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Development of a cashew nut shell liquid (CNSL)-based polymer for antibacterial activity

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ABSTRACT: A biobased polymer derived from cashew nut shell liquid (CNSL) as a renewable resource was investigated for use as an antibacterial material. CNSL is a mixture of aromatics containing cardanol as the main component and cardol and 2-methylcardol as minor components. CNSL composition analyses showed that the minor components (i.e., cardol and 2-methylcardol) in CNSL had higher contents of unsaturated structures than cardanol. These higher unsaturated contents promoted the thermal polymerization in the preparation of an epoxy CNSL prepolymer (ECNP). The biobased polymer film was fabricated by the reaction of amine compounds and ECNP without any organic solvent. The ECNP film took less than 2.0 h to reach a hardened dry condition at room temperature because of the crosslinking reaction between epoxy and amine groups. The antibacterial activities of the biobased polymer against *Escherichia coli* and *Staphylococcus aureus* were evaluated. CNSL showed antibacterial activity against *S. aureus*, whereas epoxy CNSL and ECNP alone showed no significant antibacterial activity against *E. coli* or *S. aureus*. This indicated that the antibacterial activity was based on the phenolic and catechol hydroxyl groups of CNSL. In addition, a biobased polymer film derived from CNSL and diamine showed antibacterial activity against both *E. coli* and *S. aureus*, even with alcohol conditioning. This suggested that the antibacterial activity was certainly fixed in the structure of the ECNP-based polymers after the standard antisepsis treatment in medical facilities. Therefore, this biobased polymer could be useful in antibacterial materials as a coating and resin for health care applications. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42725.

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INTRODUCTION

Recently, biobased polymer materials derived from renewable resources, such as plant and nonfood materials, have received much attention because of the increasing price of petrochemical products and growing environmental concerns. The use of natural renewable resources would be one effective solution for this problem and would be beneficial in the development of novel environmentally friendly materials.

CNSL is a plant-based natural phenolic resource.[1] CNSL contains chemicals such as the linear unsaturated phenolic derivatives anacardic acid, cardanol, 2-methylcardol, and cardol.[1–3] Until this point, CNSL-based functional resins with formaldehyde have been widely investigated for use in industrial applications.[4–11] These biobased polymer materials are expected to be used as adhesives, coatings, and composites because of their excellent adhesion properties and thermal resistance. However, these materials have been fabricated with toxic chemicals, such as formaldehyde and isocyanate. From the aspect of green chemistry, more environmentally friendly processes without such toxic chemicals have been expected in recent years.[12–16] Recently, we prepared a formaldehyde-free, solvent-free epoxy resin from an epoxy cardanol prepolymer and amine compounds.[17] In that previous work, we studied with a purified cardanol extracted from CNSL to clarify the reaction mechanism of the prepolymer and its crosslinking reaction with amine compounds. A simpler and more effective process for fabricating biobased polymers from an industrial point of view is to use CNSL directly without any purification process. We expected that the reactivity of CNSL would be higher than that of pure cardanol because cardol and 2-methylcardol in CNSL contain two hydroxyl groups, which are modification groups.

Escherichia coli (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) have emerged as major causes of community- and

Table I. Composition of CNSL

Compound	Average molecular weight (g/mol)[a]	Weight ratio (%)	Content ratio (%)[a]			
			Saturate	Monoene	Diene	Triene
Cardanol	300.19	91.92	2.71	39.21	23.04	35.04
Cardol	329.43	6.73	0.23	9.97	29.61	60.19
2-Methyl cardol	315.00	1.35	1.79	19.22	27.72	51.27
CNSL	302.09	100	2.49	36.36	23.68	37.47

[a] Determined by gas chromatography/mass spectrometry.

hospital-acquired infections at medical treatment and elder-care facilities. For example, there are nosocomial infections, such as suppurative arthritis and food poisoning. Actively antibacterial materials and products and alcohol antisepsis in these facilities have an important role in eliminating this issue. The antibacterial, antioxidant, and anticarcinogenic properties of phenolic and catechol compounds have been reported.[18,19] Recently, the antibacterial activity of versatile substrate-coated biocidal materials based on catechol chemistry was reported.[20] On the other hand, CNSL also has biological properties.[21–23] Therefore, a CNSL-based polymer material can be expected to be an antibacterially active material.

Herein, we describe the synthesis of an environmentally friendly CNSL-based polymer film for use as an antibacterial material. This CNSL-based film was evaluated in terms of its film properties, including its drying and physical characteristics and antibacterial activity against *E. coli* and *S. aureus*.

EXPERIMENTAL

Chemicals

Distilled CNSL was kindly provided by Cashew Co., Ltd. (Saitama, Japan) and was used without further purification. The composition of the CNSL was characterized by silica-gel column chromatography (FL60D, Fuji Silysia Chemical, Ltd., Aichi, Japan) with a mixed solvent of *n*-hexane and ethyl acetate, as described in our previous work.[17] The composition of CNSL used in this study is summarized in Table I. The CNSL was mixture of cardanol, cardol, and 2-methylcardol (Figure 1). These chemical structures were analyzed by NMR and Fourier transform infrared (FTIR) spectroscopy, as shown in Figure 1. As shown by these results, this CNSL contained 91.92% cardanol, 1.35% 2-methylcardol, and 6.73% cardol; this was very consistent with our previous results.[17] Interestingly, the triene and diene structures in the cardol and 2-methylcardol, which represent the unsaturated degree, were higher than those in the cardanol, the main component of CNSL. These minor components promoted the thermal polymerization more because of the higher ratio of unsaturated structures. Epichlorohydrin (>99% purity), potassium hydroxide (>85% purity), and dimethyl sulfoxide (>98% purity) were purchased from Nacalai Tesque, Inc. (Japan). Diethylenetriamine (DETA; >98% purity) was purchased from the Tokyo Chemical Industry Co., Ltd. (Japan). These chemicals were used as received.

