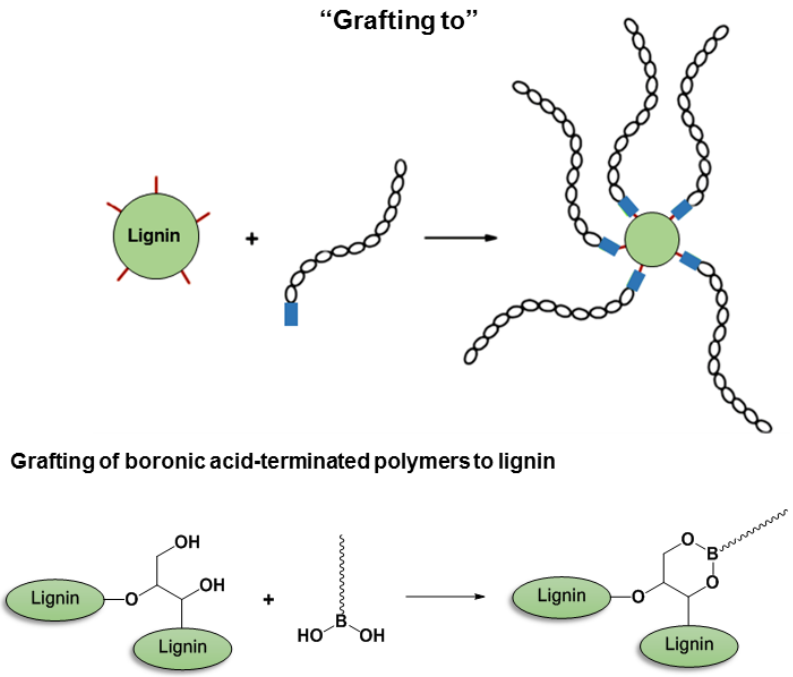

Fig. 8 Schematic representation of the lignin-graft copolymers synthesis by "grafting to" method.

5. Industrial Applications of Lignin

Lignin can be used for the starting point of different industrial applications such as high-value lignin derivatives both before and after any chemical modification. Generally, lignin-based materials can be mainly used as fuels, chemical reagents and polymers (**Fig. 9**). For example, lignin can be used to modify and control the release of fertilizers and herbicides in agriculture, as dispersant agents, as sizing agents in the paper industry and as binders **[14, 22, 31]**. The main industrial applications of lignin derivatives will be discussed in the next subsections.

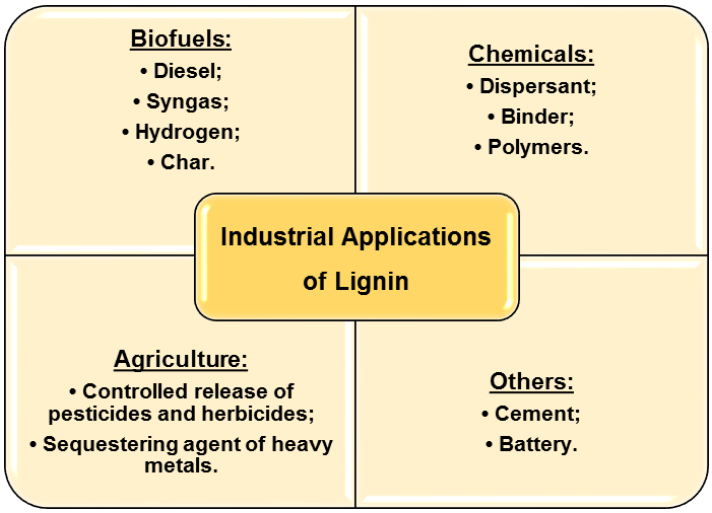

Fig. 9. Main industrial applications of lignin derivatives.

5.1. Biofuels

Lignin and its derivatives have been used as fuel sources in different forms, and these include char, syngas, hydrogen and aromatic hydrocarbons (**Fig. 10**) **[31]**. The main processes to obtain biofuels from biomass begin with pyrolysis, followed by catalytic upgrading of the resulting materials. This pyrolytic breakdown of the biomass produces a large number of chemical substances that can be used as substitutes for conventional fuels **[163]**. Pyrolysis can be fast pyrolysis to obtain oil and slow pyrolysis to obtain char **[164, 165]**. Furthermore, the gasification process can transform lignin into gases that can be used in fuel cells, gas turbines, and as synthesis gas **[105]**.

The char formed by slow pyrolysis can be combusted to produce heat, which is the principal use for the isolated lignin **[31]**. The conditions of pyrolysis will determine the chemical composition and the yield of the solid products, and these conditions include the reactor temperature, heating rate, porosity, initial particle size and initial temperature **[166, 167]**.

The manufacturing of high-value products, such as hydrogen and syngas, is also a promising use of biomass. Hydrogen has been used as an alternative to conventional fuels in order to reduce the dependence on fossil fuels and reduce fossil carbon based emissions, whereas syngas is an important building block for the petrochemical industry **[168]**. The syngas can be combusted to

generate heat and electricity. Pure hydrogen can also be obtained from the upgrading of syngas via the water-gas shift (WGS) reaction followed by gas separation using pressure swing adsorption. The resulting hydrogen can be used as a clean energy carrier for fuel upgrading in petroleum and biorefineries **[31]**.

Lignin has also been used as a source of aromatic monomers, such as guaiacol, phenol, benzene and toluene, and their production depends on the reaction temperature and presence of catalysts **[169]**.This phenolic oil separated from the bio-oil obtained by pyrolysis of lignin is also called pyrolytic lignin **[164]**. Certain properties of the bio-oil, such as its low heating value, incomplete volatility, acidity, instability, and incompatibility with standard petroleum fuels, restrict its application. However, catalytic upgrading of the pyrolytic lignin can be performed in order to increase the stability of the product **[164, 170]**. The pyrolytic products can be blended into transportation fuels, but they can also be utilized in the polymer/chemical and electronic industries **[164]**.

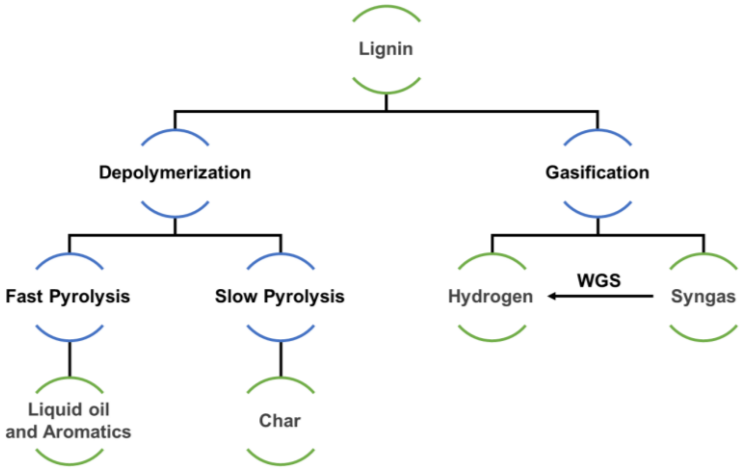

Fig. 10. Overview of the processes to convert lignin into different biofuels.

5.2. Chemicals/Polymers

Lignin has been extensively studied in the field of the material science because of its abundance, low cost, high UV-absorbance, biocompatibility, biodegradability and antioxidant properties **[4, 57]**. In addition, lignin can be used as a phenol substitute in the production of phenol formaldehyde

resins that are to be used as binders because of the presence of phenol groups in its structure **[6]**. As discussed above, different chemical modifications can be applied in order to improve the reactivity of the lignin. However, there is some variability in the properties and thereby in the uses of lignin phenol formaldehyde resins that depend on the source and the extraction method of the original lignin **[6]**. For example, some lignin phenol formaldehyde resins have been effectively used as binders, others as water-barrier coatings for cardboard substrates and yet others can be incorporated into wood adhesives **[171-173]**. Furthermore, the use of phenolic lignin resins as foams reduce thermal conductivity and, consequently, they can act as a thermal isolating foam **[6, 174]**. Conventional phenol formaldehyde resins are toxic, whereas the lignin phenol substitution is environmentally friendly and less expensive than the more conventionally used phenol **[175]**.

The type of lignin used for epoxy resins and its extraction process will influence the properties of the resulting composite **[176]**. For example, epoxy-lignin blends that are derived from the commercial hardwood lignin have enhanced adhesion to epoxy resins when compared to a softwood lignin, which correlates with the density of hydrogen-bonding groups in the material. A commercial soda lignin had the highest adhesive joint shear strength **[6, 177]**. In addition, some reports have suggested that epoxy resins can be used to improve the anti-aging properties of asphalt **[178, 179]**. Additionally, the lignin-based epoxy resins have a good stability when used in electric circuit boards **[176, 180]**.

Polyurethanes are block copolymers constituted by polyesters or polyethers that are covalently bonded by a urethane group. Polyurethanes have unique properties, such as low density, low thermal conductivity and moisture permeability, and high strength and dimensional stability, thus it is used in the production of foams that can be classified as flexible, semi-rigid or rigid foams **[181]**. However, the incorporation of lignin in the synthesis of lignin-based polyurethane has been studied in order to improve the mechanical properties, thermal stability, curing rates, and also the flame

resistance of the lignin-based polyurethane foams **[181, 182]**. In some cases, the resulting lignin-based foams were found to be comparable or even better than the conventional polyurethanes **[181]**.

Carbon fibers are materials that have properties, such as being lightweight, having high strength, great flexibility, dimensional stability, heat resistance and good electric conductivity **[10, 53, 183]**. Having these properties enable the use of carbon fibers in several industries, including spacecraft, aircraft, marine products, sport equipment and automobiles **[10, 183, 184]**. Currently, the main precursor used for the carbon fibers production is polyacrylonitrile, but high production costs has limited its high-end industrial applications **[53]**. Therefore, lignin can be used as a starting material for the production of carbon fibers because it is a low cost precursor, and also because of its high carbon content **[22, 53]**. The production of carbon fibers from hardwood kraft lignin via thermal fiber spinning was first reported by Kadla *et al* **[185]**. This method was used in order to remove impurities from lignin and decrease the hydroxyl content, which enable the lignin-based fibers to keep a characteristic fiber form during the subsequent carbonization. The addition of 3–5% of poly(ethylene oxide) facilitated the fiber spinning. Another study reported the production of carbon fibers from softwood kraft lignin for which the stabilization and carbonization was executed as a one-step process, which indicates great potential to reduce the costs and production time **[186]**. Therefore, the production of lignin-based carbon fibers can provide mechanical properties that are comparable to the conventional carbon fibers that uses polyacrylonitrile as a starting material, and which also show an increased energy efficiency and reduced environmental burden **[10]**.

5.3. Other Applications

Lignosulphonates are strong sequestering agents that trap different metal ions and form complexes with iron, calcium, copper, nickel, aluminum and zinc. Thus, lignosulfonates can be used in the treatment of poor soils by either adding deficient minerals or removing harmful metals

[14]. In addition, the controlled release of herbicide, pesticides and fertilizers can be achieved by using lignin-based biopolymer matrices **[187, 188]**.

Lignin and its derivatives have been utilized for other types of applications in addition the abovementioned applications. For example, amphiphilic lignosulfonates conjugated with poly(ethylene glycol) diglycidyl ether had promising cement dispersant properties without losing any mechanical strength even in the wintertime **[189]**. Moreover, the modification of alkali lignin *via* sulfonation enhanced the dispersion effect to the cement matrix **[129]**.

Lignin can enhance the performance of energy storage devices such as sodium ion batteries, which have been extensively explored as electrical energy storage devices for large-scale and sustainable energy storage applications. In this case, lignin-based materials have been produced in order to be used as precursors in the fabrication of low-cost high-performance carbon electrode materials for sodium ion batteries **[190]**. Another study showed that the mixture of silicon nanoparticles and lignin could be used to replace a conventional polymer binder and carbon black additive. A copper substrate was coated with the mixture and then heat-treated to form a composite electrode with good electrochemical performance. This method produced a high-performance and durable silicon nanoparticle-based negative electrode **[191]**.

6. Preparation of Lignin-Based Nanomaterials and their Applications

Different chemical reactions that can be used to modify lignin in order to produce high-value materials, which were discussed in *section 4*. Lignin is still an underexploited natural source despite its availability and its good structural potential, although the increasing potential of using lignin in the development of nanomaterials is only just emerging. However, its application is limited by the complex macromolecular structure, which is dependent on its source and extraction method. One way to overcome this limitation is to transform raw lignin into nanoparticles of uniform size and

shape **[16]**. Recently, lignin has been successfully used to produce different nanomaterials, such as nanoparticles, nanotubes, nanofibers and hydrogels for different kind of applications **[3, 11, 192]**.

The development of lignin nanoparticles (LNPs) presents key advantages, such as improving the properties of polymer blends and higher anti-oxidant activity due to higher surface-area-to-volume ratios **[15, 193, 194]**. Furthermore, LNPs possess functional groups that can be chemically modified, which significantly increase their application potential. Different approaches such as anti-solvent precipitation, interfacial crosslinking, polymerization, solvent exchange and sonication have been used to produce LNPs all of which have characteristically different shapes (**Fig. 11**) **[11]**. **Table 3** summarizes the preparation methods of LNPs and their main applications. Lignin-based nanoparticles can have potential uses in functional surface coatings (*e.g.*, nanocomposites), and also in biomedical applications, such as drug/gene delivery and tissue engineering.

