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Article

Natural Sunscreens Based on Nanoparticles of Modified Kraft Lignin (CatLignin)

Petri Widsten,* Tarja Tamminen, and Tiina Liitiä

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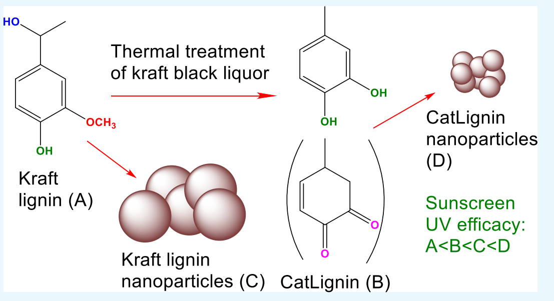
ABSTRACT: Commercial chemical sunscreens have a high content of synthetic ultraviolet (UV) actives that have caused widespread damage to marine ecosystems and may have adverse health effects in humans. In the present work, safer bio-based sunscreens with lignin UV absorbers were developed to address this issue. Partly demethylated and otherwise altered kraft lignins, the so-called CatLignins with abundant phenolic hydroxyl auxochromes and catechol units, outperformed regular kraft lignins as sunscreen UV absorbers in terms of sun protection factor (UVB−SPF) and UVA−UVB transmittance. Converting lignins to nanoparticles significantly enhanced sunscreen performance. The best lignin sunscreen, containing nanoparticles of hardwood CatLignin, had a UV transmittance of only 0.5−3.8% over the entire UVA−UVB region compared to 2.7−51.1% of a commercial SPF 15 sunscreen. Lignin-based sunscreens are particularly suitable for dark-tinted SPF cosmetics.



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■ INTRODUCTION

The Sun emits electromagnetic radiation in three ultraviolet (UV) wavelength areas. The rays with the shortest wavelengths (vacuum-UV and UVC, 100−290 nm) are captured by the Earth's atmosphere, but both medium-wavelength (UVB, 290−320 nm) and long-wavelength (320−400 nm) UVA radiation reach its surface.[1] Excessive exposure of skin to UVB rays causes sunburn, while UVA rays penetrate more deeply into the skin, causing it to tan and accelerating its aging.[1,2] Both UVA and UVB rays can cause DNA damage and skin cancer.[1,2]

Sunscreens have long been used to protect skin against sunburn.[3] Depending on their composition, they may either offer protection against both UVA and UVB radiation (broad-range sunscreens) or mostly just against UVB rays.[4] The UV actives in sunscreens may include physical sunblocks or chemical UV absorbers or both. Broad-range chemical sunscreens contain specific synthetic UVB absorbers (e.g., derivatives of *p*-aminobenzoic acid, cinnamates, and salicylates) and UVA absorbers (e.g., benzophenones and acetophenone) for broad-spectrum UV protection.[4] Physical sunblocks, usually based on titanium dioxide and zinc oxide, effectively deflect and absorb UVA and UVB rays.[4] Unfortunately, pure physical sunscreens are not very comfortable to use on the skin. The so-called herbal or natural sunscreens are free of synthetic UV absorbers and contain various plant extracts and oils[5−7] with typically low UV absorptivity compared to synthetic UV absorbers.[6] The UV blocking of these sunscreens is mainly based on physical sunblocks, but many of the plant-based ingredients are good antioxidants and emollients that may replace many synthetic ingredients and make the sunscreens more comfortable to wear.

Sunscreens are classified according to their sun protection factor (SPF) that can reach up to 50[2] in Europe. It is generally regarded that the SPF of a sunscreen should be at least 15 for it to provide sufficient daily protection against UVB rays.[1] Theoretically, when applied evenly on skin at 2 mg/cm^2, SPF 15 and SPF 50 sunscreens lengthen the time it takes a person's skin to redden in the sun by a factor of 15 or 50 while filtering out 93 and 98% of UVB rays, respectively.[1,24] However, as the SPF of a sunscreen is usually calculated based on its absorption of burning (UVB) rays (UVB−SPF), it does not indicate the level of UVA protection provided. Although many chemical sunscreens nowadays are of broad range, containing chemical UVB as well as UVA absorbers, they still tend to provide less protection against UVA than UVB, particularly when they do not contain physical sunblocks. Many of the commercial sunscreens do not meet the European standards for UVA protection, which stipulate that the UVA protection factor (PF−UVA) should be at least one-third of the claimed SPF.[8]

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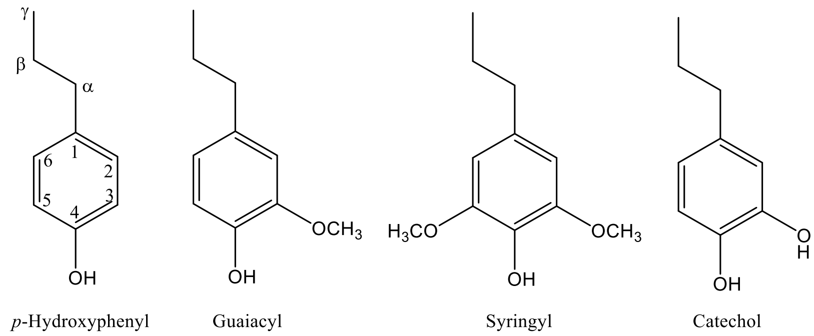

Figure 1. Lignin alkyl−aryl units in kraft lignins and demethylated/demethoxylated CatLignins and designations of their carbon atoms.