Synthesis

Epoxy CNSL (ECN). Amounts of 10.0 g (0.033 mol) of CNSL, 9.25 g (3.0 equiv) of epichlorohydrin, 4.40 g (2.0 equiv) of potassium hydroxide, and 30.0 mL of dimethyl sulfoxide solution were mixed in a 200-mL flask with stirring at room temperature for 24 h.[24,25] ECN was obtained after dehydration with magnesium sulfate and vacuum drying to give a transparent solution.

FTIR spectroscopy (NaCl, cm^{-1}): 3025, 998, 909 (C═C stretching); 2923, 2851, 1452, 723 (C—C stretching, CH_2 bending); 3005, 910, 865 (epoxy); 1602, 1585, 1481 (benzene C═C stretching); 1269, 1043 (C—O—C stretching); 778, 694 (1,3-substituted benzene).

Epoxy CNSL Prepolymer (ECNP). An amount of 5.0 g (0.017 mol) of ECN was placed in a 100-mL flask and subjected to an oil bath with stirring under the given synthesis conditions on the basis of the previous synthesis conditions of epoxy cardanol.[17] We investigated two experimental factors, the heating temperature (120, 140, and 160°C) and heating time (0, 4, 8, 12, 18, 22, and 24 h).

FTIR spectroscopy (NaCl, cm^{-1}): 3010, 990, 910 (C═C stretching); 2993, 919, 851 (C═C stretching); 2920, 2855, 1444, 729 (C—C stretching, CH_2 bending); 1726 (C═O); 1609, 1589, 1480 (benzene C═C stretching); 1263, 1045 (C—O—C stretching); 772, 691 (1,3-substituted benzene).

Film Preparation. The CNSL-based epoxy films were prepared from ECNP and DETA amine compound as control. The mixture of DETA and ECNP was degassed with a vacuum pump to remove the dissolved air in the mixture. The degassed mixture was uniformly coated onto a glass plate at 23 ± 1°C by the applicator. The amounts of amine compound were 5, 10, and 15 wt % to optimize the amount of amine compound.

Characterization

Structural Analysis. ^{1}H-NMR and ^{13}C-NMR spectroscopy was conducted on a JNM-ECA500 spectrometer (JEOL, Ltd., Tokyo, Japan) with a deuterated chloroform solvent with chemical shifts referenced from tetramethylsilane. FTIR spectroscopy was performed on an FT/IR-4100 (Jasco Co., Tokyo, Japan) spectrometer with an NaCl plate at 23 ± 1°C. Each spectrum was

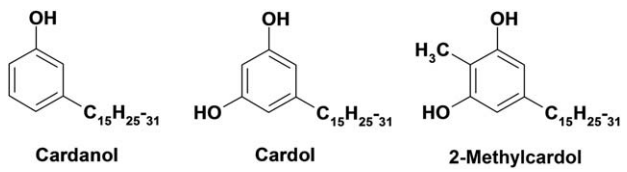

Figure 1. Chemical structure of the CNSL compound used in this study.

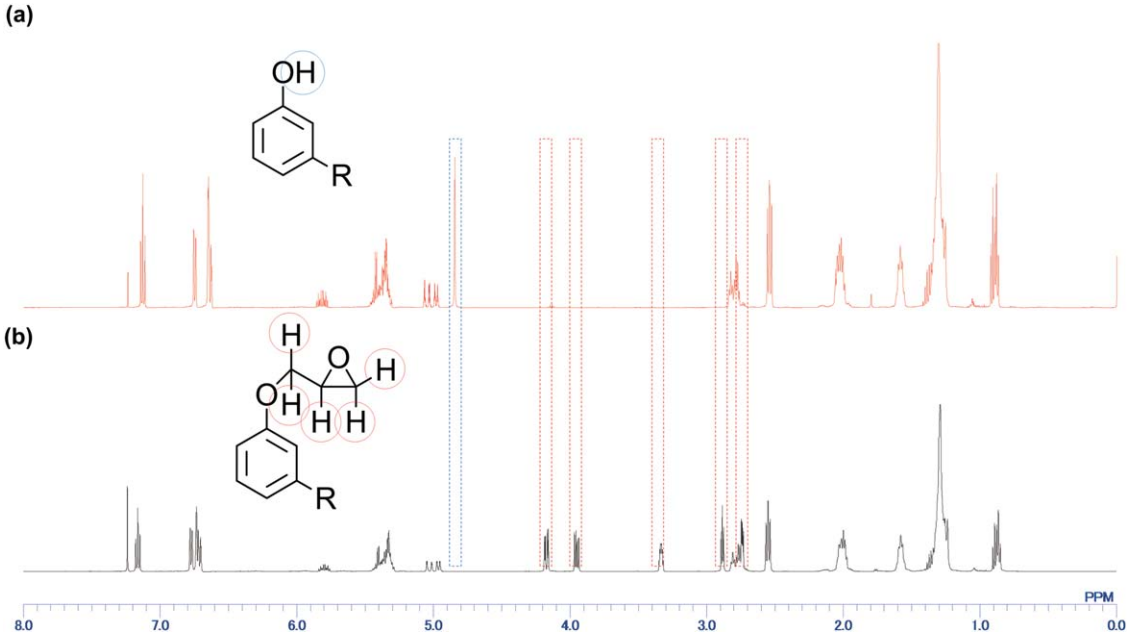

Figure 2. ^{1}H-NMR spectra of (a) CNSL and (b) ECN. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

averaged over 64 scans at a resolution of 2 cm^{-1}. The molecular weight of ECNP was determined at 40°C by aqueous phase gel permeation chromatography (TSK-gel columns α-3000, α-4000, and α-M, ϕ 7.8 mm × 300 mm × 3, Tosoh Co., Ltd., Japan) with dimethylformamide as an eluent with 0.01 mol of LiBr) on a high-performance liquid chromatography system with a refractive-index detector (RI-8012) with polystyrene standards. The elution rate was 0.8 mL/min. The viscosity of the ECNP was determined with a Brookfield programmable DV-II+ viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA). The spindles were CPE-40 and CPE-51, the rotation speed was 5–20 rpm, and the measurement sample was 0.5 mL. This measurement was conducted at room temperature.