The development of hydrogels has changed from the adaptation of synthetic polymers to the modification of natural polymers due to the biocompatibility, biodegradability, low toxicity, susceptibility to enzymatic degradation and eco-friendly properties inherent to natural polymers. Lignin, among other biopolymers, is a promising candidate for the development of hydrogels because of its properties, which have already been discussed above **[3]**. Lignin has been used in different types of hydrogels to facilitate their formation and improve their properties. Hydrogels have been used for producing contact lenses, wound dressings, and for drug delivery and tissue engineering **[217]**.

In this section, we will describe the main applications and some methodologies used in the preparation of lignin-based nanomaterials.

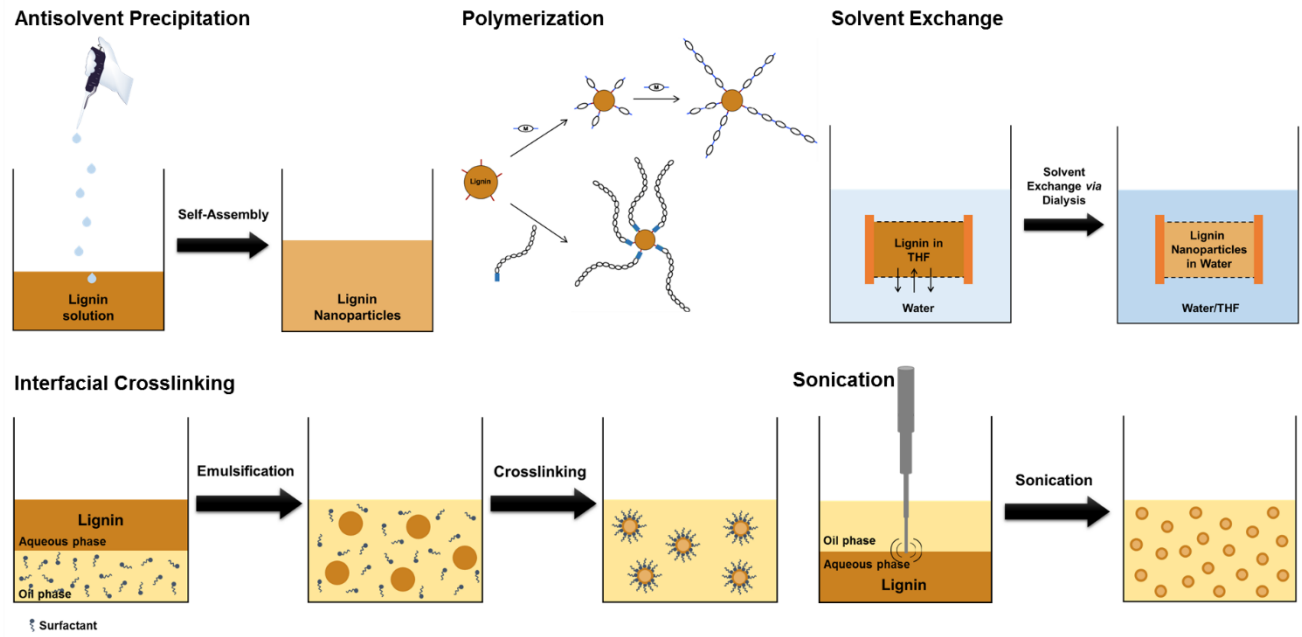

Fig. 11. Schematic representation of the different methodologies to produce LNPs.

Table 3 – Summary of the preparation methods and application of the lignin-based nanoparticles.

Preparation method	Morphology	Description of the preparation method	Potential Applications	References
Anti-solvent Precipitation	Spheres	Organosolv lignin was dissolved in acetone and supercritical CO_2 was used as an anti-solvent.	Possible applications in food processing and pharmaceutical industries.	**[193]**
Anti-solvent Precipitation	Spheres	Lignin was dissolved in acetone/water (9:1 v/v) and added to Milli-Q water.	Possible applications in food, pharmaceutical and cosmetic industries.	**[194]**
Anti-solvent Precipitation	Cluster-forming aggregate	Lignin was dissolved in ethylene glycol and precipitated by HCl.	Hybrid nanocomposites.	**[195]**
Anti-solvent Precipitation	Non-spherical	Lignin was dissolved in ethylene glycol and precipitated by HCl.	Antibacterial effects; drug delivery vehicles and as sorbents for heavy metal ions.	**[196, 197]**
Anti-solvent Precipitation	Quasi-spherical nanoparticles	Lignin was dissolved in DMF, and the solution was sprayed into the precipitator using compressed liquid CO_2 as an anti-solvent.	Potential applications in cosmetics, drug delivery systems, and nanocomposites materials.	**[198]**
Anti-solvent Precipitation	Micelles	Alkali lignin was dissolved in dioxane and cyclohexane was added into the solution.	Lignin/high-density polyethylene blends.	**[199]**
Anti-solvent Precipitation	Colloidal spheres	Acetylated lignin was dissolved in THF and water was gradually added to the solution.	Potential application as drug delivery systems or encapsulation of pesticides for controlling release.	**[200]**
Anti-solvent Precipitation	Nanocapsules	A solution of lignin in ethanol was prepared and the ultra-pure water was dropped into this solution until the water content of the solution reached 90%.	Suitable for different applications.	**[201]**
Interfacial Crosslinking	Hollow nanocapsules	Kraft lignin was dissolved in Milli-Q water (with sodium chloride), mixed with cyclohexane (with a biocompatible surfactant polyglycerol polyricinoleate), and then a solution of toluene diisocyanate in cyclohexane was added.	Encapsulation of hydrophilic compounds for agricultural applications.	**[202]**
Interfacial Crosslinking	Spherical Micro/nanocapsules	The emulsification of an oil in water phase containing lignin using high-intensity ultrasonic technology, creating the crosslinking of lignin at the water/oil interface.	Encapsulation of hydrophobic compounds for biomedical applications.	**[203]**
Interfacial Crosslinking	Micro/nanoparticles	A microemulsion was prepared after adding the lignin solution to the octane containing a mixture of surfactants (Span 80, Tween 80 and 1-pentanol), and subsequent crosslinking of the internal lignin-rich phase by adding epichlorohydrin.	Surfactant for Pickering emulsions; Loading of silver nanoparticles.	**[204]**
Interfacial Crosslinking	Nanocapsules	First, lignin was grafted with allyl groups *via* etherification and dispersed in an oil-in-water miniemulsion by ultrasonication, and then reacted with a thiol-based crosslinking agent at the interface of miniemulsion droplets to form nanocapsules.	Drug delivery and controlled release of hydrophobic compounds (*e.g.*, drugs, essential oils, and antioxidants).	**[205]**
Polymerization	Nanotubes	Lignin reacted nanopores surface of the alumina membranes;	Vehicles for gene delivery into human cells.	**[206]**

		polymerization of the monolignols after deposition of multiple layers of dehydrogenation polymer onto the base layer, and dissolving the templates in phosphoric acid to obtain the lignin nanotubes.		
Polymerization	Polyplexes	PDMAEMA-lignin copolymers synthesized by ATRP.	Gene delivery system.	**[207]**
Polymerization	Not specified	PDEAEMA-grafted lignin nanoparticles prepared *via* ATPR.	Surfactant for Pickering emulsions.	**[208]**
Polymerization	Nanofibers	Grafting of PLA onto the alkylated lignin via ring opening polymerization to form PLA−lignin copolymers. Blending of copolymers with poly(L-lactide) to form nanofibrous composites by electrospinning.	Antioxidant activity; Promising as biomedical materials in tissue engineering scaffolds.	**[209]**
Polymerization	Nanofibers	Preparation of lignin-based copolymers (lignin-poly(ε-caprolactone-co-lactide) via ring-opening polymerization. Blending of lignin-based copolymers with polycaprolactone and poly(ι-lactic acid) via electrospinning.	Antioxidant activity; Promising as biomedical materials in tissue engineering scaffolds.	**[210]**
Polymerization	Nanofibers	Grafting of poly(methyl methacrylate) with lignin by atom transfer radical polymerization. Blending of this copolymer with poly(ε-caprolactone) using electrospinning.	Promote the proliferation, attachment and interactions of human dermal fibroblasts. Promising for tissue engineering.	**[211]**
Sonication	Irregular shape	Sonication of an aqueous suspension of alkali lignin for 60 min.	–	**[212]**
Sonication	Not specified	Lignosulfonate and chitosan solutions were combined in the presence of an organic phase (vegetal oil) and the ultrasonic probe was placed at the aqueous–organic interface.	Antibacterial effect; drug delivery applications.	**[213]**
Solvent Exchange	Spherical nanoparticles	Kraft lignin was dissolved in THF and water was subsequently introduced into the system *via* dialysis.	Potential application in composites, Pickering emulsions and antimicrobial materials. Drug delivery carrier for cancer therapy.	**[214, 215]**
Solvent Exchange	Different nanoparticles morphologies	Condensation reaction of iron isopropoxide with lignin in THF solution and further hydrolysis and dialysis in water.	Potential application to produce materials with enhanced magnetic, antibacterial and catalytic properties.	**[216]**
Solvent Exchange	Spherical nanoparticles	A mixture containing 50:50 w/w of lignin solution and oleic acid-coated Fe_3O_4 NPs in THF was prepared and dialyzed against Milli-Q water.	Promising for cancer therapy and diagnosis, such as magnetic targeting and magnetic resonance imaging.	**[215]**
Aerosol assisted self-assembly	Hollow/solid lignin nanoparticles	Dioxane soluble fraction of alkali lignin at different concentrations was dissolved in DMSO and sprayed upon a liquid nitrogen cooled copper plate using an ultrasonic nebulizer	Sunscreen cosmetics (UV-absorbing ability) and drug delivery applications.	**[217]**
Aerosol Flow Reactor	Spherical nanoparticles	After dissolving alkali, kraft and organosolv lignin with an appropriated solvent, the droplets were generated during the flow-through a collision-type jet atomizer with nitrogen gas, and collected as solid particles.	Surfactant for Pickering emulsions.	**[218]**

6.1. Lignin-Based Nanomaterials as Antioxidant

The complex chemical structure that contains aromatic rings of the methoxy and the hydroxyl groups enables lignin to be incorporated into different materials to produce antioxidant products that can be used in several applications. These functional groups lead to the termination of oxidative propagation reaction *via* hydrogen donation **[17]**. Lu *et al*. prepared LNPs with an average size of ca. 144 nm by using a supercritical anti-solvent precipitation method **[193]**. That study used the nanoscale lignin at 12.4 times higher concentration than the bulk lignin and, consequently, the resulting product had higher antioxidant and free radical scavenging activities and enhanced reducing power. Thus, the resulting nanoscale lignin product could be used in food processing and pharmaceutical industries. Yearla and Padmasree fabricated dioxane LNPs that had an average size of approximately 104 nm by an anti-solvent precipitation method whereby a solution of lignin was dissolved in acetone and water (9:1 v/v) and then the solution was added to water **[194]**. The LNPs manifested greater antioxidant and UV-protection properties than the bulk lignin after monitoring the survival rates of *Escherichia coli* upon UV-irradiation-induced mortality. The resulting LNPs could be further exploited in food, pharmaceutical and cosmetic industries because of these beneficial properties.

6.2. Lignin-based Nanomaterials for Nanocomposites

LNPs have also been exploited as reinforcing agents in polymer matrix and nanocomposites, for which the resulting copolymers have better biocompatibility, mechanical and thermal properties than the original polymers. For example, Gupta *et al*. synthesized LNPs that had an average size of 181 nm by using the anti-solvent precipitation method. Those authors dissolved the lignin in ethylene glycol and then precipitated the LNPs by adding HCl **[195]**. These LNPs were used in the preparation of bio-poly(trimethylene terephthalate) hybrid nanocomposites, that contained 1.5% by weight of LNPs and 7.0% by weight of vapor-grown carbon fibers. Their study reported an

improvement in the mechanical properties of the nanocomposites such as tensile strength, tensile modulus and impact strength in the thermal properties and biodegradation characteristics. The resulting nanocomposites were found to be suitable for several end-use applications. Qian *et al*. used a self-assembly method to prepare lignin reverse micelles after adding cyclohexane into a solution of alkali lignin dissolved in dioxane **[199]**. When further quantities of cyclohexane were added, the lignin micelles tended to flocculate and precipitate. The rendered nanoparticles ranged in size from 120 nm to micrometers, depending on the cyclohexane content, and these had better UV-absorbance properties than the alkali lignin (**Fig. 12**). In addition, the nanoparticles had good miscibility with hydrophobic plastics, such as high-density polyethylene (HDPE), and had improved UV-absorbing and mechanical properties of the resulting lignin/HDPE blends.

