JEOL

Solutions for Innovation

Applications note

Learning Polymer Material Analysis from Natural Lacquer (Urushi)

UrushiNote

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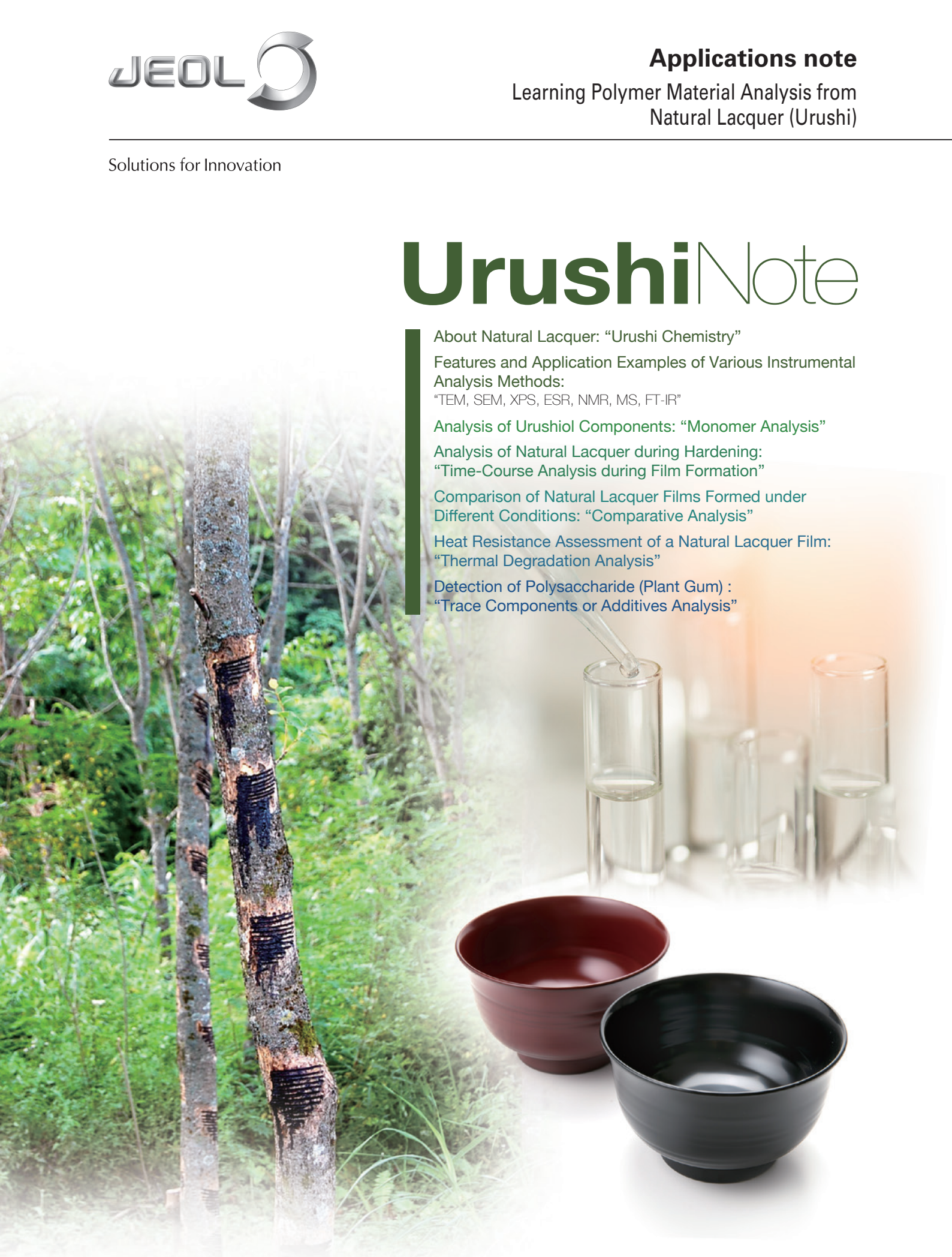

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UrushiNote

Learning Polymer Material Analysis from Natural Lacquer (Urushi)

- Characterization of Urushi -

1.Introduction

In recent years, polymer materials have become more complex due to increased composition and diversification so that a one-sided analysis is insufficient and multifaceted observations and analyses are required.