Film Characterization. The drying process of the biobased epoxy film at 23 ± 1°C was divided into three stages: dust-free drying, touch-free drying, and hardened drying. Each stage was measured with an automatic drying time recorder (an RC autorecorder of painting drying time, TaiYu Co., Ltd., Osaka, Japan) at 23 ± 1°C and 60% relative humidity. The hardness of pencil lead is determined with designations consisting of letters and numbers according to the current national standard GB/T6739-1996. H and B designate how hard or soft the tested films were, respectively. A higher number with H or B expressed the hardness or softness of the tested films, respectively. F and HB indicate medium hardness. However, F is slightly harder than HB. In this study, pencil lead hardness was determined with a C-221 (Yoshimitsu Seiki, Tokyo, Japan) at 23 ± 1°C. The gel content of the epoxy film was determined by immersion in acetone at 23 ± 1°C for 24 h. The nonsoluble parts were filtered and dried in an oven for 1 h at 50°C, cooled, and subsequently examined at room temperature to remove residual solvent before weighing. The gel content was calculated with the following equation:

$$\text{Gel content } (\%) = \frac{M_1}{M_0} \times 100 \quad (1)$$

where M_1 and M_0 are the weight of the insoluble fraction and the original weight of the completely dry polymer film, respectively. A rigid-body pendulum physical property testing (RPT) instrument (RPT-3000W, A&D, Ltd., Tokyo, Japan) was used to determine the viscoelasticity of samples. The glass-transition temperature (T_g) of the samples was also analyzed by this RPT. The heat of the RPT oven was programmed to rise at a rate of 4.3°C/min from −35 to 200°C.

Antibacterial Activity. The bacterial strains *E. coli* ATCC25922 and *S. aureus* ATCC29213 were used for the antibacterial assays. In assays with the liquid precursor compounds, 1 × 10^5 colony-forming units (cfu) of the bacterial strain were inoculated into 1 mL of Mueller–Hinton broth (Becton Dickinson and Co.,

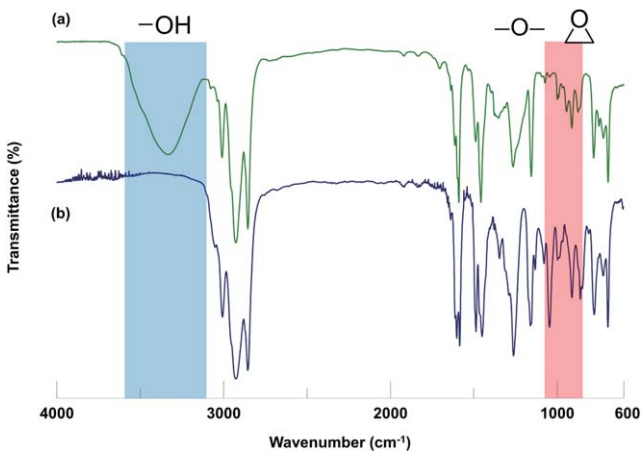

Figure 3. FTIR spectra of (a) CNSL and (b) ECN. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table II. Effect of Heating Time on Synthesis of CNSL Prepolymer at Each Heating Temperature

			Content ratio (%)[a]			Molecular weight[b]				
Entry	Heating temperature (°C)	Time (h)	Monomer	Oligomer	Polymer	M_n	M_w	M_w/M_n	Unsaturation	Viscosity (mPas)
0[c]	-	0	100	0	0	390	420	1.1	1.64	39
1	-	0	86.17	13.76	0.07	440	620	1.4	1.78	28.0
2	120	4	75.15	23.11	1.74	420	1160	2.8	1.45	96.8
3	120	8	63.04	33.30	3.66	530	2110	4.0	1.12	242.2
4	120	12	58.40	36.56	4.99	530	2650	5.0	0.99	514.5
5	120	18	59.78	34.16	6.07	560	3160	5.7	0.97	444.7
6	120	22	58.76	35.65	5.59	560	3250	5.8	1.01	565.1
7	120	24	53.16	36.88	9.95	550	8010	14.6	0.98	732.5
8	140	4	79.65	19.22	1.13	480	920	1.9	1.54	52.8
9	140	8	72.33	25.34	2.33	490	1650	3.0	1.33	85.3
10	140	12	64.90	32.03	3.07	530	1900	3.6	1.22	204.0
11	140	18	56.24	37.54	3.23	570	3540	6.2	1.04	622.6
12	140	22	52.43	38.48	9.09	610	5980	9.9	0.90	898.2
13	140	24	48.20	38.57	13.23	690	11,040	16.0	0.74	2113
14[d]	160	28	—	—	—	—	—	—	—	—
15	160	4	66.70	28.08	5.23	500	2650	5.3	1.42	123.8
16	160	8	59.09	33.69	7.22	570	4470	7.8	1.27	353.2
17	160	12	45.68	36.28	18.03	680	38,300	56.7	0.79	7111
18[d]	160	18	—	—	—	—	—	—	—	—

[a] Oligomer: dimer ≤ molecular weight < 10,000 g/mol; polymer: molecular weight ≥ 10,000 g/mol.
[b] M_n: number-average molecular weight, M_w: weight-average molecular weight, M_w/M_n: molecular weight distribution (polydispersity).
[c] Previous epoxy cardanol.[17]
[d] Polymer gelation.