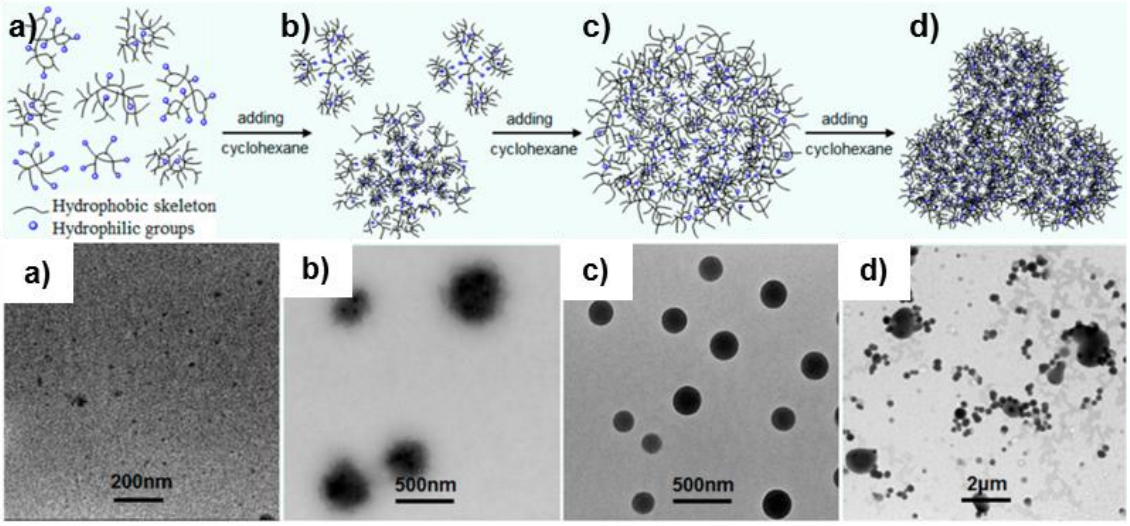

Fig. 12. Schematic representation of the manufacturing process of lignin reverse micelles in dioxane-cyclohexane medium and transmission electron microscopy (TEM) images of the samples from the dispersions with different cyclohexane content: (a) 0−4% vol; (b) 4−7% vol; (c) 7−10% vol, and (d) >10% vol. Adapted from ref. 199 with permission from American Chemical Society, copyright 2015 **[199]**.

Qian *et al*. synthesized *N*,*N*-diethylaminoethyl methacrylate (DEAEMA)-grafted LNPs *via* ATRP, which had sizes that ranged from 237–404 nm **[208]**. These lignin-DEAEMA nanoparticles

were used as surfactant for CO_2/N_2-switchability Pickering emulsions that was correlated with the graft density and chain length of the DEAEMA. A study, by Ago *et al*., described the preparation of spherical LNPs by using an aerosol flow reactor, a high-yield and high-throughput manufacturing approach that rendered particles with sizes that ranged from ca. 30 nm to 2 µm, depending on the collector number (fraction), lignin source, and concentration of the precursor solution **[218]**. The obtained particles were used to stabilize oil-in-water Pickering emulsions with variable droplet size, which depend on the size of the particles, the particle concentration and the origin of the lignin. Nypelö *et al*. fabricated spherical particles of sizes that varied from 90 nm to 1 µm *via* microemulsion and further crosslinking **[204]**. This process required that the water-in-oil microemulsion was prepared by adding a lignin solution to a mixture of surfactants, such as Span 80, Tween 80 and pentanol dissolved in octane, which resulted in a hydrophilic–lipophilic balance of ca. 7. This formulation allowed a spontaneous emulsification, and the solid particles were produced after the crosslinking of the internal lignin-rich phase by the addition of epichlorohydrin and the subsequent separation by removing the continuous oil phase. These particles can be used in the stabilization of oil-in-water Pickering emulsions, in which the larger particle size provided an increased emulsion drop size and stability.

Salas *et al.* produced nanofibers with diameter ranging from 124 to 400 nm by electrospinning aqueous alkaline solutions containing different mass ratios of soy protein and lignin **[219]**. Poly(ethylene glycol) was added as coadjutant in order to facilitate the formation of better-quality nanofibers, and the unfolding of the soy protein improved the interaction with the lignin macromolecules, contributing to the successful electrospinning process. Thus, the mixture of two major renewable resources with exciting chemical features was found to be appropriate for the development of composites.

6.3. Lignin-based Nanomaterials as Delivery Systems

Lignin has the potential to produce nanoparticles for encapsulation of different compounds for different pharmaceutical applications due to its low cost and eco-friendly properties. For example, Frangville *et al*. used an anti-solvent precipitation method, in which the lignin was dissolved in ethylene glycol and precipitated with HCl to form LNPs with sizes that varied from 100 nm to a micrometer scale, depending on the initial concentration of lignin and the concentration of HCl added **[196]**. The LNPs did not have any apparent cytotoxicity for yeast and microalgae, thus, they were considered promising vehicles for drug delivery, stabilizers of cosmetic and pharmaceutical formulations. In addition, these LNPs can also be used as sorbents for heavy metal ions and other environmental pollutants. Later, Richter *et al*. also prepared LNPs by using the same precipitation method referred above, and the LNPs were subsequently infused with silver ions and coated with poly(diallyldimethylammonium chloride) (PDAC) (**Fig. 13**) **[197]**. The coating promoted the adhesion of the biodegradable and environmentally green LNPs infused with silver ions to the bacteria membranes, a process that could efficiently kill a wide-range of bacteria. Thus, by synthetizing such nanoparticles with biodegradable cores and high antimicrobial activity, it is possible to diminish the environmental impact associated with metallic silver nanoparticles.

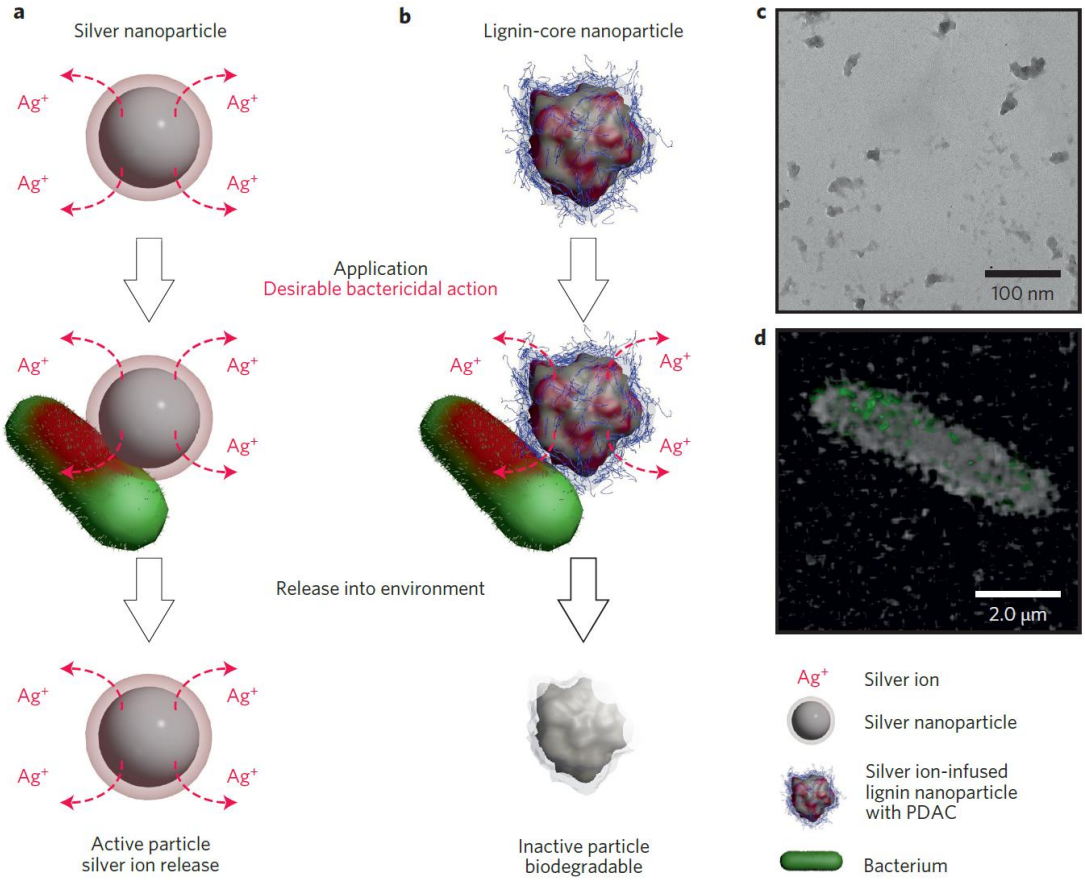

Fig. 133. A schematic representation of (a) general principle of the bactericidal effect of common silver nanoparticles via the release of Ag^+ ions and (b) Ag^+-infused environmentally benign lignin-core nanoparticles with PDAC that promotes electrostatic attraction between the nanoparticles and the negatively charged cell wall. (c) The TEM images of the prepared LNPs. (d) Confocal fluorescence microscopy image of the LNPs with PDAC coating adhering to the cell membrane of *Escherichia coli*. Reproduced from ref. 197 with permission from Nature Publishing Group, copyright 2015 **[197]**.

Qian *et al*. prepared colloidal lignin spheres using the self-assembly method by dissolving acetylated lignin in tetrahydrofuran (THF) and adding water gradually to the solution. This caused the lignin molecules to start associating with each other due to hydrophobic interactions: a process that was completed by adding a water to the extent of 67% by volume of the solution **[200]**. The nanoparticles that formed in water after the evaporation of THF had an average size of around 106

nm and they could be potentially used as drug delivery systems or microencapsulation of pesticides for their controlled release in an environment. In another study, Yiamsawas *et al*. produced hollow nanocapsules *via* interfacial crosslinking, in which lignin was dissolved together with sodium chloride in water and mixed with cyclohexane containing the biocompatible surfactant polyglycerol polyricinoleate **[202]**. Then, after a miniemulsion had been produced by ultrasonication, a polyaddition reaction was started by adding a solution of toluene diisocyanate in cyclohexane solution to the miniemulsion. The obtained nanoparticles had diameters that ranged from 311 to 390 nm in water. In addition, this reaction allowed the encapsulation of hydrophilic compounds such as drugs, fertilizers, and pesticides that can be released by enzymatic degradation of the lignin, which makes these nanocontainers attractive for agricultural applications. In order to form an oil phase in water emulsion with lignin, Tortora *et al*. used a high-intensity ultrasonic technology to synthesize oil-filled microcapsules of kraft lignin of sizes that ranged from 300 nm to 1.1 μm **[203]**. This induced the crosslinking of lignin at the water/oil interface, whose stability was improved by adding a cross-linking agent. This technique also allowed the loading of hydrophobic compounds dissolved in the oil phase and the products had low cytotoxicity and they could be efficiently internalized into Chinese hamster ovary cells.

An ultrasonic spray-freezing method was used by Mishra and Wimmer who prepared hollow/solid lignin colloidal nanoparticles, without chemical modification **[217]**. For this to occur, different concentrations of dioxane soluble fraction of alkali lignin were dissolved in DMSO and sprayed onto a liquid-nitrogen-cooled copper plate using an ultrasonic nebulizer, producing LNPs with sizes in the 96–193 nm range. The morphology of the resulting nanoparticles was dependent on two different processes that can occur in parallel, namely: 1) the time taken by the solvent to freeze, and 2) the time taken by the lignin to diffuse. When the freezing of solvent is enough for the solute to diffuse, a solid particle should be expected, otherwise a hollow particle should be produced (**Fig. 14**). Furthermore, other parameters such as droplet size, nature of polymers and

concentration of solute (higher concentration favors solid particle formation) that can affect the morphology of the nanoparticles. In addition to that, six layers of lignin colloids were deposited on a quartz slide assisted by a negligible UV-absorbing aqueous solution of PDAC, which improved the UV absorbing ability of the lignin product by increasing the number of layers. Thus, the obtained nanoparticles could be used in sunscreen cosmetics due to their UV-absorbing ability, or they could also be used for drug delivery applications.