In response to this need, JEOL has engaged in applied research under the keyword of "YOKOGUSHI" (multifaceted or cross-instrumental) using various instruments organically.

In this Urushi Note, multifaceted analysis methods for polymer materials are illustrated using the examples of natural lacquer (urushi) analysis.

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1-1 About Natural Lacquer: "Urushi Chemistry"

Since the enactment of the Sustainable Development Goals (SDGs) by the United Nations in September 2015, the international movement towards a sustainable society has achieved increased momentum. In Europe, with the backing of Environment, Society and Governance (ESG) investments, there has been an acceleration of the applications of renewable bioresources based on the bioeconomy and, in Japan, the development and dissemination of technologies supporting sustainability have been promoted under the "Fifth Environmental Basic Plan." In this situation, active research is being conducted into natural lacquer (urushi), which has been used as paint, as adhesive materials for thousands of years.[1-①~⑦] As a representative Japanese craft, lacquerwork has been highly appreciated in different parts of the world for its elegant appearance and excellent durability, and has become known as "japan."[1-⑧] Also, it is currently drawing attention as a subject of environment and human-friendly, highly functional polymer research alongside the growing trend of biomimetic material development.[1-⑨⑩]

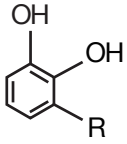
The composition of Toxicodendron vernicifluum lacquer sap, which is commonly used for Japanese lacquerwork, is shown in Table 1-①.

Table 1-① Composition of *Toxicodendron vernicifluum* lacquer sap

Components	Content (%)
Urushiol	60-65
Water	20-25
Polysaccharide (plant gum)	5-7
Glycoprotein (nitrogen-containing material)	2-5
Laccase enzyme	1-2

As shown in Fig.1-①, natural lacquer sap is a water-in-oil (w/o) emulsion (dispersion solution) containing urushiol as a main component (see Table 1-②), water, polysaccharide (plant gum) (see Table 1-③), glycoprotein (nitrogen-containing material), and laccase enzyme.

Table 1-② Composition of urushiol



R	*Mr*	%
$C_{15}H_{31}$	320	4.5
8(Z)-$C_7H_{14}CH{=}CHC_6H_{13}$	318	15.0
10(Z)-$C_9H_{18}CH{=}CHC_4H_9$	318	1.5
8(Z),11(E)-$C_7H_{14}CH{=}CHCH_2CH{=}CHC_3H_7$	316	4.4
8(Z),11(Z)-$C_7H_{14}CH{=}CHCH_2CH{=}CHC_3H_7$	316	6.5
8(Z),11(E),13(E)-$C_7H_{14}CH{=}CHCH_2CH{=}CHCH{=}CHCH_3$	314	1.7
8(Z),11(E),13(Z)-$C_7H_{14}CH{=}CHCH_2CH{=}CHCH{=}CHCH_3$	314	55.4
8(Z),11(E),14-$C_7H_{14}CH{=}CHCH_2CH{=}CHCH_2CH{=}CH_2$	314	7.4
10(Z)-$C_9H_{18}CH{=}CHC_6H_{13}$	346	1.5
8(Z),11(Z)-$C_7H_{14}CH{=}CHCH_2CH{=}CHC_5H_{11}$	344	1.8

Table 1-③ Composition of constituent monosaccharide components of the polysaccharide

Monosaccharide	Content (%)
D-galactose	66.1
4-O-methyl-gulucuronic acid	24.1
D-gulucuronic acid	3.0
L-arabinose	5.0
L-rhamnose	3.1

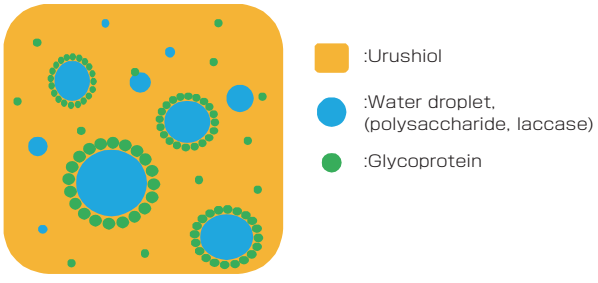


Fig. 1-① Schematic image of lacquer sap (w/o emulsion)