Sparks, MD) supplemented with 1 mg/mL of the test compound. After 20 h of incubation at 35°C under aerobic conditions, the viable cells in the culture were counted by the modified Miles and Misra method.[26] In the assay with the polymer films, the bacterial cells were suspended in a 500-fold diluted Tripticase Soy broth (Becton Dickinson) to yield 1×10^6 cfu/mL for *E. coli* and 1×10^8 cfu/mL for *S. aureus*, respectively. An aliquot of 100 μL of the bacterial suspension was applied to the autoclave-sterilized polymer film coated on a glass plate (70×70 mm^2) and incubated in a box with 100% humidity at 35°C for 20 h. For the control, the same volume of bacterial suspension was placed on a noncoated glass plate. After incubation, the bacterial cells on the plate were washed out with 10 mL of saline, and the number of viable cells in the wash was determined with the same method described previously. The chemical stability of 80% ethanol was evaluated to ensure the retention of the antibacterial activity. The film was immersed into an 80% ethanol solution for 5 min. Eighty percent ethanol is widely used in many medial facilities as standard disinfectant conditions.

RESULTS AND DISCUSSION

Synthesis of ECN

The conversion of the phenolic hydroxyl group to the epoxy group proceeded completely, with a conversion from the hydroxyl group to the epoxy group of over 99% on the basis of ^{1}H-NMR analysis. Figures 2 and 3 present the ^{1}H-NMR and FTIR spectra of ECN, respectively. The peaks of the phenolic hydroxyl group were observed at 4.80 ppm in the ^{1}H-NMR spectrum and at 3346 and 1265 cm^{-1} in the FTIR spectrum. On the other hand, new peaks was observed at 2.75, 2.9, 3.35, 3.95, and 4.15 ppm in the ^{1}H-NMR spectrum and at 1261 and 1046 cm^{-1}, which corresponded to ether, and 911 cm^{-1}, which corresponded to the epoxy group.

Synthesis of ECNP

The molecular weight, degree of unsaturation, and viscosity of the ECNP synthesized in air under the given conditions are summarized in Table II. ECN contained 86.17% as monomers, 13.76% as oligomers, and 0.07% as polymers; this indicated that around 14% ECN reacted and developed higher molecular weights. Previously, we reported that epoxy cardanol was 100% monomer content.[17] This was because the oligomers and polymers in ECN probably came from the original CNSL raw material. The degree of unsaturation of ECN was calculated to be 1.78 on the basis of NMR, and the viscosity was 28 mPa s; these values were similar to values previously reported for epoxy cardanol.[17] The unsaturation degree of ECN was about 8.5% higher than that of epoxy cardanol because of the higher contents of unsaturated structures (i.e., diene and triene) in the ECN, as shown in Table I. Figure 4 presents the ratio of the monomer, oligomer, and polymer in the ECNP as determined

by gel permeation chromatography as a function of the heating time at each temperature. The monomer content normally decreased, whereas the oligomer and polymer contents increased with increasing heating time and heating temperature. The polymer content and viscosity increased significantly after 22 h at 120°C, 18 h at 140°C, and 8 h at 160°C; this suggested a typical autoxidation polymerization. The monomer and oligomer contents in CNSL were lower than that in the previous cardanol, whereas the polymer content in CNSL was higher than that in the cardanol. This result indicates that the high contents of triene and diene in the cardol and 2-methylcardol promoted the autoxidation polymerization by thermal treatment. Figure 5 also presents the viscosity and degree of unsaturation of ECNP as functions of the heating time. The viscosity increased exponentially as the heating time and temperature increased, whereas the degree of unsaturation decreased. In particular, the viscosity increased significantly after 22 h at 140°C and 8 h at 160°C. As the heating temperature and/or heating time increased, excess crosslinking reactions in the unsaturated parts of the side chain and cleavage of the epoxy groups occurred. In this case, the gelation of ECN was observed at 160°C after 18 h because of the previous reasons. The previous epoxy cardanol prepolymer showed that the gelation happened at 160°C after 28 h. In addition, the contents of monomer and oligomer in the epoxy cardanol prepolymer prepared at each temperature were lower than those in ECN; this suggested that the polymer content in ECNP was higher than that in the cardanol. On the basis of these results, the cardol and/or 2-methylcardol, which were other components in CNSL, promoted the thermal polymerization than the case of cardanol. These components contained higher contents of unsaturated groups of diene and triene than cardanol, as presented in Table I. Figure 6 presents the changing ratios of aromatic rings, epoxy groups, and unsaturation degree in ECN as a function of the heating time. There was no structural change in the epoxy group for up to 24 h at 120°C, up to 24 h at 140°C, and up to 12 h at 160°C according to the ^{1}H-NMR analysis, whereas the degree of unsaturation decreased linearly with time at 140 and 160°C. This suggested that the higher heating temperature promoted the oxidative reaction of ECN at side chains before the gelation and cleavage of epoxy groups. On the basis of these results, a higher temperature or longer heating time caused polymer gelation because of excess crosslinking reactions at the side chains, as observed in the previous cardanol study.[17] ^{1}H-NMR and FTIR analyses showed that ECNP proceeded at the side chains in CNSL. In this case, the reaction at the side chain could have been more complicated and promoted than in the previous case of pure cardanol because CNSL contained three compounds. In this case, the double bond in the side chain was autoxidized by oxygen to produce allyl radicals and/or radical transfer in double bonds to give a stable radical site under thermal treatment. The radical should have reacted with oxygen to form peroxy radicals, and the crosslinking reaction also occurred when the peroxy radicals attached to other double bonds or allyl radicals in the other side chains.[17] This autoxidation happened among three components, cardanol, cardol, and 2-methylcardol, and produced a more complicated crosslink structure in this case.