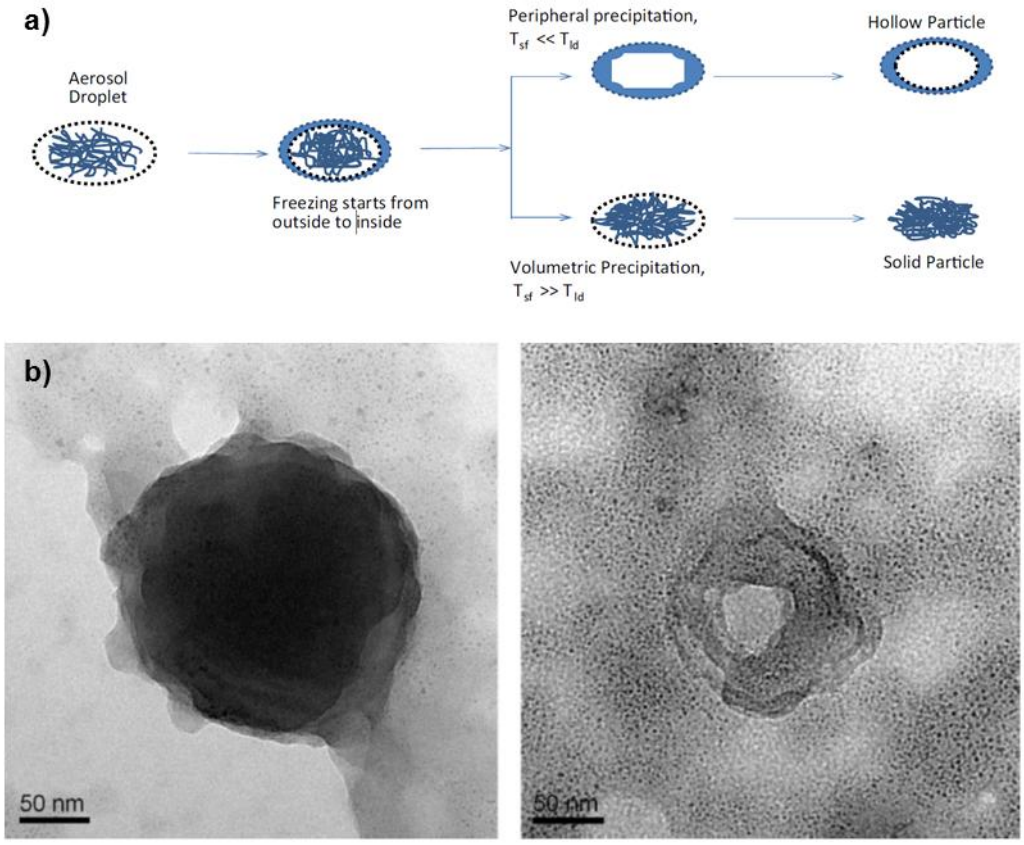

Fig. 14. (a) Schematic representation of the LNPs morphology according to the mechanism followed for their synthesis. T_{sf} – characteristic time for solvent freezing, T_{ld} – typical time for diffusion of lignin. (b) TEM pictures of the solid and hollow LNPs. Adapted from ref. 217 with permission from Elsevier, copyright 2017 **[217]**.

LNPs can also be used for gene delivery applications. Ten *et al*. fabricated lignin nanotubes using nanopore alumina membranes as a template **[206]**. In this method, lignin was first reacted

with the amine groups on the nanoporous surface of the alumina membranes. Then, after the deposition of multiple layers of dehydrogenation polymer onto the base layer, the polymerization of the monolignols occurred with horseradish peroxide as a catalyst. Finally, the templates were dissolved with phosphoric acid in order to obtain lignin nanotubes. The physicochemical properties of the resulting nanotubes depend on the composition, origin and extraction method of the lignin, which affected the size and reactivity of lignin. The lignin nanotubes showed good biocompatibility and a high DNA binding capacity, both of which are good attributes for making these nanotubes a good vehicle for gene delivery into human cells (**Fig. 15**).

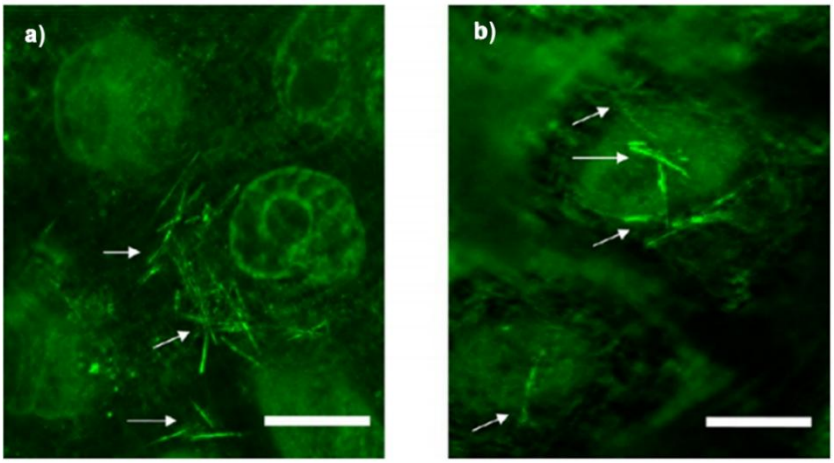

Fig. 15. Confocal microscopy images of lignin nanotubes made of (a) lignin nanotube that had been isolated from PINE with thioglycolic acid and (b) lignin that had been isolated from PINE with NaOH, located inside HeLa cells. The scale bar represents 15 μm. White arrows point to lignin nanotubes. Reproduced from ref. 206 with permission from American Chemical Society, copyright 2014 **[206]**.

Liu *et al*. synthesized poly(2-dimethylaminoethyl methacrylate) (PDMAEMA)-lignin copolymers by ATRP **[207]**. After the synthesis, the copolymers were used to form polyplexes with plasmid DNA by electrostatic interactions between the negatively charged plasmid DNA and the positively charged PDMAEMA-lignin copolymers whose sizes ranged from 100 to 200 nm. The cytotoxicity and gene transfection efficiency of these copolymers were dependent on the chain

length of the grafted PDMAEMA. The copolymers that had very short arm length (i.e. with a mean of 5.5 DMAEMA units) exhibited an excellent *in vitro* transfection efficiency, and is therefore a promising gene delivery system.

An ultrasonication method was used by Kim *et al*. who fabricated chitosan-lignosulfonate nanoparticles after combining chitosan and lignosulfonate solutions in the presence of an organic phase (vegetal oil) and placing the ultrasonic probe at the aqueous–organic interface **[213]**. In this formulation, lignosulfonates act as a counter polyion for the stabilization of chitosan nanoparticles. Different parameters were tested during this process, including the pH of chitosan solution, the sonication time and the presence of surfactant (poloxamer 407) in order to obtain monodisperse and small particles that had a mean size of 230 nm. Furthermore, the obtained nanoparticles were found to be biocompatible with human cells and also had an efficient RNase A loading and enhanced antimicrobial effect compared to the chitosan nanoparticles alone, which makes them promising candidates for cosmetic and drug delivery applications. Gilca *et al*. also reported the preparation of lignin nanoparticles after the sonication of an aqueous suspension of alkali lignin for 60 min, which yielded nanoparticles whose sizes ranged from 10 to 50 nm **[212]**. This method is more attractive for future applications because hazardous solvents were not used in the process.

Uniform and quasi-spherical LNPs were developed by Myint *et al*. *via* a compressed fluid anti-solvent method **[198]**. This method entailed dissolving the kraft lignin in DMF, the solution was then sprayed into the precipitator using compressed liquid CO_2 ($clCO_2$) as an anti-solvent, which produced uniform and quasi-spherical LNPs. The precipitated nanoparticles had a mean particle diameter of around 38 nm, which had higher UV-absorbing, dispersion stability and improved solubility in water when compared with the raw lignin. Furthermore, they could be a good candidate for applications in cosmetics, health and drug delivery systems, and for nanocomposite materials due to the biodegradability and biocompatibility of the LNPs.

Lignin nanocapsules were produced by Li *et al*. *via* self-assembly, which was achieved by taking a solution of kraft lignin that had been dissolved in ethanol and then adding water until the water content of the solution reached 90% **[201]**. The size of the obtained nanocapsules were tunable in the range of tens to hundreds of nanometers, and this together with their biocompatibility, biodegradability, environmentally friendly properties, ease and low cost of preparation make these nanocapsules suitable for multiple applications. In another study, Chen *et al*. synthesized lignin nanocapsules with pH-responsive properties by interfacial miniemulsion polymerization and further crosslinking **[205]**. First, an oil-in-water miniemulsion was obtained *via* ultrasonication, after mixing the oil phase, containing butyl acetate, hexadecane (co-stabilizer), azobisisobutyronitrile (AIBN, oil soluble initiator) and the cross-linker trimethylolpropane tris(3-mercaptopropionate) with the water phase containing lignosulfonate (with and without sodium dodecyl sulfate (SDS)), under vigorous stirring. Then, a cross-linking agent was reacted with lignin at the interface of the miniemulsion droplets to form nanocapsules. Nanocapsules were prepared using the same methodology in the encapsulation process with coumarin-6 dissolved in butyl acetate. The obtained nanocapsules had a mean size range of 50 to 300 nm, and the release of the hydrophobic coumarin-6 was controlled by the variation in pH due to the presence of acid-labile β-thiopropionate cross-linkages in the capsule shell (**Fig. 16**). These lignin nanocapsules are promising vehicles for delivery and controlled release of hydrophobic molecules (*e.g.*, drugs, essential oils and antioxidants).

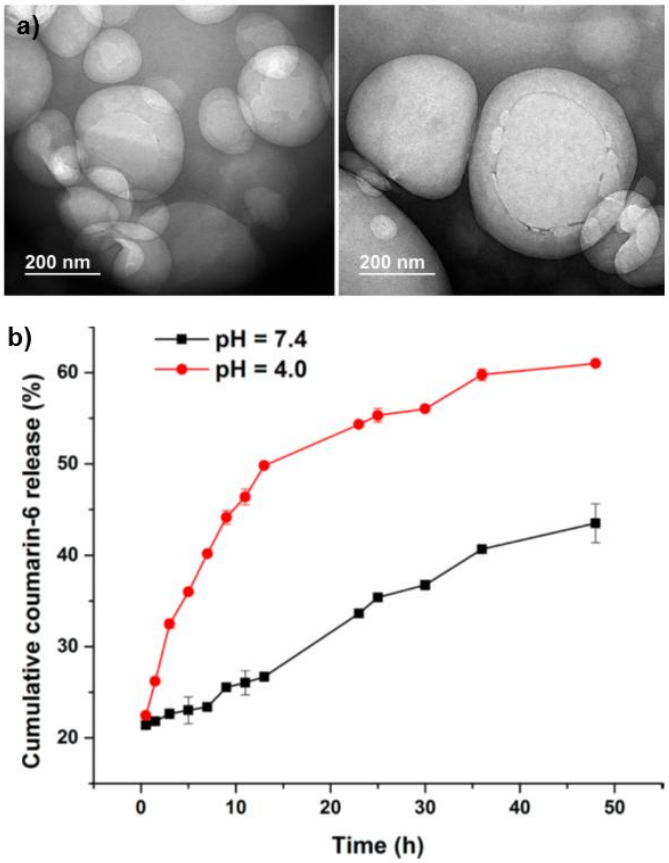

Fig. 16 (a) TEM pictures of lignin nanocapsules in miniemulsion. (b) Coumarin-6 release profile of lignin capsules in a 5% SDS solution at pH 7.4 and at pH 4.0. Reproduced from ref. 205 with permission from American Chemical Society, copyright 2016 **[205]**.

A solvent exchange method was used by Lievonen *et al*. to prepare spherical LNPs, in which kraft lignin was first dissolved in THF and then water was introduced into the system *via* dialysis, without chemical modification of lignin **[214]**. The diameter of the obtained nanoparticles ranged from 200 to 500 nm, depending on the pre-dialysis lignin concentration. In addition, the surface of the LNPs was modified by adsorption of an oppositely charged PDAC by gradually adding a nanoparticle suspension into a solution of PDAC. The obtained LNPs could be used in nanocomposites, Pickering emulsions, antimicrobial materials and drug delivery systems. Using the same solvent exchange process, Lintinen *et al*. also prepared different iron-complexed LNPs by changing the condensation and hydrolysis reaction parameters to yield particles whose sizes ranged

between 50 to 400 nm, with a tunable morphology (**Fig. 17**) **[216]**. This method entailed that lignin was first dissolved in THF and then mixed with an iron isopropoxide [$Fe(OiPr)_3$] solution, which led to a condensation reaction. Subsequently, the hydrolysis reaction was completed by adding water to the previous solutions, and finally, the obtained iron(III)-complexed lignin nanoparticles were solvent exchanged in water. Additionally, nanoparticles synthesized with $Fe(OiPr)_2$ or a mixture of $Fe(OiPr)_2$/$Fe(OiPr)_3$ showed paramagnetic or superparamagnetic behaviors, depending on the size and morphology of the nanoparticles. Thus, depending the obtained nanoparticles, these LNPs could be used to produce biocompatible materials with magnetic, antibacterial and catalytic properties.