The percentage of actives required to achieve an SPF of 15−50 is very high—for example, an oxybenzone or octinoxate content of 1% gives a sunscreen an SPF of 1.5. In fact, a commercial SPF 15 sunscreen contains at least 20% chemical UV actives,[9] and the higher SPF sunscreens considerably more. Some of the chemical and physical actives harm marine ecosystems by causing coral bleaching[8,10−12] and oxidative damage to phytoplankton.[13] Annually, thousands of tons of sunscreen actives are washed off from people's skin while bathing or washing, the small molecules passing through wastewater treatment plants[14] and ending up in natural bodies of water. Some of the most harmful chemical actives, oxybenzone and octyl methoxycinnamate (octinoxate), were recently banned from sunscreens used in Hawaii where 7000 tons of sunscreen enter the ocean annually and cause widespread devastation. In addition, the synthetic UV actives or their photodegradation products can cause unwanted side effects on the skin such as allergies and irritation.[8] Thus, there is a need for more environmentally safe and healthier broad-range sunscreen actives.

The plant-based ingredients of herbal sunscreens[5−7] may be expensive to extract and purify and be limited in supply. Technical lignins, however, are inexpensive polyphenolic compounds available in huge quantities as a byproduct of chemical pulping of lignocellulosic raw materials.[15] Kraft lignin, the most abundant of the technical lignins, has more potent antioxidant properties[16] than the commercial antioxidant BHT and shows low cytotoxicity toward normal animal cells but promising biological activity against cancerous cells.[16,17] These properties indicate that technical lignins should be safe to use in cosmetic preparations and sunscreens, which would then require no additional antioxidants. The chromophores responsible for the light absorption of technical lignins in the UVA−UVB region include quinones and aromatic rings, which may be conjugated with double bonds and carbonyl groups in the lignin side chain.[18−20] The aromatic units also contain phenolic hydroxyl and methoxyl groups as auxochromes[20] whose free pairs of electrons are conjugated with the chromophoric aromatic rings, shifting chromophore absorption to the UVA−UVB region and intensifying it.[19] For example, while phenol is a chromophore that has absorption in the UVC region (200−290 nm) but not in the UVA−UVB region (290−400 nm), phenols with additional phenolic hydroxyl or methoxyl groups also absorb light in the UVA−UVB area.[21] In addition, charge-transfer complexes between electron-accepting *ortho*-quinones and electron-donating phenolic groups are known to occur in technical lignins, strongly increasing lignin absorptivity in the UV/vis region.[18]

Natural bodies of water contain dissolved lignins, and the ecosystem has adapted to them, while from a human health point of view, they have been found neutral or beneficial.[22−24] These characteristics make lignins attractive as potential bio-based substitutes for harmful synthetic sunscreen UV actives.

In the past few years, technical lignins[21,25−31] and milled wood lignins (MWLs)[28] have been investigated as sunscreen actives as such or as lignin nanoparticles (LNPs). Technical lignins directly incorporated into moisturizing creams at a concentration of 5−10% imparted the creams with an in vitro SPF of 3.7−8.6, depending on the type of lignin.[21,25,27,28] As for MWL-based sunscreens, adding 10% softwood MWL to a base cream gave an SPF of only 2.6, while 10% grass MWL produced an SPF of 7.3. The reason for the different performances of the MWLs was that while both had low lignin-based phenolic hydroxyl contents, the grass MWL also contained UVB-absorbing hydroxycinnamic acids. Higher SPF values (up to 19.7 for a sunscreen with 5% technical lignin) were reported by Gordobil et al.[26] who used a much higher than the usual amount of sunscreen in their SPF testing (5.1 instead of 2.0 mg/cm^2). To improve the efficacy of technical lignins in sunscreens, Qian et al.[19] converted them to LNPs of various sizes.[19] Their sunscreens formulated with 10% LNPs had an SPF of up to 15.[19] The SPF was inversely proportional to the LNP size, but the sunscreens with larger LNPs had lower UV transmittance at a higher UVA wavelength of 380−400 nm. The distribution of chromophores and auxochromes between the surface and core of LNPs has been reported to depend on particle size and affect their UV absorption spectrum.[19,32]

Although in vivo testing of sunscreens is standardized, there is no official standard method for in vitro sunscreen testing, and therefore, the results of different in vitro lignin sunscreen investigations are often not directly comparable. For example, some researchers applied sunscreen directly on quartz plates[26,27] and others[19,21,25,28] on a 3M Transpore tape (simulating the skin surface) attached to a quartz plate. The amount of sunscreen in in vitro testing was usually the same as that used in in vivo testing on human subjects (2.0 mg/cm^2), but higher doses have also been used.[26] Further, two different equations have been used for calculating SPF: the Mansur equation[33] based on the absorbance in the UVB region and another based on the entire UVA−UVB region.[34]

The goal of the present work was to produce safe and eco-friendly broad-range sunscreens with UVB−SPFs >15 and overall low UVA−UVB transmission with lignin as the sole UV active. To achieve this, modified kraft lignins with high phenolic hydroxyl contents produced by CatLignin technology based on the heat treatment of black liquor[35,36] were used in

Table 1. Structural Information on Lignins

^{31}P NMR spectroscopy, mmol/g molar mass distribution									
OH/COOH	OH_{Al}	cond. G + S–OH	G-OH	cat-OH	*p*-H-OH	OH_{Ph}-T	COOH	M_w	M_n
ppm	150–145	145–140.5	140.5–139.5	139.5–138.5	138.5–137	148–134	136–134		
S	1.99	2.05	2.46	0	0.23	4.74	0.43	5010	2277
H	1.3	3.4	0.49	0.5	0.2	4.59	0.3	2050	1170
SC	0.9	2.4	1.2	1.2	0.7	5.5	0.7	3700	1700
HC	0.29	3.05	0.67	1.87	1.17	6.76	0.83	2330	1370

quantitative ^{13}C NMR spectroscopy[a], mmol/g											
C	COOH, COOR	C_{Ar}-O	C_{Ar}-C	C_{Ar}-H	C_{Ar}-T	C_{Al}–O–Ar, α-O–C_{Al}	γ-O–C_{Al}, C_{Al}–OH_{sec}	C–OH_{Pri}	C_{Al}-O-T	OCH_3[b]	C_{sat}[c]
ppm	185–165	162–142	142–125	125–102	162–102	90–77	77–65	65–58	90–58	58–54	54–0
S	1.26	9.78	8.73	12.54	31.05	0.74	1.14	1.17	3.06	4.55	3.68
H	0.69	7.44	7.01	7.24	21.70	0.75	1.30	1.22	3.26	5.60	3.25
SC	1.40	8.44	9.44	10.64	28.51	0.47	0.48	0.66	1.61	2.19	4.40
HC	1.10	10.86	14.95	13.48	39.28	0.00	0.00	0.27	0.12	2.75	4.81