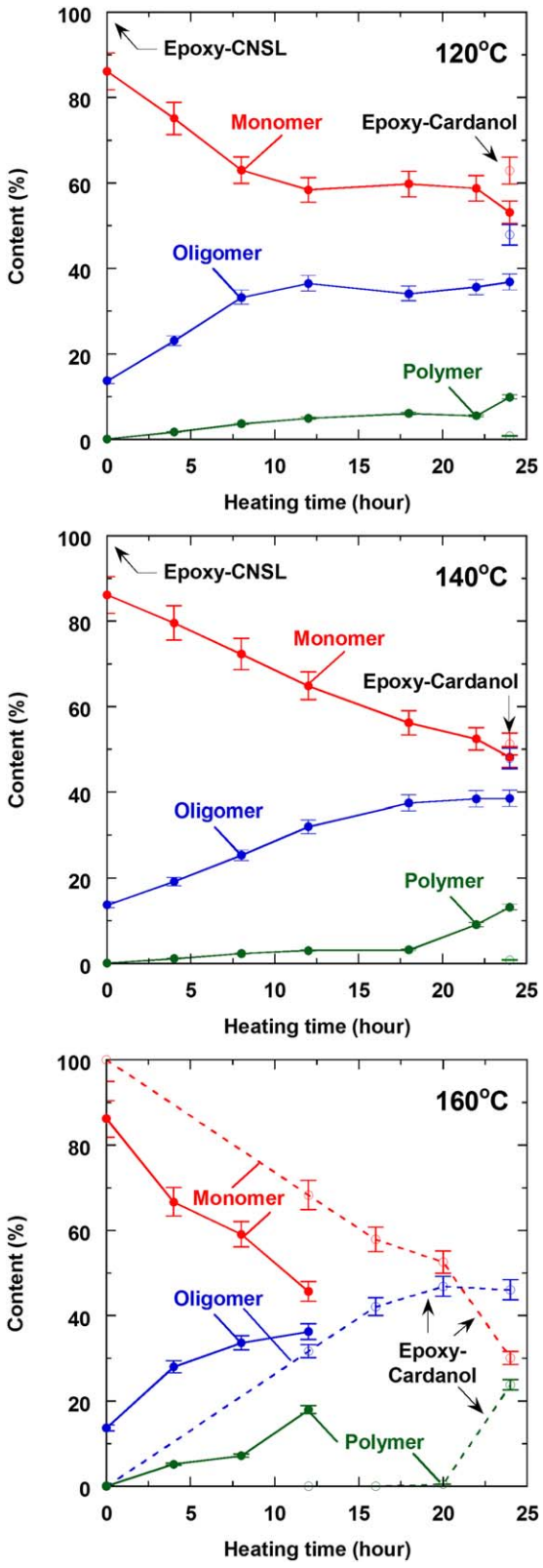

Figure 4. Changes in the compositions of the monomer, oligomer, and polymer of ECNP. The solid plots and lines present the results of this study, and the blank and dotted lines present the results of a previous work (epoxy cardanol).[17] [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

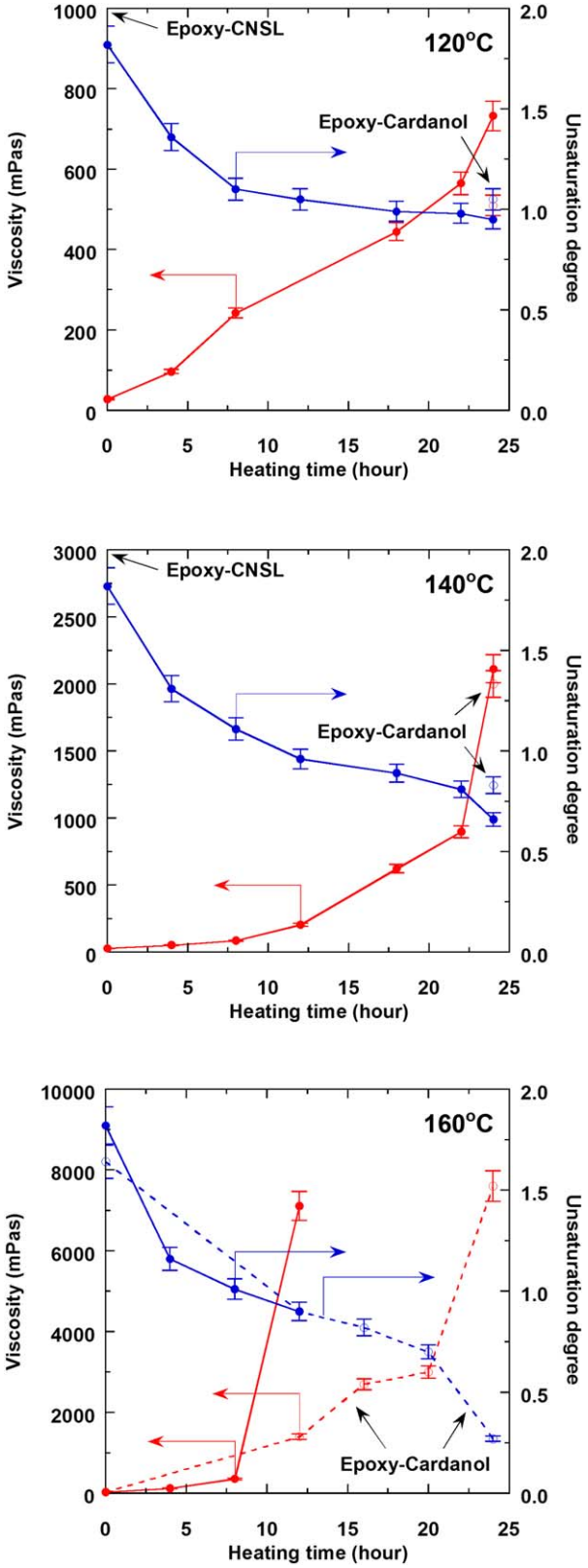

Figure 5. Changes in the viscosity associated with the decrease in the unsaturation degree of ECNP. The solid plots and lines present the results of this study, and the blank and dotted lines present the results of a previous work (epoxy cardanol).[17] [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