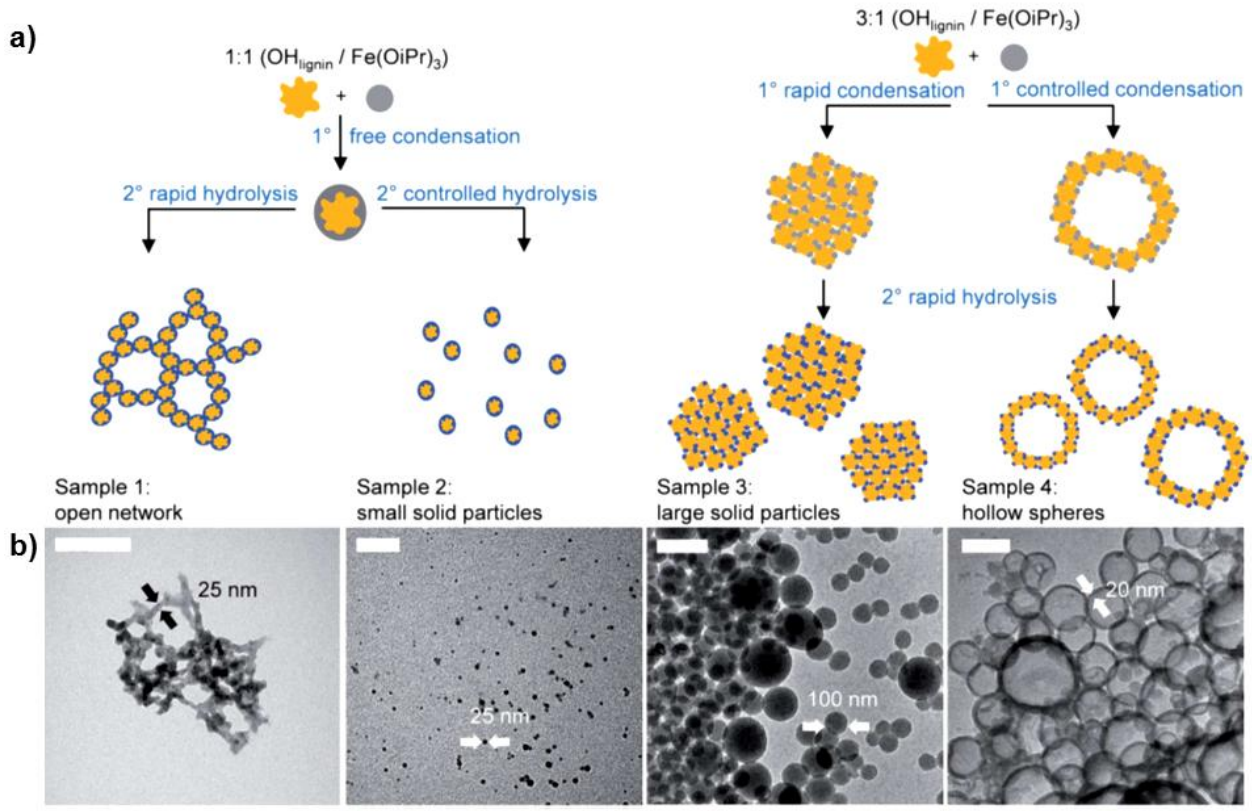

Fig. 177. Morphology of the different nanoparticles obtained by varying the reaction parameters. (a) Schematic representation of the parameters used to obtain nanoparticles with different morphologies. (b) TEM pictures of the obtained LNPs. Scale bars in all TEM images are 200 nm. In Sample 1, a rapid hydrolysis fuses Fe(OiPr) coated Fe:LNPs into fishnet-type open network structures. For Sample 2, a controlled hydrolysis keeps the Fe:LNPs from fusing together and forms

small solid particles. In Sample 3, when less $Fe(OiPr)_3$ is used than the accessible OH groups in lignin, the lignin clusters are partially covered with Fe(OiPr) moieties, which fuse together to form larger (ca. 200 nm) particles. Finally, for Sample 4, when less $Fe(OiPr)_3$ is used than the accessible OH groups and the solutions are slow, the fusing of Fe:LNPs create hollow spheres. Reproduced from ref. 216 with permission from the Royal Society of Chemistry, copyright 2016 **[216]**.

Recently, Figueiredo *et al*. developed and evaluated *in vitro* three types of spherical LNPs that had been prepared by solvent exchange (**Fig. 18) [215]**. These three types were pure lignin nanoparticles (pLNPs) **[214]**, iron(III)-complexed lignin nanoparticles (Fe-LNPs) **[216]**, and iron oxide (Fe_3O_4) nanoparticle infused lignin nanoparticles (Fe_3O_4-LNPs). The Fe_3O_4-LNPs were synthesized by mixing a lignin solution with oleic acid-coated Fe_3O_4 nanoparticles in THF (50:50 w/w) and dialyzed against water. Their superparamagnetic behavior makes them promising for cancer therapy and diagnosis **[215]**. All of the LNPs had low cytotoxicity for different cancer cell lines, and also low hemolytic rates with red blood cells after 12 h incubation. Then, the pLNPs were used to encapsulate hydrophobic drugs, which improved their release profiles and consequently enhanced their anticancer activity. Overall, these LNPs showed important features related to drug delivery and biomedical applications, and their surface chemistry can allow the modification with targeting moieties in order to increase the cellular uptake into specific cells for cancer therapies **[215]**.

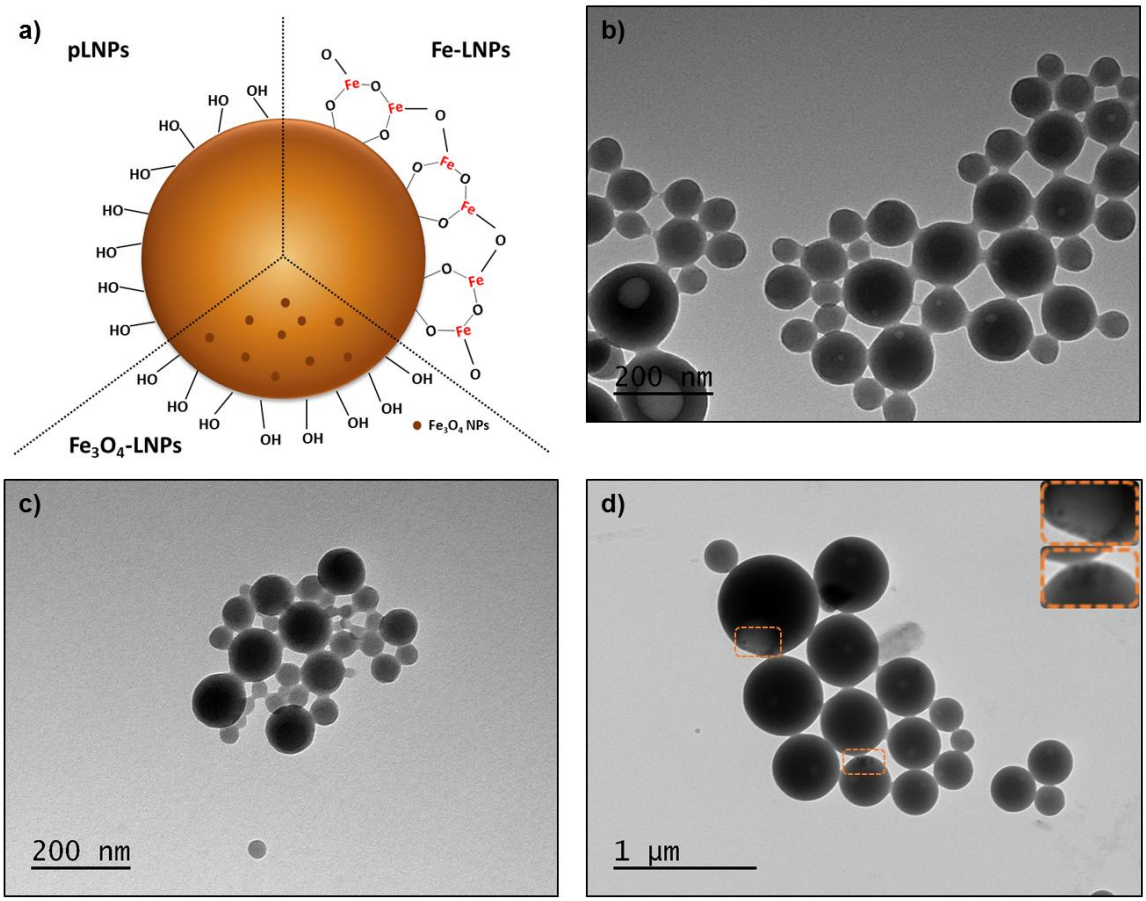

Fig. 18. (a) Schematic representation of LNPs used in the study. TEM images of (b) pLNPs, (c) Fe-LNPs and (d) Fe_3O_4-LNPs and magnification of the Fe_3O_4 NPs inside the LNPs. Reproduced from ref. 215 with permission from Elsevier, copyright 2017 **[215]**.

Besides the lignin-based nanoparticles, hydrogels can also be produced for delivery of different molecules. Superabsorbent cellulose-lignin hydrogels were synthesized by Ciolacu *et al*. by dissolving the cellulose in alkali solution and then mixing with lignin, followed by the chemical crosslinking with epichlorohydrin **[220]**. The hydrogels can incorporate polyphenols and their subsequent release from the hydrogels increased with the lignin content in the matrices. Thus, the swelling process and drug release from hydrogels could be controlled by the composition of the hydrogels, which made the prepared hydrogels potential candidates for biomedical applications as delivery systems.

Wang *et al*. produced sodium lignosulphonate-grafted poly(acrylic acid-*co*-poly(vinyl pyrrolidone)) hydrogels *via* radical polymerization that was assisted by ultrasonic treatment **[221]**. Wang's group also used amoxicillin as a model drug, which exhibited a favorable pH-sensitivity and controllable release behavior *in vitro*. The amoxicillin-loaded hydrogels had better release rates in simulated intestinal fluids than those in simulated gastric fluids.

6.4. Lignin-Based Nanomaterials for Tissue Engineering

Tissue engineering has emerged as a promising tool in the regenerative medicine field for repairing, replacing and enhancing the function of a specific tissue or organ, using biocompatible and biodegradable polymers **[222, 223]**. Several studies explored the potential use of lignin in the development of functional materials for tissue engineering, including hydrogels, aerogels and nanofibers **[210, 211, 224]**.

A study performed by Diao *et al*. reported the synthesis of a temperature sensitive lignin-based thermogelling graft copolymers by ATRP technique **[225]**. They used a lignin core and multiple graft polymer chains composed by a block of poly(*N*-isopropylacrylamide) (PNIPAAm) and block of brush-like random copolymer of PEG and poly(propylene glycol) (PPG) in that method. In aqueous solutions, the copolymers displayed thermogelling behaviors, going from a solution at low temperatures to a hydrogel at temperatures between 32 and 34 °C, and further to a dehydrated gel at higher temperatures (**Fig. 19**). The block architecture of the copolymers grafted onto the lignin is crucial to hydrogel formation in such cases, because the hydrogel is produced when the lignin-based PNIPAAm-*block*-PEG/PPG copolymer is used. However, when a lignin-based PNIPAAm-*random*-PEG/PPG copolymer was used, with the same monomer ratio, the formation of hydrogels was not observed at any temperature. Furthermore, the thermogelling copolymers presented very low critical gelation concentrations that ranged from 1.3 to 2.5% by weight, in which the PNIPAAm block is important for gelling at the critical gelation temperature, and the PPG segments can assist

in water retention by balancing the hydrophilicity/hydrophobicity of the hydrogel network at the temperatures up to 52 °C. Thus, the resulting copolymer solutions experienced a thermogelling transition at a temperature higher than room temperature and lower than human body temperature, suggesting that the hydrogels can be useful for biomedical applications, such as drug delivery and tissue engineering (*e.g.*, stem cells culture and differentiation).

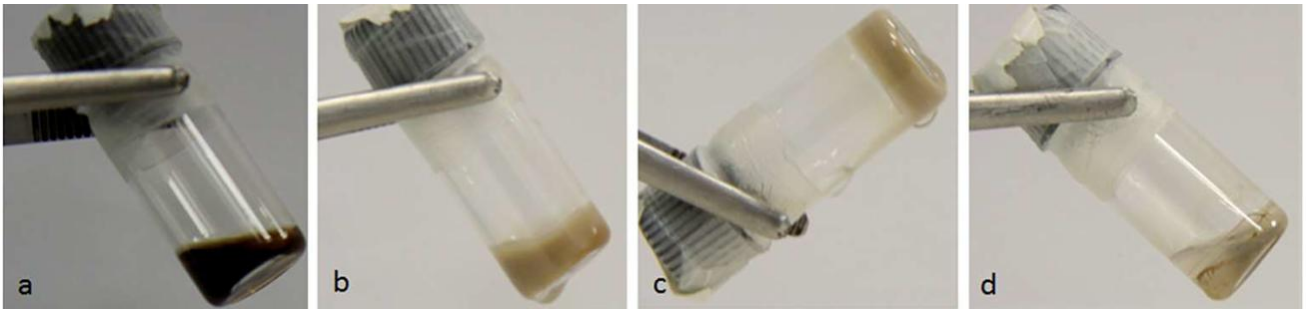

Fig. 1919. A sequence of pictures showing the lignin-based PNIPAAm-*block*-PEG/PPG copolymer behavior at different temperatures: (a) at 25 °C, (b) at 33 °C, (c) at 35 °C, and (d) at 52 °C. Reproduced from ref. 225 with permission from Royal Society of Chemistry, copyright 2014 **[225]**.

Xanthan/lignin hydrogels were prepared by Raschip *et al.*, using epichlorohydrin as a crosslinking agent in an alkaline medium and different types of lignin **[226]**. Compared to xanthan gums, the hydrogels had higher stability due to the incorporation of lignin. The morphology and thermal decomposition of the hydrogels were influenced by the type of lignin used. In another study by Raschip *et al.*, they prepared biodegradable and superabsorbant xanthan/lignin hydrogels by crosslinking between xanthan gum and different types of lignin, and they also used epichlorhydrin as a crosslinking agent **[227]**. The hydrogels that were produced had combined antimicrobial and antioxidative properties, with an increased thermal stability, hydrophilicity and biocompatibility of hydrogel-films by incorporating lignin. Owing to these properties, the resulting hydrogels can be used in pharmaceutical and biomedical field.