[a]Signals for syringyl C4 appear in the region 138–134 ppm.[39] *p*-H = *p*-hydroxyphenyl; G = guaiacyl; S = syringyl; cond = condensed; cat = catechol; Al = aliphatic; Ph = phenolic; Ar = aromatic; T = total; Pri = primary; sec = secondary; and sat = saturated (not connected to oxygen). [b]Content determined by gas chromatography[40] and then used as an internal standard to quantify other types of carbons in the ^{13}C NMR spectra. [c]Lignin C-$_{Sat}$ resonance from 44 to 35 ppm not integrated because of its overlap with the DMSO peak at 39.5 ppm.

sunscreen formulations, while regular kraft lignins were included for comparison. In the CatLignin process, the lignin is partially demethylated, demethoxylated, and depolymerized via cleavage of alkyl–aryl ether bonds. The lignins were also converted to LNPs prior to their application in sunscreens to boost the UV absorptivity and photostability of lignin sunscreens, and LNPs were investigated by exposing them to UV radiation.

RESULTS AND DISCUSSION

Characterization of Lignins. The types of alkyl–aryl units comprising the technical lignins of the present work are illustrated in Figure 1. It should be noted that in kraft lignins, many of the original propyl side chains of native lignin are shortened because of reactions occurring during kraft pulping.[37] During thermal treatments of kraft black liquors to produce CatLignins, such modifications may be amplified. The functional groups of lignins (Table 1) include aliphatic hydroxyl, carboxyl, and carbonyl groups on the alkyl side chains,[20,37,38] and some of the phenolic units may have been converted to quinones.[18] Probably as a result of this, the CatLignins SC and HC (Table 2) are a darker shade of brown than the regular kraft lignins S an H. In technical lignins in general, the alkyl–aryl units are connected mostly by carbon–carbon and carbon–oxygen bonds such as C5–C5, 4-O-5, and β-5 ("condensed" lignin structures).[20,37,38] Some alkyl–aryl ether bonds (mostly β-O-4) of native lignin survive the kraft pulping conditions[20,37,38] but not necessarily subsequent thermal treatments used to produce the catechol-rich CatLignins SC and HC.

Table 2. Technical Lignins Used in Sunscreen Formulations[a]

Material	As received	LNPs (Method 1b)	smaller LNPs (Method 2)
Softwood kraft lignin	S	SNP	SNPS
Softwood CatLignin[b]	SC	SCNP	SCNPS
Hardwood kraft lignin	H	HNP	HNPS
Hardwood CatLignin[b]	HC	HCNP	HCNPS

[a]The Nivea lignin sunscreens are designated as N-"lignin". [b]Partially demethylated, demethoxylated, and otherwise during black liquor heat treatment-altered kraft lignin.[36]

The sunscreen performance of lignins is to a large extent determined by their auxochromic phenolic hydroxyl and methoxyl groups.[19,21,25−28] However, when lignin is used as a UV absorber at a certain percentage of weight, aliphatic hydroxyls that probably play a negligible direct role in UV absorption will nevertheless have an indirect negative effect on it by adding to the lignin mass. Therefore, the auxochrome content of lignin is increased by elimination of aliphatic hydroxyls. Demethylation and cleavage of β-O-4 or any other alkyl–aryl ether bonds in lignin[20,37,38] will also add to the phenolic hydroxyl content. Analytical data on lignins are presented in Table 1 and the parts relevant to UVA–UVB absorption are discussed below.

S and H are softwood and hardwood kraft lignins, respectively. CatLignins (SC and HC) were prepared from softwood and hardwood kraft black liquors by thermal treatment to increase the content of phenolic hydroxyls of the kraft lignins via demethylation of their methoxyl groups.[36] New phenolic units are also formed because of the cleavage of the remaining native aryl ether linkages.[36] Indeed, the total phenol contents of SC and HC are higher and their methoxyl contents over 50% lower compared to S and H (Table 1). ^{31}P NMR spectral analysis shows that both demethylation and demethoxylation of guaiacyl units took place during the thermal treatments, affording catechol and *p*-hydroxyphenyl type phenolic units that are much more abundant in SC and HC CatLignins than in S and H kraft lignins. The fact that HC nonetheless has a higher content of guaiacyl-type phenolic units than H suggests that part of them originate from demethoxylation of syringyl units. ^{31}P NMR spectroscopy also shows that the thermal treatments eliminated most of the aliphatic hydroxyls as evidenced by their much lower content in SC and HC compared to S and H.

The total phenolic hydroxyl and methoxyl contents of the softwood lignins S and SC agree fairly well with the values for oxygen-bonded aromatic carbons (C_{Ar}–O) determined by

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quantitative ^{13}C NMR (Table 1).[20,37,38] The reason that the C_{Ar}–O values are slightly higher may be that they include the nonphenolic aromatic units etherified at C_4 (4-O-5 and *β*-O-4). The fact that the ratio of C_{Ar}–O to total aromatic carbons (C_{Ar}-T) in SC (0.25) is lower than in S (0.29) is consistent with the higher content of *p*-hydroxymethyl units in SC. SC also has a higher content of saturated carbons (C_{sat}; not directly bonded to oxygen) and lower content of oxygen-bonded aliphatic carbons (C_{Al}–O) than S. This indicates an extensive loss of side-chain aliphatic hydroxyls (e.g., by dehydration), which can be seen clearly from the ^{31}P NMR data, during the production of SC. It should be noted that only part of the C_{sat} signals of each of the four lignins, located between 54 and 0 ppm,[20] were able to be integrated because of the solvent [dimethyl sulfoxide (DMSO)] peak at 39.5 ppm.