Natural lacquer is a complex natural coating material based on the interaction and polymerization of these constituents. The film forming process was reported to be primarily advanced by the laccase-catalyzed oxidative polymerization (see Fig. 1-②) and autoxidation of urushiol in the air (see Fig. 1-③).[1-⑨] However, there are still many unclear points about the more detailed film formation process and the process of expressing high durability.

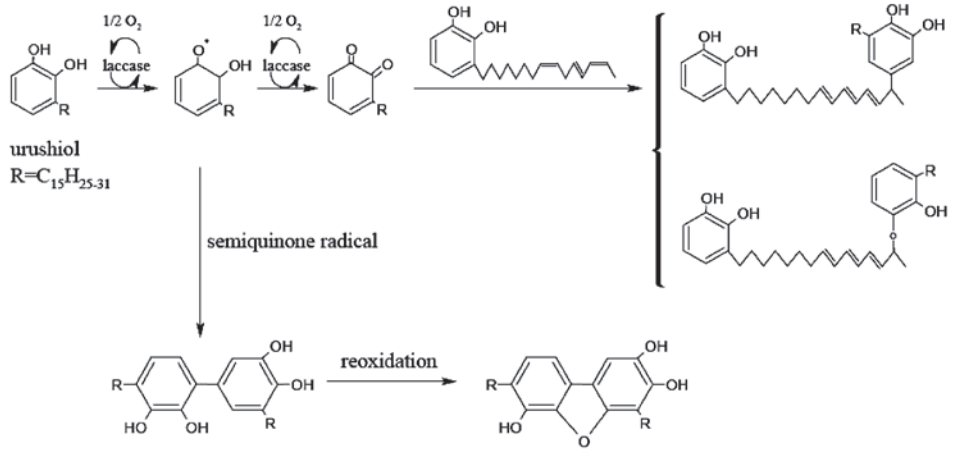


Fig. 1-② Dimerization mechanism of urushiol catalyzed by laccase enzyme

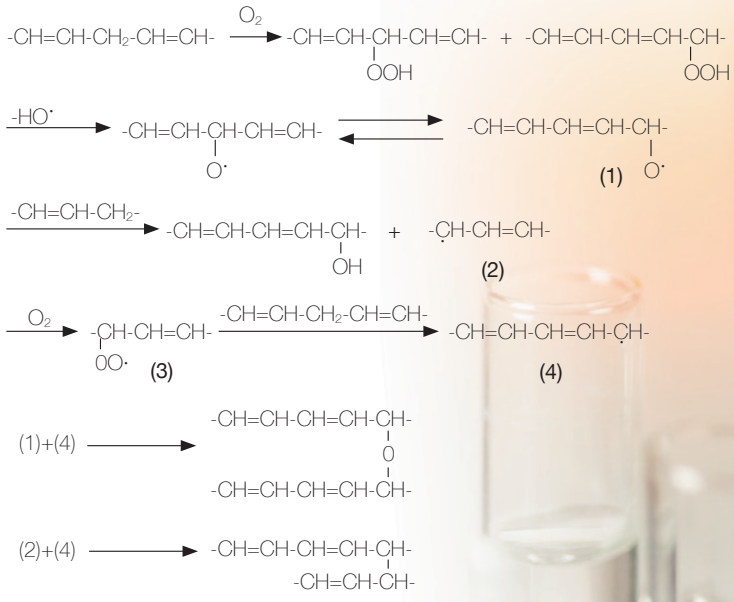


Fig. 1-③ Autoxidation of urushiol side chain

References
1-① T. Miyakoshi, H. Kobuchi, N. Niimura and Y. Yoshihiro, *Bull. Chem. Soc. Jpn.,* 64, 2560 (1991).
1-② N. Niimura, Y. Iijima and T. Miyakoshi, *Analytical Chemistry* (in Japanese), 42, 605 (1993).
1-③ N. Niimura, T. Miyakoshi, J. Onodera and T. Higuchi, *Int. J. Polym. Anal. Charact.,* 4, 309 (1998).
1-④ N. Niimura, Y. Kamiya, T. Sato, I. Katano and T. Miyakoshi, *J. Jpn. Oil Chem. Soc.,* 47, 171 (1998).
1-⑤ N. Niimura and T. Miyakoshi, *Surf. Interface Anal.,* 29, 381 (2000).
1-⑥ Y. Kamiya, N. Niimura and T. Miyakoshi, *Bull. Chem. Soc. Jpn.,* 73, 2621, (2000).
1-⑦ N. Niimura, T. Miyakoshi and Y. Iijima, *Analytical Science 2001 Supplement,* 17, i155, (2001).
1-⑧ N. Niimura, T. Miyakoshi, et al., "Japanese and European Lacquer ware", Arbeitsheft des Bayerischen Landesamtes fur Denkmalpflege, Munich, 2000.
1-⑨ T. Miyakoshi, *Lacquer Studies* (in Japanese), Maruzen Publishing (2016).
1-⑩ H. Uyama, *Chemistry and Biology* (in Japanese), 44, 815 (2006).

1-2 Features and Application Examples of Various Instrumental Analysis Methods:

"TEM, SEM, XPS, ESR, NMR, MS, FT-IR"

JEOL is offering wide varieties of analytical instruments for morphological observations, spectroscopy, mass spectrometry, separation analysis, physical property measurements, and pre-treatments. This section describes features of the main instruments used to analyze natural lacquers, which are natural polymer materials, and the applications of these instruments to polymer materials.

1-2-1 Transmission Electron Microscopy

Transmission electron microscopes (TEMs) are instruments designed to observe transmission images of a sample using electrons transmitted through the sample (transmission electrons). TEM can cover magnifications from tens of times to the atomic resolution range. As electrons lose energy through strong interaction with materials, thin samples should be used for TEM (generally 100 nm or thinner).