Quraishi *et al.* developed alginate-lignin aerogels using CO_2, as an acidifier to release Ca^{2+} ions for the crosslinking of alginate–lignin mixture and promote the gelation, followed by solvent exchange and supercritical drying **[228]**. Foaming by rapid expansion of CO_2 showed to be an effective way to introduce pores of few hundred microns into the aerogels. The obtained alginate-lignin aerogels presented textural and morphological properties (**Fig. 20**), cytocompatibility and good cell adhesion that fulfill the requisites for tissue engineering and regenerative medicine applications.

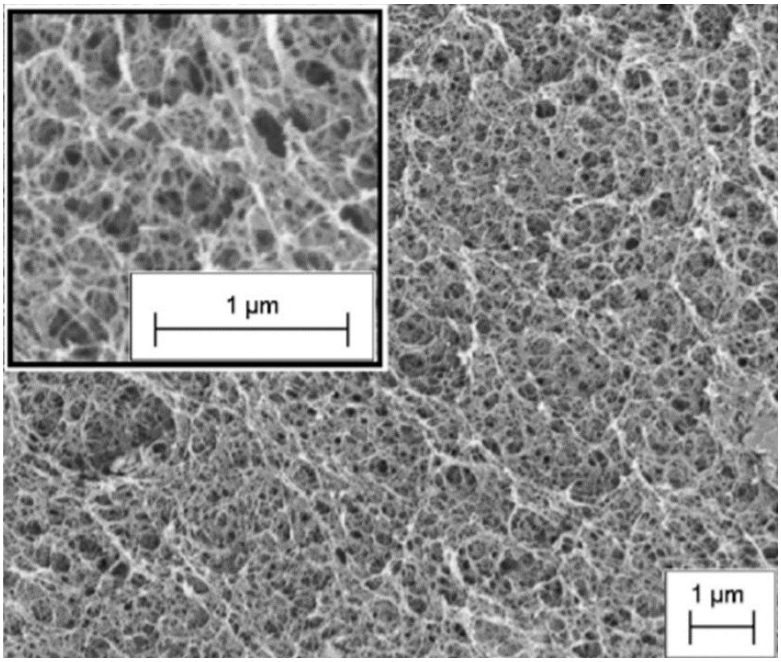

Fig. 20. SEM structure of alginate–lignin aerogel (alginate/lignin; mass ratio 4:1). Reproduced from ref. 228 with permission from Elsevier, copyright 2017 **[228]**.

Xanthan/lignin hydrogels were prepared by Raschip *et al.*, using epichlorohydrin as a crosslinking agent in an alkaline medium and different types of lignin **[226]**. Compared to xanthan gums, the hydrogels had higher stability due to the incorporation of lignin. The morphology and thermal decomposition of the hydrogels were influenced by the type of lignin used. In another study by Raschip *et al.*, they prepared biodegradable and superabsorbant xanthan/lignin hydrogels by crosslinking between xanthan gum and different types of lignin, and they also used epichlorhydrin

as a crosslinking agent **[227]**. The hydrogels that were produced had combined antimicrobial and antioxidative properties, with an increased thermal stability, hydrophilicity and biocompatibility of hydrogel-films by incorporating lignin. Owing to these properties, the resulting hydrogels can be used in pharmaceutical and biomedical field.

Using ATRP, Kai *et al.* synthesized poly(ethylene glycol) methyl ether methacrylate (PEGMA)-grafted lignin hyperbranched copolymers with hyperbranched architecture **[229]**. In the presence of α-cyclodextrin, aqueous solutions of the copolymers formed supramolecular hydrogels with a very low critical gelation concentration. The lignin biodegradable core was found to be particularly important for inducing the gelation, in which 1 wt-% copolymers were sufficient to form stable hydrogels at body temperature. Combining the rheological properties and excellent self-healing ability with the good biocompatibility, the resulting lignin–PEGMA/cyclodextrin hydrogels were proposed as promising smart biomaterial for biomedical applications.

Scaffolds are widely used for tissue engineering purposes due to their similarity to the native extracellular matrix in terms of both chemical composition and physical structure. Polymeric nanofiber matrices constituted by lignin are also candidates to mimic the extracellular matrix, because of its nanoscaled size, being a potential candidate for the development of future tissue engineering scaffolds **[209, 230]**. In this way, Kai *et al*. prepared poly(lactic acid) (PLA)−lignin copolymers by grafting the PLA onto the alkylated lignin *via* ring opening polymerization, using different proportions of lignin (10–50%) **[209]**. They found that the PLA chain length of the resulting copolymers varied from 5 to 38. These copolymers were then blended with poly(L-lactide) (PLLA) to form nanofibrous composites by electrospinning with fiber diameters of 350–500 nm (**Fig. 21**). The resulting nanofibers showed a good antioxidant activity and biocompatibility when they were evaluated by incubation with three different cell lines (PC12, human dermal fibroblasts, and human mesenchymal stem cells).

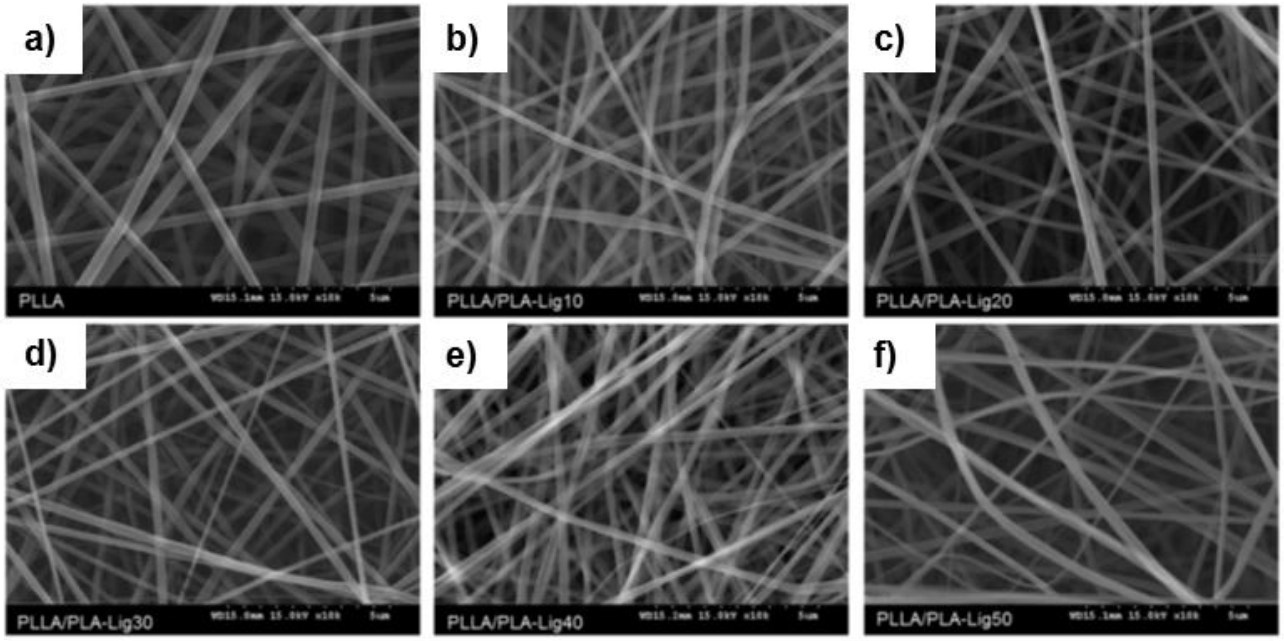

Fig. 21. Scanning electron microscopy (SEM) images of electrospun nanofibers: (a) PLLA; (b) PLLA/PLA–10% Lignin; (c) PLLA/PLA–20% Lignin; (d) PLLA/PLA–30% Lignin; (e) PLLA/PLA–40% Lignin; and (f) PLLA/PLA–50% Lignin. Scale bars = 5 μm. Reproduced from ref. 209 with permission from American Chemical Society, copyright 2016 **[209]**.

The same group also fabricated poly(methyl methacrylate) (PMMA) grafted lignin copolymers by atom transfer radical polymerization, and further blending with poly(ε-caprolactone) to produce nanofiber structures by electrospinning, with reinforced mechanical properties **[211]**. These biocompatible electrospun nanofibers were found promote the proliferation, attachment and interactions of human dermal fibroblasts. More recently, Kai *et al.* synthetized new lignin-based copolymers (lignin-poly(ε-caprolactone-co-lactide), lignin-PCLLA) via ring-opening polymerization **[210]**. Then, the lignin-PCLLA copolymers were blended with polycaprolactone and poly(ι-lactic acid) via electrospinning, originating uniform nanofibers with diameter ranging from 300 to 500 nm. The mechanical properties of the nanofibers were significantly improved after adding the lignin copolymers. As in the previous study, these nanofibers also showed antioxidant

activity and *in vitro* cytocompatibility, suggesting that these nanofibers could be used as biomedical material in tissue engineering scaffolds for protecting cells from oxidative stress.

6.5. Lignin-based Nanomaterials for Other Applications

Lignin-based hydrogels have also been developed for other kind of applications, such as water-storing soil conditioner for agriculture, forestry, and soil rehabilitation, as well as for controlled release of fertilizers.

Lignin-modified alginate hydrogels were synthesized by Flores-Céspedes *et al*., with high entrapment efficiency (99.5%) of azadirachtin **[231]**. The addition of lignin to the alginate formulation allowed a slow release and improved stability against photodegradation of the botanical insecticide azadirachtin, which enhanced the storage period of the insecticide. Peng *et al*., prepared pH-responsive hydrogels by the chemical crosslinking of acetic acid lignin with isocyanate group-terminated polyurethane ionomers, in which the swelling ratios increased with the rising of the pH values **[232]**. In addition, the thermal stabilities of the resulting hydrogels were improved and the release profiles of ammonium sulfate indicated that the hydrogels could be used as coating materials to control the release of fertilizer for different agricultural and horticultural applications. Gao *et al*. also prepared pH-responsive lignin-based hydrogels by blending lignin with PDMAEMA and poly(ethylene oxide). The components formed hydrogels at neutral pH and turned to liquid solutions under both acidic and alkaline conditions **[233]**.

Green polymer hydrogels were synthesized by El-Zawawy, by graft polymerization of acrylamide and poly(vinyl alcohol) with alkali or kraft lignin, and then mixing with acrylamide monomer **[234]**. The alkali lignin hydrogel has high swelling ratios and slower water uptake and deswelling rates than the kraft lignin hydrogel. The slower deswelling rate is due to the hydrogel's compatible network structure, whereas the high swelling ratio was attributed to the cooperative interactions between the sponge particles and the bulk matrix. Later, El-Zawawy and co-workers

prepared lignin-based hydrogels using black liquor, a mixture of lignin/carbohydrates that had been obtained from alkaline pulping of rice straw as the backbone material **[235]**. The hydrogels were prepared by two different approaches: 1) by graft copolymerization of poly(vinyl alcohol) and polyacrylamide involving a radical polymerization in the presence of an initiator and mixed with black liquor, and 2) by an addition reaction in which the same reagents were mixed with black liquor in the absence of an initiator. The hydrogels prepared by radical polymerization exhibited higher swelling capacity (60%) when compared to the hydrogels that had been prepared by an addition reaction (27%). Thus, the black liquor containing lignin can also be used to prepare hydrogels, with potential to solve the environmental water pollution.

Lignosulfonate-*g*-acrylic acid hydrogels with superabsorbent capacity were synthesized by Yu *et al*., by copolymerization of acrylic acid with lignosulfonates using *N,N′*-methylene-*bis*-acrylamide as a cross-linker and laccase/tert-butyl hydroperoxide as an initiator **[236]**. The absorption capacities of the obtained hydrogels were evaluated using methylene blue, a cationic organic dye that can be found in the wastewater of textile mills. The absorbing capacity and removal efficiency of methylene blue were improved by introducing carboxylic acid groups from the acrylic acid, enhancing the interaction between the lignin-based hydrogel and cationic dyes. These hydrogels can be successfully used for absorbing contaminants. In another study, Xue *et al*. prepared acrylamide-based hydrogels with different amounts of ethanol organosolv lignin as a reactive filler in the presence of *N,N′*-methylene-*bis*-acrylamide as a cross-linker and ammonium persulfate (APS) as an initiator, and they used the crosslinking copolymer network (**Fig. 22**) **[237]**. The water swelling capacity increased with the increase of the lignin content. In addition, the obtained hydrogels showed excellent properties, including high tensile modulus and elongation at break, which make them promising materials for water retention applications.