For the hardwood lignins H and HC, reconciling the analytical results is more complicated than for the softwood lignins. The C_{Ar}-O/C_{Ar}-T ratio is much lower for HC (0.24) than for H (0.32), consistent with the high *p*-hydroxymethyl content of HC. However, the content of C_{Ar}–O bonds in H according to ^{13}C NMR is considerably lower than its combined phenolic hydroxyl and methoxyl content. A major reason for this is the appearance of syringyl C4 signals outside the integrated C_{Ar}–O region (162–142 ppm) at 138–134 ppm.[39] In the case of HC, there is a reasonably good agreement between the C_{Ar}–O and phenolic and methoxyl contents. Factors that contribute to this are conversion of syringyl units to other types of phenolic units, reducing signals from syringyl C4 carbons falling outside the integrated C_{Ar}–O region 162–142 ppm, and cleavage of alkyl–aryl ether bonds adding to the phenolic hydroxyl content. The ^{13}C NMR spectra show a virtual absence of side-chain alkyl–aryl ethers in HC that are present in H. The degree of aromaticity (C_{Ar}-T) of HC is very high compared to H, which agrees with its much lower combined content of methoxyl groups, side-chain oxygens (C_{Al}–O), saturated carbons (C_{sat}), and free or esterified carboxyl groups (8.0 mmol/g for HC vs 13.4 mmol/g for H).

Preparation of LNPs. LNPs were produced by dissolving lignin in aqueous acetone and then evaporating off the acetone. Acetone and water are required to fully solvate the hydrophobic and hydrophilic moieties of lignin, respectively, promoting lignin dissolution.[41] As the water content is increasing and the medium thus becoming increasingly more polar during the evaporation, molecules start to cluster together, the loose aggregates eventually giving rise to LNPs, where the polar functional groups (hydroxyl and carboxyl) are concentrated at their surface and the less polar (side chain) moieties in the middle.[34] Apparently, as long as the lignins are fully dissolved at the beginning, the initial acetone content of the solvent does not affect LNP formation: the acetone-to-water ratio had no effect on the size of the SCNPs produced by Method 1a when the initial volume and lignin concentration were kept constant (Figure 2). The SNPs prepared similarly using 80% acetone were larger in size than the SCNPs, possibly because SC had 25% more polar (OH and COOH) functional groups than S (Table 1) and thus less hydrophobic moieties to pack inside the LNPs.

Method 1a was scaled up in volume (Method 1b) to produce enough LNPs for sunscreen formulations. The mean size of the SNPs and SCNPs increased (Figure 3) compared to those obtained from the lower-volume experiments. The rate of acetone removal from the two different volumes of solution (100 and 500 mL) may have been different and affected LNP formation. As was the case with Method 1a, a higher polar functional group content (Table 1) was associated with a reduction in LNP size. The size of LNPs prepared from the four lignins was highly correlated with their content of hydroxyl and carboxyl groups (the correlation coefficient r^2 was 0.96 for a linear trend line).

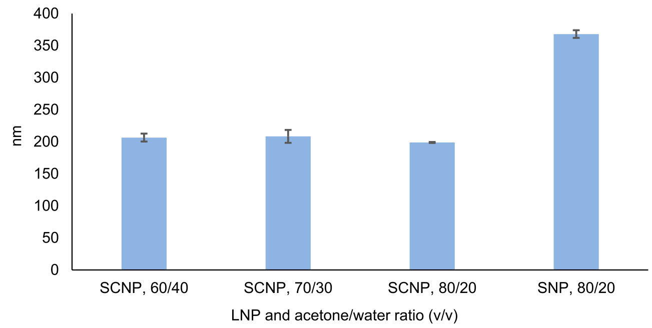

Figure 2. Unimodal mean size of LNPs prepared at different acetone/water ratios (Method 1a). Error bars show standard deviation of triplicate size determinations. NP = nanoparticles.

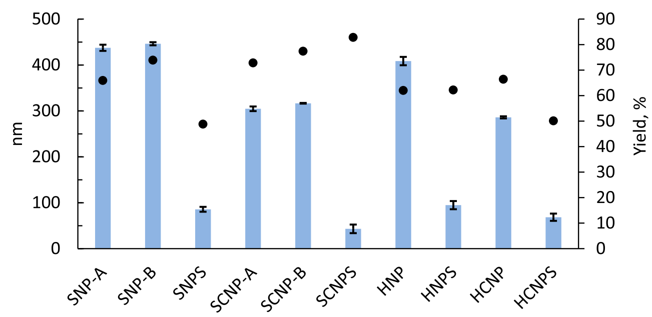

Figure 3. Unimodal mean size (columns) and yield (circles) of LNPs prepared for sunscreens (Methods 1b and 2). NP = nanoparticles; NPS = smaller nanoparticles. Two batches each of SNPs (SNP-A and SNP-B) and SCNPs (SCNP-A and SCNP–B) were prepared. Error bars show a standard deviation of triplicate size determinations.

Batches of smaller LNPs were prepared (Method 2) based on the hypothesis that adding small amounts of dissolved lignin into a large volume of vigorously stirred water would force the lignin to assemble into smaller LNPs. The hypothesis was verified as the LNPs produced were on average 80% smaller than the LNPs prepared according to Method 1b. The smallest were SCNPS and HCNPS, prepared from the CatLignins.