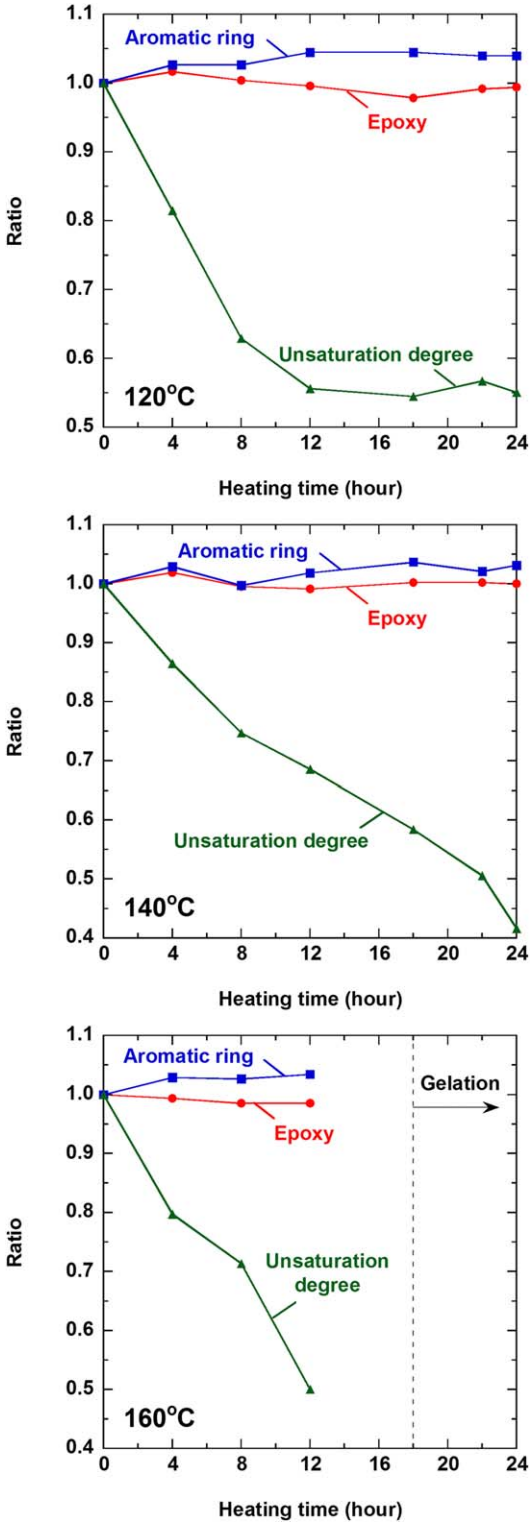

Figure 6. Changes in the ratios of the aromatic ring, epoxy, and unsaturation degree of ECNP based on NMR. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Film Preparation and Characterization

The optimal amount of DETA for the ECNP films was investigated in terms of the drying properties, hardness, gel content, and T_g. The properties of the ECNP films prepared with DETA

Table III. Drying Properties and Hardness of the CNSL-Based Polymers

				Drying properties (h)[a]			Hardness[b]				
Entry	Heating temperature (°C)	Content (wt %)	Gel content (wt %)[d]	Dust-free drying	Touch-free drying	Hardened drying	1 day	2 days	7 days	14 days	T_g (°C)
1	140	5	73.8	0.6	1.3	2.0	<6B	<6B	<6B	<6B	0.7
2	140	10	85.5	0.6	1.1	2.0	<6B	4B	3B	3B	17.8
3	140	15	79.8	0.6	0.9	1.6	6B	4B	3B	3B	17.1
4	160	5	69.2	0.4	1.0	2.0	<6B	<6B	<6B	<6B	3.6
5	160	10	80.1	0.5	1.1	1.8	<6B	4B	3B	3B	17.8
6	160	15	70.9	0.4	1.0	1.6	<6B	6B	4B	4B	19.4

[a] After 7 days.
[b] Based on JIS-K-5400.
Pencil hardness: Hardened drying << 6B < B < HB < F < H << 9H.

are summarized in Table III. All of the ECNP-based films required about 2.0 h to cure until hardened drying, regardless of the amine content. This time was shorter than that of the previous ECN-based film; this suggested that the mixture (i.e., cardanol, cardol, and 2-methylcardol) promoted the reaction with the DETA amine compound. The hardness of the films prepared with 5 wt % DETA was below 6B, whereas the hardnesses for the 10 and 15 wt % DETAs reached 3B or 4B after 7 days.

Figure 7 presents the gel contents of the ECNP-based film (10 wt % DETA) after 1 day as a function of the heating time. As the heating time increased, all of the gel contents increased with increasing temperature. As mentioned before, we could not test the ECNP prepared at 160°C for 18 h because of gelation. Therefore, we used ECNPs prepared at 140°C for 24 h and 160°C for 12 h as basic conditions in terms of molecular weight distribution and viscosity to prepare the biobased film. It should be noted that further optimization for the improvement of the crosslinking reaction is required because the gel content of this film was not 100%. Figure 8 also presents the ECNP-based film as a function of the time at each DETA content. The gel contents of both ECNPs prepared with 5 wt % DETA were lower than those of the ENCPs prepared with 10 and 15 wt % DETA; this indicated that a 5 wt % amine content was not sufficient to react with the epoxy group in the prepolymer.