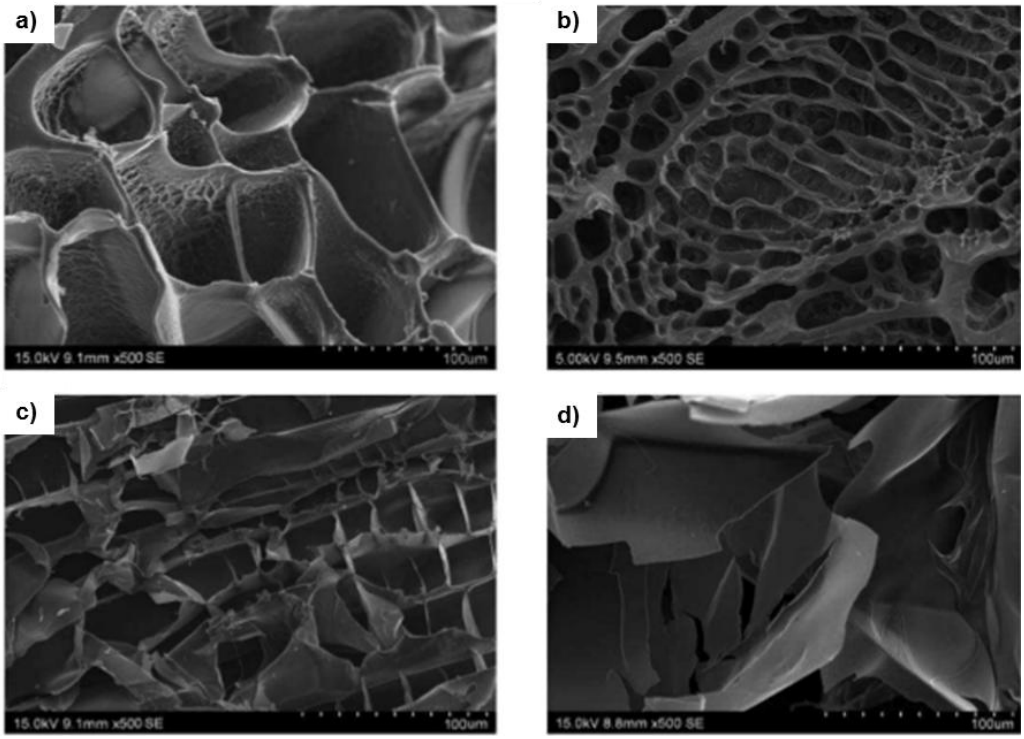

Fig. 202. SEM images of the morphology of acrylamide-based hydrogels that contain an organosolv lignin content of: (a) 0 mg, (b) 10 mg, (c) 20 mg, and (d) 30 mg. Reproduced from ref. 237 with permission from Wiley, copyright 2015 **[237]**.

Passauer *et al*. prepared highly swellable lignin-based hydrogels by crosslinking organosolv lignin with PEG diglycidyl ether (PEGDGE). The swelling properties of the hydrogels and the moisture sorption of the resulting xerogel were strongly dependent on the degree of chemical modification with PEGDGE **[238]**. The total and free water content of the hydrogels decreased by increasing the crosslinking density. The water bound in hydrogels and moisture sorption of xerogels also increased, because of the hydration/dissolution and plasticization that was mediated by the hydrophobic oligo(oxyethylene) and oligo(oxyethylene) glycol substituents, which resulted in moisture diffusion into the xerogel matrix, a plasticization, and swelling of the gels. The hydrogels that were obtained can be used for various applications, particularly as water-storing soil conditioner for agriculture, forestry, and soil rehabilitation.

7. Conclusions and Future Perspectives

Green biomaterials are becoming increasingly in demand due to their low cost of production and eco-friendly properties, which include biocompatibility and biodegradability. For this increased usage to be achieved, bioengineering and nanotechnology are being developed in order to produce promising vehicles for different applications. Thus, lignin shown great potential as a component of new green materials, because of its key characteristics, such as antioxidant, antimicrobial and stabilizing properties that make this biopolymer promising for end-use applications. The emerging valorization of lignin by the chemical and pharmaceutical research fields represents an important stimulus towards the development of high-value materials, such as chemicals and biopolymers, from an economical and renewable aromatic source. Without any chemical modification, lignin is an attractive polymer to reduce the dependence on fossil resources, and it can also be used as a source of carbon for carbon fibers, as well as additive and stabilizer in polymer blends. However, the development and usage of lignin for broader application areas present some challenges, because of its highly variable chemical structure that depends on the extraction method and the source of lignin. To overcome these limitations, different chemical modifications have been proposed to increase the potential use of lignin for the development of advanced lignin-based polymers and nanostructures. For example, esterification or alkylation of the hydroxyl groups on the lignin structure can be performed to increase the compatibility of the lignin polymer with non-polar polymer matrices. Another challenge that lignin faces is the high polydispersity, which increases the complexity to control its macromolecular structure after chemical modification. The development of nanosized lignin-based materials allows the control of the lignin macromolecular architecture, leading to an improvement on the properties of polymer blends and nanocomposites, as well as their application in the biomedical field.

Most of the existing literature is focused on the extraction processes and chemical modifications of lignin for the development of chemicals and polymers with improved properties. This review

covers the topics previously mentioned, and also addresses the main preparation methods of lignin-based nanostructures towards their biomedical applications. The use of natural polymers (such as lignin) over the synthetic polymers is preferable in the biomedical field due to their unique properties (*e.g.*, biocompatibility, biodegradability and abundance in nature). Studies that explored the potential of using lignin in the production of nanosized nanomaterials for drug/gene delivery and other biomedical applications are still limited. Furthermore, *in vitro* and *in vivo* assessments of the LNPs is even more scarcely studied with only few reports on the cytotoxicity evaluation. Apart from nanomaterials, the development of lignin-based hydrogels has promising potential to replace conventional synthetic materials for biomedical and other industrial applications. Given the improvements on the modification chemistry, lignin valorization and its increasing potential at the nanoscale is expected to emerge as an important research field in the near future.

Acknowledgements

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Figure

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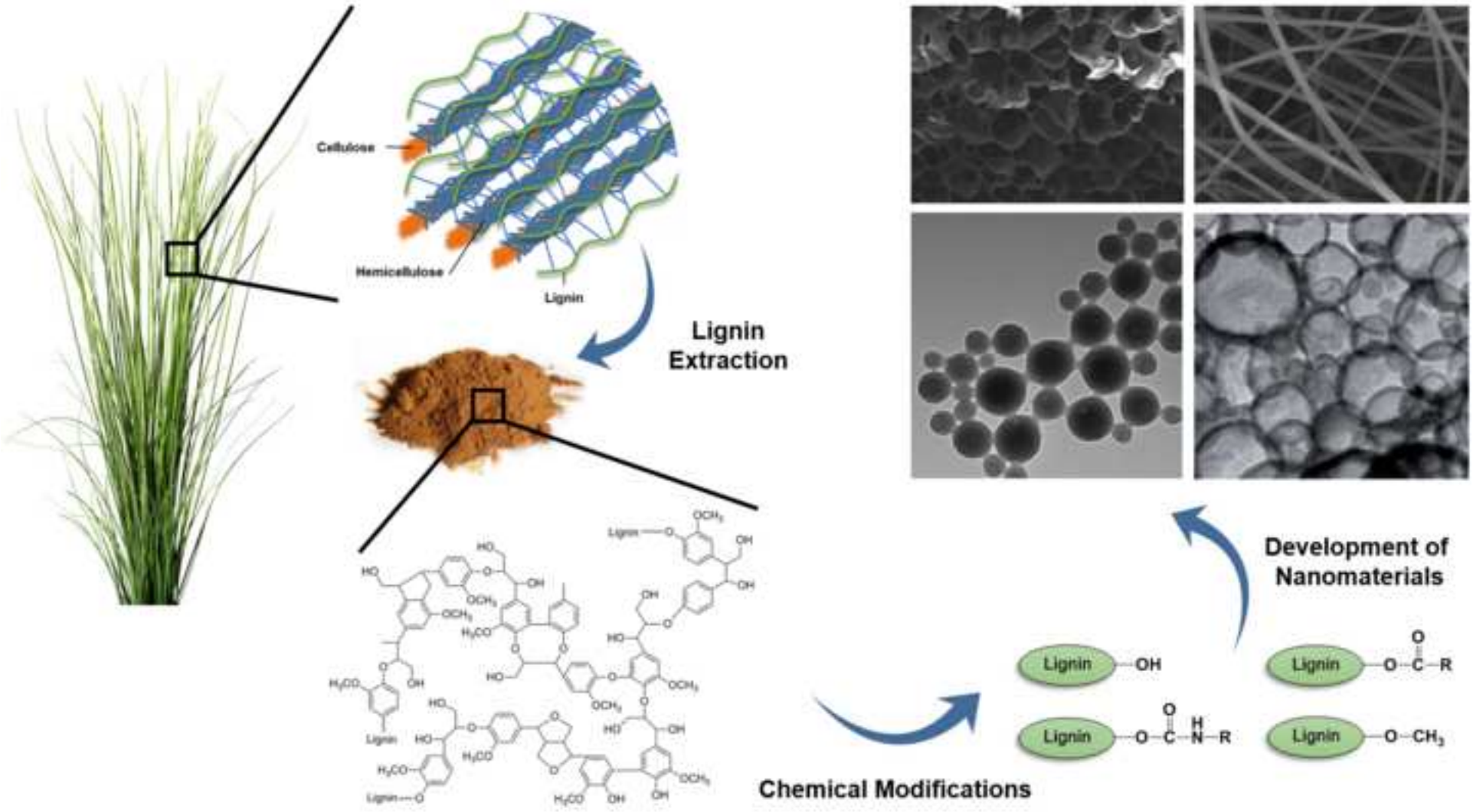

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RESPONSE TO THE REVIEWERS' COMMENTS

REVIEWER #1

In this review, the authors summarized the current state of art for the preparation of lignin-based nanomaterials. Recent advances in the application of lignin-based nanomaterials in drug delivery and gene delivery vehicles were also discussed in the manuscript. However, this review is premature for publication in Progress in Materials Science. Some specific comments are listed as follows.

R.: We thank the reviewer for the time spent revising our manuscript. All comments and suggestions were very welcome and helped to improve the quality of our manuscript. We are pleased to clarify the requested points below.

1. In the section of processing methods for lignin extraction, it lacks an insightful analysis of the technologies and pros and cons of different processing methods for lignin extraction. It just involves four kinds of classification, description and simple list of the studies. Hence, the authors should add some comprehensive analyses and compare these processing methods.

R.: The advantages and disadvantages of the different processing methods for the lignin extraction were addressed all over the section 3:

3.1. "*Similarly to other isolation processes, the native lignin is submitted to some chemical and structural changes [31]. Nevertheless, the production of large amounts of lignin using this process is important for its transformation into high-value lignin-based materials [12].*"

3.2. "*However, the sulfite process presented some disadvantages, including the formation of new C–C bonds that alter the lignin structure, and the presence of high content of ash and other impurities after isolation [39].*"

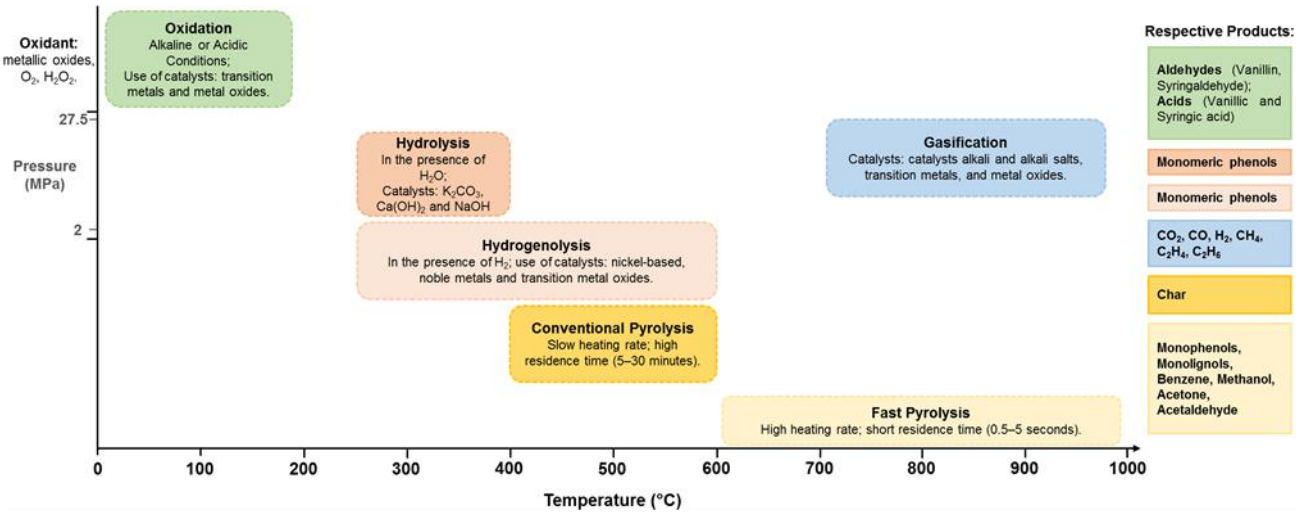
3.3. "*However, comparing to the previous extraction methods, the soda lignin contains no sulfur that makes it a good potential for the preparation of high-value products [6].*"

3.4. "*Moreover, this process is relatively clean and less aggressive compared to the other processes. The obtained lignin is sulfur-free, with lower ash-content, it has higher purity due to the lower carbohydrate content and lower MW (500–5000 Da) [39, 49, 54, 55]. However, despite these advantages, this delignification process is not widely used because of the extensive corrosion of the equipment and the lower quality of the pulp produced compared to the soda and kraft pulping methods [6]. Also, under acidic conditions, random condensation reactions can occur, instead of the depolymerization reactions. For example, the formation of benzylic cations under acidic conditions can take place, leading to the formation of new C–C bonds [39]*."