UV Transmittance and SPF of Lignin Sunscreens (Non-NP Lignin). The UV transmittance of the Nivea-lignin sunscreens (hereafter designated as N-"lignin type") increased slowly and linearly from 290 to 400 nm (Figure 4). The overall UV transmittance was reduced and the UVB–SPF[42,43] increased as a function of increasing phenolic hydroxyl content of the lignins (Table 1), although there was little difference between N–HC and N–SC. N–S and N–H showed UVB–SPFs comparable to those reported for other lignin sunscreens containing 10% lignin in the non-NP form.[21,25,27,28] Although the transmittance of the commercial sunscreen (N15) was slightly lower in the UVB region than that of N–HC and N–SC, it climbed sharply from 375 to 400 nm with both N–HC and N–SC clearly outperforming it in this long-wave UVA region. Its measured UVB–SPF (15.7) was consistent with its SPF15 rating. The performance of sunscreens with catechol-enriched CatLignins (N–SC and N–HC) was superior to that

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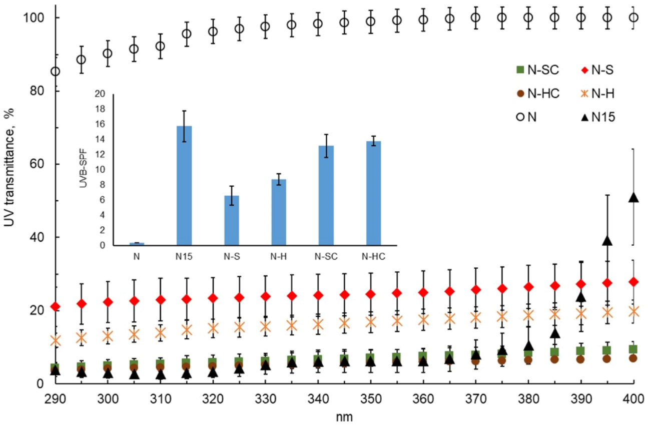

Figure 4. UVA−UVB transmittance and UVB−SPF (inset) of non-NP lignin sunscreens, their Nivea base cream (N), and a commercial SPF15 sunscreen (N15). Error bars indicate standard deviation of five measurements.

of sunscreens formulated with regular kraft lignins (N−S and N−H). The difference between N−SC and N−HC was small and possibly not statistically significant. A comparison of the sunscreen test results (Figure 4) and the phenolic hydroxyl and methoxyl auxochrome contents of the lignins (Table 1) indicates that phenolic hydroxyls were more strongly associated with lignin sunscreen performance than methoxyls. The importance of phenolic hydroxyls was also demonstrated by Qian et al.[19]—blocking them by acetylation halved the lignin sunscreen SPF values. Because of the syringyl lignin units found in hardwood lignin, the methoxyl contents are higher for H and HC than for S and SC, respectively.

Because of the potentially complex interactions between chromophores and auxochromes, the occurrence of charge-transfer complexes, and differences in particle size, it is not possible to attribute the observed different UV absorptivities of lignins only to any particular lignin characteristics. However, certain earlier discussed changes in lignin structure during production of SC and HC that increase their content of phenolic hydroxyl auxochromes are likely to play a significant role in their higher UV absorptivity compared to S and H. While new phenolic hydroxyls are formed via demethylation and cleavage of alkyl−aryl ether bonds (between lignin side chains and aromatic units), the content of phenolic hydroxyls and aromatic ring chromophores is further increased by elimination of methyl, methoxyl, and aliphatic hydroxyls groups.

The sunscreen base cream (N) had very high UV transmittance and an UVB−SPF of 0.4 based on the UV area of 290−320 nm.[42,43] The same cream was also previously[25,28] found to have a low SPF of 1.0−1.1 based on the entire UVA−UVB wavelength area of 290−400 nm. The minor amount of UVB absorptivity of N may be mostly due to benzyl salicylate, a weak UVB absorber listed among the ingredients.

UV Transmittance and UVB−SPF of Sunscreens Containing UV-Irradiated Lignin. Other researchers found the UV absorptivity of commercial sunscreens with added lignin to increase as they were exposed to UV radiation.[19,28] Their results suggested that it might be possible to exploit UV irradiation to increase the UV absorptivity of lignins prior to their incorporation into sunscreens. Indeed, the shortest UV irradiation time of 0.5 h improved the UVB−SPF and lowered the UV transmittance of N−SC, while irradiation times longer than 1 h were detrimental to sunscreen performance (Figure 5). Although the differences between

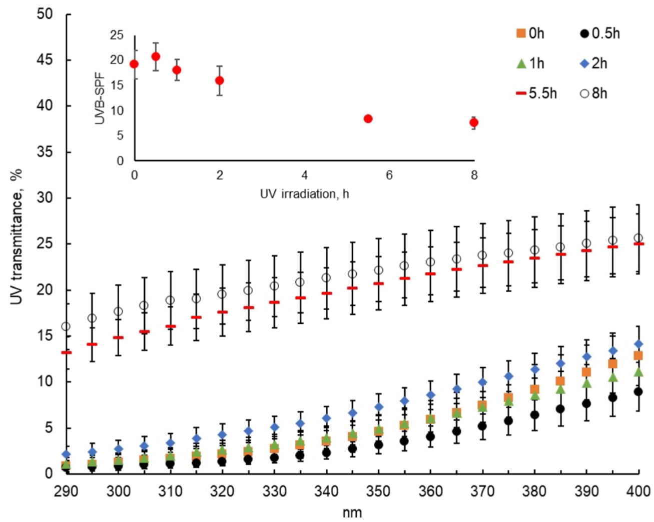

Figure 5. UVA−UVB transmittance as a function of UV irradiation time and UVB−SPF (inset) of N−SC (dissolved in ethanol/water during irradiation). Error bars indicate standard deviation of five measurements.

the 0 and 2 h samples were not statistically significant, the results show that SC could be subjected to a considerable amount of UV radiation before the performance of N−SC deteriorated significantly, thus showing good photostability.