Figure 9 presents the RPT spectrum of the ECNP-based film prepared at 140°C for 24 h with each DETA contents. The T_g values of each film were determined from the RPT spectra and are summarized in Table III. The spectra shows that the T_g of 5 wt % DETA film was also lower than those of the 10 and 15 wt % DETA films. Because the T_g determined from RPT represented the density of crosslinking, the densities of the ECNPs prepared with 10 and 15 wt % DETA were higher than that of the ENCP prepared with 5 wt % DETA. On the other hand, 10 wt % DETA showed the highest gel contents at over 80%; this suggested that 10 wt % DETA was suitable for reacting with epoxy groups. Hence, we found that an amine content of around 10 wt % DETA was suitable for reacting with epoxy groups in ECNP. Therefore, antibacterial activity experiments were conducted with the ECP film prepared with the 10 wt % DETA compound.

Antibacterial Activity

The antibacterial activities of the liquid precursors against *E. coli* and *S. aureus* are summarized in Table IV. None of the compounds, ECN, ECNP, nor DETA, showed antibacterial activity against *E. coli* or *S. aureus* at a concentration of 1 mg/mL. Natural CNSL inhibited the growth of *S. aureus* at the tested concentration but did not inhibit the growth of *E. coli*. The genus *Escherichia* is a Gram-negative bacillus, and the genus *Staphylococcus* is a Gram-positive coccus. The major difference between Gram-positive and Gram-negative bacteria is the structure of the cell wall. The cell wall of Gram-positive bacteria consists of a thick peptidoglycan layer, whereas that of Gram-negative bacteria has an outer layer of lipopolysaccharide and a thin inside layer of peptidoglycan. In our preliminary study, the Gram-stained cells of the CNSL-treated *S. aureus* turned from

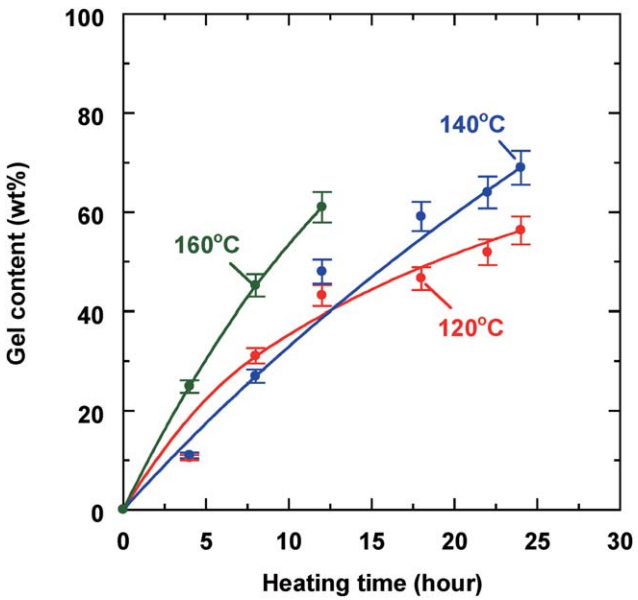

Figure 7. Gel content of the ECNP–DETA films after 1 day as a function of the heating time. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

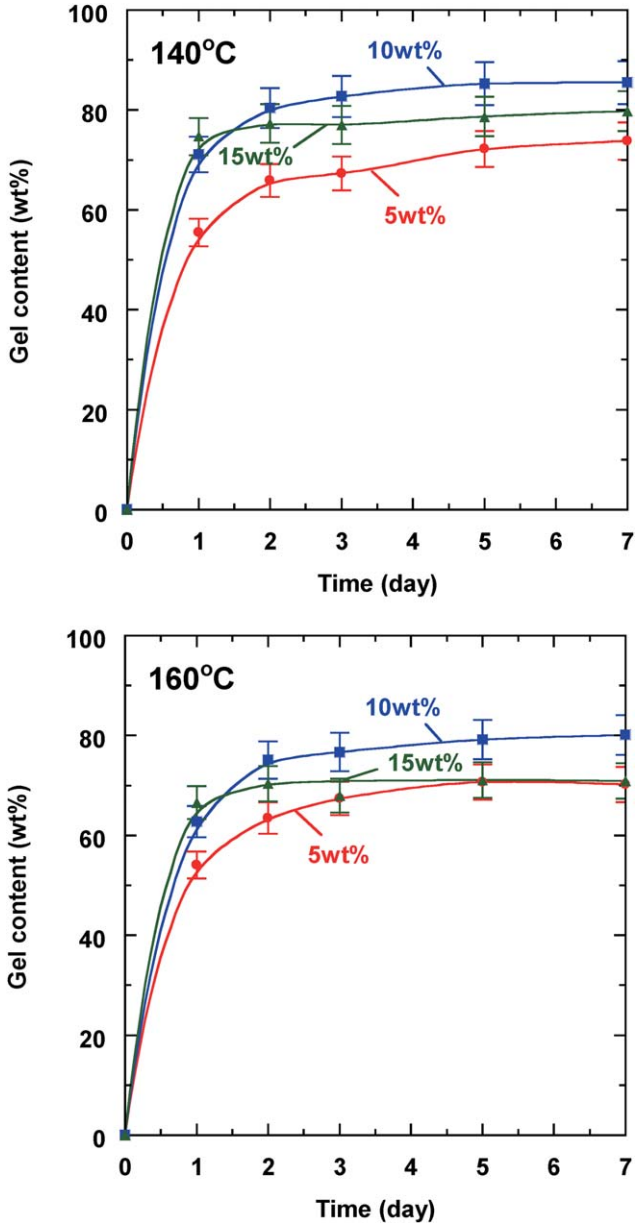

Figure 8. Gel content of ECNP prepared with various DETA contents as a function of time. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