To conclude the section 3, the following paragraph was also added:
"*Taking all of this into consideration, the native structure of lignin undergoes a chemical transformation after the extraction process. Besides the limitations abovementioned, the structure of kraft, organosolv, sulfate and soda lignin can also vary according to the plant source. Thus, the introduction of lignin into new markets will strongly depend on its structure, reactivity and physicochemical properties [56]*."

2. "4.1 Lignin Depolymerization": There are too many descriptive statements. In order to improve the understanding of different lignin depolymerization processes, main reaction equations (similar to fig. 4) should be added in this section.

R.: As each depolymerization process can originate different products, with different chemical structures, we decided to added a figure (Fig. 4) that summarize the different conditions used for each lignin depolymerization process, with the different products that can obtained from each process.



3. "4.4 Production of Lignin Graft Copolymers": This section is too long to read. The authors should divide it into several parts and provide some subtitles here. In this way, the readers can easily follow.

R.: In order to facilitate the reading, the following subsections were created in the "4.4. Production of Lignin Graft Copolymers" section, according to the processes described in the Figures 7 and 8:

4.4.1. "Grafting from" Technique

4.4.1.1. Ring Opening Polymerization

4.4.1.2. Radical Polymerization

4.4.1.3. Atom Transfer Radical Polymerization

4.4.2. "Grafting to" Technique

4. In section 6, the authors claimed that lignin has been successfully used to produce different nanomaterials including nanoparticles, nanotubes and hydrogels. The reviewer believes that nanofiber is also one of the important parts of lignin-based nanomaterials. Though the authors introduced PLA-lignin copolymer nanofibers in this section, the reviewer believes they are less extensive than nanoparticles and hydrogels. For example, Gao et al. prepared lignin nanofibers with ionic-responsive shells (Biomacromolecules, 2012, 13, 3602). Salas et al. synthesized soy protein-lignin nanofibers (Reactive and Functional Polymers, 2014, 85, 221). The authors should expand this part and provide more comments/insights on the progress of this field.

5. For a broader impact, the authors should also provide a section discussing the application of lignin-based nanomaterials in other biomedical fields. For example, the authors should provide more insights on how the development of lignin-based nanomaterials can promote their application in tissue engineering and regenerative medicine.

R.: We recognize that the lignin-based nanofibers are underexplored among the other nanomaterials in this review. Only two studies were lignin nanofibers were referred: Kai et al. (ACS Sustainable Chemistry & Engineering. 2016; 4: 5268-76) about the PLA-lignin copolymer nanofibers, and the study conducted by Gao et al. (Biomacromolecules. 2012; 13: 3602-10) about the lignin nanofibers with ionic-responsive shells, as an example of the atom transfer radical polymerization (section 4.4).

In order to emphasize the potential use of lignin-based materials in other biomedical fields such as tissue engineering, and also include additional studies in which nanofibers were used (namely for

tissue engineering), we decided to reorganize the section of the lignin-based nanomaterials according to the types of application, instead of the division previously established. Thus, the section 6 is now divided by:

6.1. Lignin-based Nanomaterials as Antioxidant

6.2. Lignin-based Nanomaterials for Nanocomposites

6.3. Lignin-based Nanomaterials as Delivery Systems

6.4. Lignin-based Nanomaterials for Tissue Engineering

6.5. Lignin-based Nanomaterials for Other Application

The following studies were added to the section 6:

Quraishi *et al.*, 2015 (https://doi.org/10.1016/j.supflu.2014.12.026)

Kai *et al.*, 2015 (DOI: 10.1021/acssuschemeng.5b00405)

Kai *et al.*, 2017 (DOI: 10.1021/acssuschemeng.7b00850)

Kai *et al.*, 2015 (DOI:10.1039/C5TB00765H)

Salas *et al.*, 2014 (https://doi.org/10.1016/j.reactfunctpolym.2014.09.022)

6. In the conclusion part, the statements about the current challenges in development and usage of lignin are very descriptive and lack in-depth discussion. For example, the authors only said several chemical modifications could be used to increase the potential use of lignins in the development of advanced lignin-based nanoparticles. The authors should provide more insights and discussions on the challenges.

7. Additionally, in conclusions and perspectives, the authors need to highlight the importance of lignin-based nanomaterials in broader application fields.

8. Finally, the difference and novelty of this review in comparison with previously reported works needs to be discussed.

R.: In order to answer the three comments raised by the reviewer, the conclusion and perspectives section was completely modified:

"*Green biomaterials are becoming increasingly in demand due to their low cost of production and eco-friendly properties, which include biocompatibility and biodegradability. For this increased usage to be achieved, bioengineering and nanotechnology are being developed in order to produce promising vehicles for different applications. Thus, lignin has shown great potential as a component of new green materials, because of its key characteristics, such as antioxidant, antimicrobial and stabilizing properties that make this biopolymer promising for end-use applications. The*

emerging valorization of lignin by chemistry and pharmaceutical research fields represents an important stimulus towards the development of high-value materials, such as chemicals and biopolymers, from an economical and renewable aromatic source. Without any chemical modification, lignin is an attractive polymer to reduce the dependence on fossil resources, and it can also be used as a source of carbon for carbon fibers, as well as additive and stabilizer in polymer blends. However, the development and usage of lignin for broader application areas present some challenges, because of its highly variable chemical structure that depends on the extraction method and the source of lignin. To overcome these limitations, different chemical modifications have been proposed to increase the potential use of lignin for the development of advanced lignin-based polymers and nanostructures. For example, esterification or alkylation of the hydroxyl groups on the lignin structure can be performed to increase the compatibility of the lignin polymer with non-polar polymer matrices. Another challenge that lignin faces is the high polydispersity, which increases the complexity to control its macromolecular structure after chemical modification. The development of nanosized lignin-based materials allows the control of the lignin macromolecular architecture, leading to an improvement on the properties of polymer blends and nanocomposites, as well as their application in the biomedical field.

Most of the existing literature is focused on the extraction processes and chemical modifications of lignin for the development of chemicals and polymers with improved properties. This review covers the topics previously mentioned, and also addresses the main preparation methods of lignin-based nanostructures towards their biomedical application. The use of natural polymers (such as lignin) over the synthetic polymers is preferable in the biomedical field due to their unique properties such as biocompatibility, biodegradability and abundance in nature. Studies that explored the potential of using lignin in the production of nanosized nanomaterials for drug/gene delivery and other biomedical applications are still limited as yet. Furthermore, in vitro assessment of the LNPs is even more scarcely studied with only few reports on the cytotoxicity evaluation. Apart from nanomaterials, the development of lignin-based hydrogels has promising potential to replace conventional synthetic materials for biomedical and other industrial applications. Given the improvements on the modification chemistry, lignin valorization and its increasing potential at the nanoscale is expected to emerge as an important research field in the near future."

9. Some grammar and typographic errors need to be corrected in the manuscript.

R.: The grammar and typographic errors are now revised.

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RESPONSE TO THE REVIEWERS' COMMENTS

REVIEWER #2

The review paper by Patrícia Figueiredo et al. presents different features of lignin materials in terms of chemical modification and application. One of the main credits given to this Work is the right time selected to compile all novel original works performed on lignin materials and prepare a very comprehensive review paper. Currently, plenty of attention towards lignin materials is observed from Scientific communities and despite immensely increasing number of research works in this field, a perceptible lack of complete review paper was recognized in this field. Therefore, I believe, this scholarly presented work has enough potential to fullfill requirements of getting published in Progress in Materials Science because of its broad audience and covering the most recent research efforts on lignin materials, especially in application section.

However, there are still some minor considerations that need to be taken into account by authors before final approval of this manuscript.

R.: We thank the reviewer for the time spent revising our manuscript and for recommending our paper for publication. All comments and suggestions were very welcome and helped to improve the quality of our manuscript. We are pleased to clarify the requested points below.

1. Introduction is very short and mainly provides a general information about the composition of Lignocellulosic materials. I think authors can provide more information about these materials in terms of their potentials, interests of applications and its practical challenges, mainly in one or two paragraphs.

R.: After describing the composition of lignocellulosic materials, we addressed the following text about the potential of lignin as a starting material for different applications, and the main challenges of using lignin-based materials:

"*Thus, lignin is one of the main bio-resource raw materials that can be used for the synthesis of environmentally friendly polymers, and its high content of aromatic structures offers the possibility to replace industrially relevant aromatic polymers and fine chemicals [10]. However, only approximately 2% of the annually extracted lignin by the paper and pulp industry from woody biomass has been mainly used for low-value applications as dispersants, adhesives and fillers [11]. This is mainly due to the complex and variable lignin structure, high polydispersity and immiscibility of lignin with host polymer matrices [6, 11, 12]. The procedures that are applied to separate, to isolate and to chemically transform these three components can lead to a multifunctional array of bio-derived value-added fuels, chemicals and other materials [13, 14]. Another way to overcome these limitations is to prepare lignin nanoparticles, which improves the blending properties with host matrix, and offers a morphological and structural control of the lignin [15-17]. In addition, the preparation of nanostructured lignin also opens the possibility to use lignin-based materials for high-value applications, such as drug/gene delivery and tissue engineering [17].*

In this review, we provide an overview of the composition and properties of lignin, the processing methods for lignin extraction, chemical modifications of lignin, and the industrial applications of lignin derivatives. Finally, we discuss the preparation of lignin-based nanomaterials for different applications, highlighting the potential of these nanomaterials for biomedical applications."

2. Sentences with same meaning are presented in different ways in some parts of the paper. I believe they can be merged to provide better understanding for readers. For example, these two consecutive sentences can be merged:

Lignocellulosic materials are mainly constituted by cellulose, hemicellulose, lignin, and their compositions are dependent on their origin and species (hardwood, softwood or grass). Commonly, they contain 30-50% cellulose, a polymer of glucose; 20-35% hemicellulose, a heteropolymer containing xylose, and 15-30% lignin [3, 8, 9].

R.: The referred sentences were merged into the following sentence:

"*Lignocellulosic materials are mainly constituted by 30–50% cellulose, a polymer of glucose, 20–35% hemicellulose, a heteropolymer containing xylose, as well as 15–30% lignin, and their compositions are dependent on their origin and species (hardwood, softwood or grass).*"

3. All lignin extraction procedures for different methods are represented very well in detail except for The Organosolv Process in section 3.4. I recommend the authors to use one of the cited examples in this section to include a detailed explanation of this method rather than just mentioning the most appropriate solvents and the cleavage site for the extraction.

R.: In the section 3.4, the extraction of lignin by the organosolv process was approached by adding the following statement:

"*The delignification process is carried out by adding mixtures of organic solvent and water that dissolve part of the lignin and hemicellulose [46]. During this process, mainly the lignin-carbohydrate and the α–O–4' linkages are broken, but β–O–4' can also be cleaved to a lesser extent when the lignin oligomers are released from the lignocellulose matrix [34, 47]. The recovery and separation of the dissolved lignin and hemicelluloses can be done by precipitation of lignin or evaporation of the organic solvent, after adjusting temperature, pH and concentration of the organic solvent [46, 48]*."

4. Provide reference for this sentence in the text:

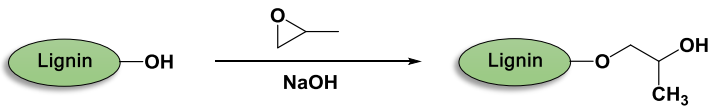
Although nitrobenzene is a very effective oxidant, it is also a proven carcinogen.

R.: The following reference (Ref. 72) was added to the manuscript to support the abovementioned sentence:

Holder JW. Nitrobenzene carcinogenicity in animals and human hazard evaluation. Toxicology and industrial health. 1999; 15: 445-57.

5. In figure 5, etherification protocol is shown by using propylene oxide or ethylene oxide to form epoxy functionalized lignin. Would the authors check the content of cited papers and provide information in the text how propylene oxide structure (glycidyl ether/epoxy) does not open in the presence of NaOH? This is a very well confirmed chemical reaction that epoxy groups opens in the alkalyne solutions (NaOH) to react with hydroxyl groups. It would be very important to double check the reaction and final product obtained after etherification in the referred papers.

R.: The etherification reaction in mentioned figure was modified to the following reaction:



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After checking some papers, we realized that during the oxypropylation reaction in alkaline solutions, the ring on propylene oxide opens. In addition, we rearranged the main text to clarify the reaction protocol:

"*The oxypropylation is the most used etherification method to modify lignin using propylene oxide in the presence of an alkaline solution, in order to prepare lignin-based epoxy resins. The resulting solution was treated with epichlorohydrin and cured using m-phenylene diamine for cross-linking [132, 133]*."

6. There are few minor typos that need to be revised…

R.: All the typos mentioned by the reviewer were corrected.

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RESPONSE TO THE REVIEWERS' COMMENTS

REVIEWER #3

The authors provide an overview on the composition and properties, extraction methods and chemical modifications of lignin and its potential applications in pharmaceutical nanotechnology and nanomedicine. Furthermore, they report different preparation methods for lignin-based nanomaterials with antioxidant UV-absorbing and antimicrobial properties that can be used as reinforcing agents in nanocomposites, in drug delivery and gene delivery vehicles for biomedical applications. It is opinion of this reviewer that the review paper can be accepted for publication in the following form without any further revision.

R.: We thank the reviewer for the time spent revising our manuscript and for recommending our paper for publication as such.