The Fourier transform infrared−attenuated total reflection (FTIR−ATR) spectra of UV-irradiated lignins (Figure 6)

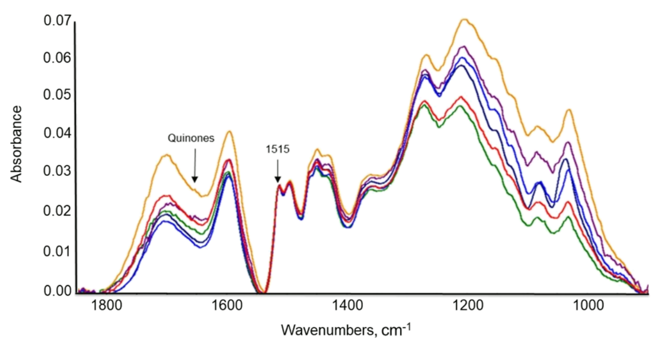

Figure 6. Partial FTIR−ATR spectra of SC irradiated with UV for 0−8 h and normalized to the aromatic band at 1515 cm^{-1}. The absorbance at ca. 1660 cm^{-1}, assigned to quinones, increases nonlinearly as a function of irradiation time: 0, 0.5, 1, 2, 5.5, and 8 h.

showed evidence of chromophore formation via partial oxidative degradation or aromatic units. The intensity of the bands assigned to carbonyl and carboxyl groups (ca. 1600−1750 cm^{-1}) relative to the lignin aromatic band at ca. 1515 cm^{-1} (to which these spectra are normalized) increased as a function of irradiation time. The oxidized structures are likely to contain chromophoric conjugated carbonyl groups such as quinones and coniferyl aldehyde at ca. 1660 cm^{-1} that increase the UV absorptivity of lignin.[18,44] Under UV light, *ortho*-quinonoids may be formed via phenoxy radicals[45] from catechol units, occurring in SC or formed via demethylation of its guaiacyl or syringyl units during the irradiation, while

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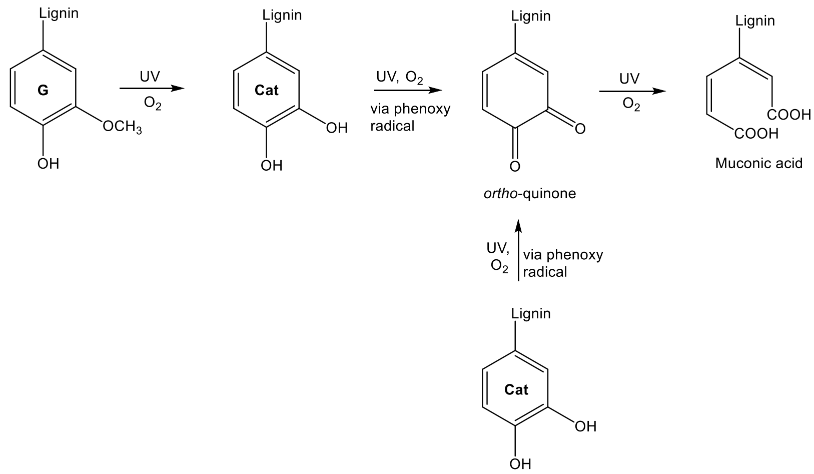

Figure 7. Possible route for the formation of quinones (via phenoxy radicals) from guaiacyl (G) and catechol (cat) units in SC under UV radiation. UV radiation of quinones can produce muconic acids.

excessive UV radiation may afford muconic acids (Figure 7). However, only levels of UV radiation that did not significantly alter the IR spectrum were beneficial—in the carbonyl region of the IR spectra, there is only a minor difference between nonirradiated SC and the SC irradiated for 0.5 h that gave the best sunscreen performance.

UV Transmittance and UVB−SPF of LNP Sunscreens. A comparison of the sunscreens containing LNPs (Figure 8) or non-NP lignins (Figure 4) shows that the particle size was an important predictor of sunscreen performance. In general, sunscreen performance improved in the order non-NP sunscreens < LNP sunscreens (LNPs: 280−450 nm) < small LNP sunscreens (LNPs: 43−95 nm) in terms of UVB−SPF and UV transmittance. The only exceptions were in the area 385−400 nm for N−SC and N−SCNP where N−SC performed better and from 365 nm to 400 nm where N−SNP showed lower UV transmittance than N−SNPS. The results agree with those of Qian et al.[19] who prepared sunscreens with lignin particles in three size ranges (mean size <50 nm, 210 nm, and ca. 2.5 μm). One reason for this behavior might be that the relative amounts of chromophores and auxochromes at the particle surface varied with their size.

Regarding the effect of lignin demethylation, the results on LNP sunscreens track those obtained for the non-NP sunscreens. The phenolic hydroxyl content of the lignins used for preparing the LNPs was thus positively correlated with sunscreen performance, although the difference in UVB−SPF between N−SCNP and N−HCNP was not statistically significant. It is noteworthy that N−SCNP and N−HNP performed similarly, which could be due to the much higher methoxyl content of H compensating for its lower phenolic hydroxyl content compared to SC. The best three sunscreens based on UV absorptivity across the entire UVA−UVB area were CatLignin based: N−SCNPS, N−HCNP, and N−HCNPS.