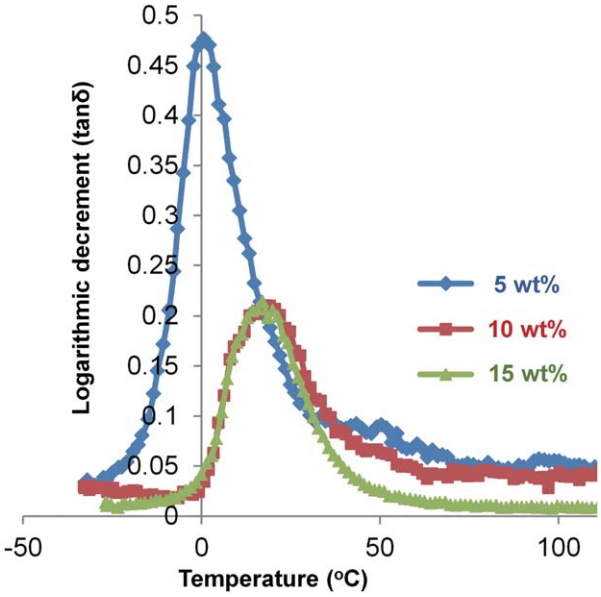

Figure 9. RPT spectrum of the ECNP prepared at 140°C for 24 h with various DETA contents. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Gram-positive to Gram-negative. This result suggests that the CNSL might have affected the synthesis of peptidoglycan in the cell wall and inhibited the proliferation of *S. aureus* cells. Phenolic and catechol compounds were reported to have antibacterial properties and antioxidant and anticarcinogenic properties.[18,19] Kim *et al.*[27] reported that urushiol, a mixture of natural phenolic compounds with a long unsaturated alkyl chain, exhibited an inhibitory effect on the growth of Gram-positive bacteria (e.g., *S. aureus*). CNSL is also a plant-based natural phenolic/catechol compound, and its antibacterial activity disappeared when the OH groups of the CNSL were converted to epoxy groups. Therefore, the inhibitory effect of CNSL against Gram-positive bacteria might be derived from the phenolic group in the compound.

The antibacterial activities of the ECNP-based polymer films are summarized in Table V. Interestingly, the viable cell numbers of *E. coli* and *S. aureus* incubated at 35°C on the ECNP–DETA polymer films for 20 h showed remarkable decreases. Furthermore, the films maintained antibacterial activity against *E. coli* even after 80% ethanol conditioning. The vial cell number of the ethanol conditioned ECNP–DETA film against *S. aureus* was greater than that of the unconditioned film but still less than that of the mock treated control. These results suggest that antibacterial properties were fixed in the polymer film. The reaction between the epoxy and amine groups produced OH and secondary amine groups, as observed in a previous work.[17] In addition, the DETA part of the polymer film retained unreacted free amine groups. Natural chitosan normally has a positively charged surface on the basis of amino groups and antibacterial properties against Gram-negative bacteria.[28] The antibacterial activity of the ECNP–DETA polymer film may have been based on the newly formed OH and secondary amine groups and/or the unreacted amine groups in DETA. Therefore, this

Table IV. Antibacterial Activities of the Precursor Against *E. coli* and *S. aureus*

			Number of viable cells (cfu/mL)	
Entry	Sample	State	*E. coli*	*S. aureus*
1	CNSL	Liquid	1.4×10^9	< 10
2	ECN	Liquid	1.3×10^9	5.8×10^7
3	ECNP	Liquid	2.0×10^9	7.0×10^7
4	DETA	Liquid	4.6×10^9	6.7×10^7
5	Control	Control	1.7×10^5	2.3×10^5

Table V. Antibacterial Activities of the CNSL-Based Polymer Against *E. coli* and *S. aureus*

			Number of viable cells (cfu/mL)	
Entry	Sample	Remark	*E. coli*	*S. aureus*
1	ECNP-DETA	As-prepared	$<10^3$	$<10^3$
2	ECNP-DETA	Alcohol-conditioned	$<10^3$	3.6×10^5
3	Control 1	Before incubation	1.5×10^6	1.2×10^8
4	Control 2	1/500TS[a] after 20 h	2.0×10^7	7.8×10^7

[a] 500-fold dilute tripticase soy broth.

ECNP-based polymer film could be interesting as a possible antibacterial material for the health care field.

CONCLUSIONS

We prepared biobased polymer films from CNSL for use as antibacterial materials. The CNSL-based films were prepared from ECNP and DETA compounds without any organic solvent. In the preparation of ECNP, the minor components, cardol and 2-methylcardol, promoted the thermal polymerization better than the previous pure epoxy cardanol. This was because the higher contents of unsaturated structure in the cardol and 2-methylcardol promoted autoxidation polymerization. This film required 2 h to dry to a hardened state at room temperature. We found that CNSL itself had antibacterial activity against *S. aureus* on the basis of the phenolic and catechol structures of cardol, cardanol, and 2-methylcardol. In addition, the biobased film reduced the number of *E. coli* and *S. aureus* bacteria relative to the original control; this suggested that this biobased ECNP–DETA polymer film had antibacterial activities. This activity was maintained even with 80% ethanol conditioning, which is standard antisepsis conditioning in medical facilities. Therefore, this CNSL-based film could be effective as an antibacterially active material in health care applications.

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