UV Treatment of SCNPs. As for the UV-irradiated (2−8 h) SCNPs, the oxidative changes at the particle surfaces are more extensive (Figure 9) than those observed for the above-discussed dissolved lignins (Figure 7). At a longer irradiation

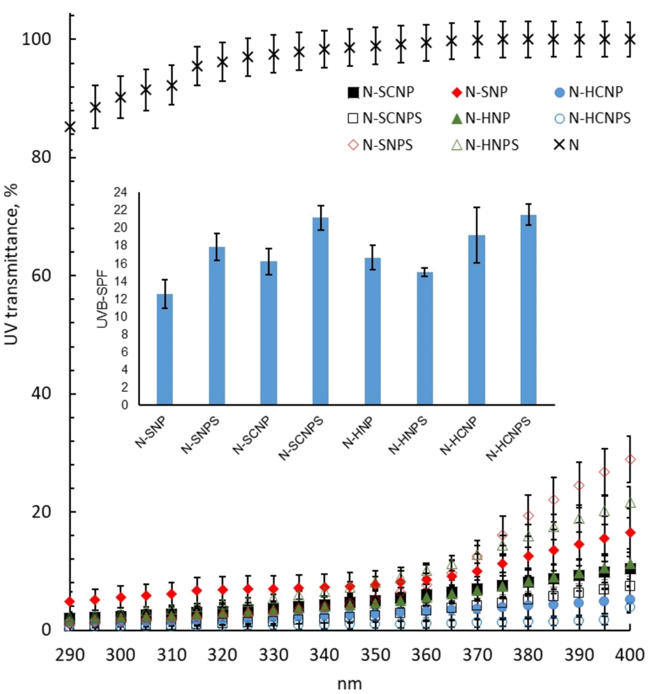

Figure 8. UVA−UVB transmittance and UVB−SPF (inset) of sunscreens prepared with larger (filled markers) and corresponding smaller (empty markers) LNPs. N = Nivea base cream; NP = nanoparticles; and NPS = smaller nanoparticles.

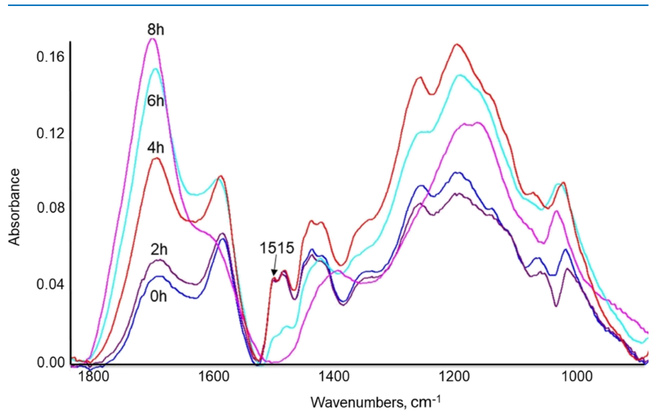

Figure 9. Partial FTIR−ATR spectra of SCNPs exposed to UV radiation for 0−8 h. The 0−4 h spectra are normalized to the aromatic band at 1515 cm^{-1}.

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time of 6−8 h, most of the phenolic aromatic units have been broken down: the aromatic band at 1515 cm^{-1} is barely visible at 6 h and has disappeared entirely at 8 h with a large carbonyl/carboxyl band dominating the spectrum. The size of the NPs remained unchanged at ca. 200 nm with a very narrow size distribution for up to 6 h, but at 8 h, they had been largely broken down, with most particles only about 5 nm in size but also larger particles remaining. To produce UV-treated SCNPs for sunscreen tests, 4 h was chosen as the irradiation time to induce significant formation of UVA-absorbing structures while preserving the NP structure and a large proportion of the phenolic units. In this case, the UV irradiation had been excessive as the UVB−SPF of the sunscreen was lower, and its UVA−UVB transmittance was higher compared to N−SCNP.

■ CONCLUSIONS

1. The UVB−SPFs of sunscreens formulated with 10% regular kraft lignin as the UV actives reached values of up to 8.7, well below the value of 15 considered adequate for a daily use sunscreen.
2. Compared to sunscreens containing regular kraft lignins, sunscreen performance in terms of UVB−SPF and overall UVA−UVB transmittance was improved by
    - Using demethylated and otherwise modified kraft lignins (CatLignins)
    - Exposing lignin to a limited amount of UV radiation prior to its application in sunscreens
    - Converting lignins to nanoparticles (LNPs)
    - Reducing the LNP size
3. The best lignin sunscreens with a lignin content of 10% showed UVB−SPFs of >21 and low UVA transmittance. This performance was significantly better than any previously published results on sunscreens with lignin as the only UV active.
4. Sunscreens formulated with 10% lignin or LNPs could be applied as UV absorbers for dark-tinted SPF cosmetics that are environmentally benign and safer to humans than current chemical sunscreens.

■ EXPERIMENTAL SECTION

Materials. The softwood (*Pinus sylvestris*/*Picea abies*) and hardwood (*Eucalyptus* sp.) kraft lignins used in this study were industrial lignins precipitated using carbon dioxide from the black liquor of pulp mills that produce paper-grade kraft pulp. The CatLignins from the same two wood species were produced at a laboratory scale from industrial black liquors using a patented CatLignin method[36] based on heat treatment of black liquor, followed by conventional precipitation using carbon dioxide and acidic washing. The hardwood kraft lignin and the black liquor from which the hardwood CatLignin was prepared were supplied by Suzano Pulp and Paper, Brazil. The samples with their designations are listed in Table 2. The base cream (N) for lignin sunscreens was Nivea Refreshingly Soft Moisturizing Cream (200 mL, Hamburg, Germany).[19] A commercial SPF 15 sunscreen (N15), Nivea Sun Protect & Moisture Sun Lotion 15 (200 mL, Hamburg, Germany), was included as a reference. Its ingredients are listed as follows: aqua, homosalate, octocrylene, glycerin, C12-15 alkyl benzoate, alcohol denat., ethylhexyl salicylate, butyl methoxydibenzoylmethane, glyceryl stearate citrate, panthenol, hydrogenated coco-glycerides, myristyl myristate, tocopheryl acetate, cellulose gum, tetrasodium iminodisuccinate, VP/hexadecane copolymer, xanthan gum, sodium acrylates/C10-30 alkyl acrylate crosspolymer, cetyl alcohol, stearyl alcohol, silica dimethyl silylate, trisodium ethylenediaminetetraacetic acid, hydroxyacetophenone, ethylhexylglycerin, linalool, limonene, benzyl alcohol, alpha-isomethyl ionone, citronellol, coumarin, and parfum.

Characterization of Lignins. The hydroxyl and carboxyl contents of lignins were determined from freshly phosphitylated lignins by ^{31}P NMR on a Bruker 500 MHz NMR spectrometer at room temperature using a previously published method.[46] Quantitative ^{13}C NMR spectra were recorded on a Bruker AVANCE III 500 NMR spectrometer with a magnetic flux density of 11.7 T and equipped with a 5 mm BB(F)O double-resonance probe head. Lignin (180 mg) was dissolved into 1 mL of DMSO-d_6, with 6 mg/mL of $Cr(acac)^3$ added as a relaxation agent. All spectra were recorded with 20 000 scans using a 90° flip angle, an rf-pulse, and a 2.0 s delay between successive scans. The pulse program did not include NOE enhancement. The spectral width for the experiments was 280 ppm, and the signal acquisition time was 0.9 s. The spectra were recorded at 22 °C and processed with TopSpin 3.6 software. A cubic spline baseline correction was performed prior to the signal integrations. Methoxyl groups were determined using the method of Baker[40] with some modifications.

For the molar mass measurements, the samples were dissolved in 0.1 M NaOH and filtered (0.45 μm). The molar mass measurements were performed with size exclusion chromatography using 0.1 M NaOH eluent (pH 13, 0.5 mL/min, T = 25 °C) and PSS MCX 1000 & 100,000 Å columns. The elution curves were detected using a Waters 2998 Photodiode Array detector at 280 nm. The weight (M_w)- and number (M_n)-average molar masses were calculated against polystyrene sulfonate standards (eight standards with a range of 3420−148,500 g/mol) using Waters Empower 3 (Milford, MA, USA) software.

Synthesis of LNPs. *Method 1a.* LNPs were synthesized by dissolving lignin (100 mg on oven-dry basis) in aqueous acetone (100 mL, acetone content 60−80% on volume basis as indicated), evaporating the bulk of the acetone off in a rotary evaporator, and then leaving the residual acetone to evaporate overnight under magnetic stirring.

Method 1b. The same as Method 1a except that 500 mg of o.d. lignin was dissolved in 500 mL of 80% acetone.

Method 2. To produce smaller LNPs, lignin (500 mg, o.d. basis) was dissolved in 50 mL of 60% acetone, and the solution was then added dropwise over 40 min into water (450 mL) and stirred magnetically at 1000 rpm. The resulting dispersion was stirred overnight to allow the acetone to evaporate.

After each method of preparation, the acetone-free LNP dispersions were vacuum-filtered (Whatman GF/F glass fiber filter, pore size 0.7 μm) to remove any micrometer-scale particles and then either used as such to determine their particle size distribution or freeze-dried for other analyses or sunscreen preparation.

Particle Size Determination. The unimodal size distributions of LNPs were determined from their original water dispersions by dynamic light scattering on a BeckmanCoulter N5 particle size analyzer that has a measuring range of 3−3000 nm.

UV Treatment of Lignin/LNPs. *Lignin.* For UV treatment, SC lignin (10 g, o.d. basis) was dissolved in 100 mL of ethanol/water (80/20, v/v) and then vacuum-filtered (What-

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man GF/F glass fiber filter, pore size 0.7 μm) to remove any undissolved material. The dissolved lignin was placed in a beaker and stirred magnetically at 100 rpm under ambient conditions. After that, a frame prepared from a cardboard box, with a hole cut in the middle for a UV lamp, was placed over the beaker, and the solution was irradiated for 0.5−8 h from a distance of approximately 15 cm from the lamp (UVAHAND 250, unfiltered) to the dispersion surface. The samples were withdrawn after designated times, and the solvent that had evaporated was replenished every 60 min. After treatment, the ethanol was evaporated off on a rotary evaporator, and the lignin was then freeze-dried for other analytical work.

LNPs. SCNPs for UV treatment were prepared and their dispersion filtered according to method 1a above. The UV treatment of SCNPs was carried out as described for the dissolved lignin except that they were dispersed in their original water medium. During the treatment, the samples were withdrawn at designated times to check for any changes in particle size or freeze-dried for other work.

FTIR−ATR Analysis of Lignins and LNPs. Freeze-dried UV-irradiated SC and SCNPs were analyzed by FTIR−ATR (32 scans) at room temperature on a Thermo Scientific Nicolet iS50 FT-IR spectrometer.

Preparation of Lignin Sunscreens. The base cream N (2.00 g) was blended with dry lignin, LNPs, in a 10 mL glass beaker so that the final lignin content in the sunscreen formulation was 10%. Any (carbohydrate) lignin impurities were not taken into consideration. The beaker was covered with a parafilm, and the sunscreens were homogenized by magnetic stirring at ambient temperature (24 h, 1000 rpm).

Measurement of UV Transmittance and SPF of Sunscreens. UV transmittance of sunscreens was measured in vitro by using a gloved (neoprene) finger to apply a layer of sunscreen (the standard dose for in vivo SPF measurements, 2.0 mg/cm^2) as evenly as possible onto a 1 × 2 cm strip of 3M Transpore tape (used to simulate skin)[47] attached to a quartz plate. A quartz plate and tape without sunscreen were used to zero the UV spectrometer (PerkinElmer Lambda900) over the UVA−UVB region 290−400 nm. The UV transmittance of the sample was then recorded over the same region. For each sunscreen, five replicate samples were prepared and the results averaged. UVB−SPF values of sunscreens were calculated based on the UVB region using the Mansur eq 1[33]

$$\mathrm{SPF} = \mathrm{CF} \times \sum_{290}^{320} \mathrm{EE}(\lambda) \times I(\lambda) \times \mathrm{Abs}(\lambda) \quad (1)$$

where CF stands for correction factor (=10), EE (erythemal effect spectrum) and I (solar intensity spectrum) are constants determined by Sayre et al.,[43] and abs is the absorbance.

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Notes

The authors declare no competing financial interest.

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