The characteristics of the measuring method using TEM are as follows:

- Structural observation and analysis from organisms and polymers to atoms (bright field and dark field).
- Electron diffraction patterns (observation of interference patterns by electron-beam interaction with a sample)
- Elemental analysis by energy dispersive X-ray spectrometry (EDS) using characteristic X-rays
- Elemental analysis and chemical state analysis by electron energy loss spectroscopy (EELS) using electrons with energy lost interacting with a sample
- Scanning transmission electron microscopy(STEM) for observing transmission images by scanning a sample with a focused electron beam
- Electron diffraction and elemental analysis in micro areas with a focused electron beam
- Observation of frozen samples using cryo-electron microscopy

etc.

For polymer materials, TEM enables ultrastructure observation, elemental analysis, and electron diffraction of thin-film samples and particle samples, etc. and its applications range from basic research to R&D and quality assurance of industrial products.

Application examples:

- Ultrastructure observations, elemental mapping, and chemical state analysis of polymer materials
- Distribution measurement and elemental analysis of additives and inorganic filler in polymer materials
- Observation of emulsion and polymer fine-particles by cryo-electron microscopy

References
Analytical Instrument Guidelines 2016 (in Japanese), JAIMA
Method of Polymer Sample Preparation by Ultramicrotome for TEM (in Japanese), JEOL

1-2-2 Scanning Electron Microscopy

Scanning electron microscopes (SEMs) are instruments designed for surface observation.

An SEM scans a sample surface with a focused electron beam, detects various signals generated from the sample, and synchronizes and displays intensities of the detected signals on a monitor to form an image.[1-2-2-①] Morphology and the composition of a sample can be observed depending on the type of signal to be detected[1-2-2-②] Normally, samples for SEM observation must be solid and conductive. Thus, in order to observe a polymer material at its native state, several techniques are required, e.g. uncoated observation with low-accelerating voltage, use of low-vacuum SEM capable of observing a sample containing a small amount of water (fat)[1-2-2-③], and use of a cryo-SEM equipped with a sample holder to observe a frozen- or liquid sample[1-2-2-④].

Also, when equipped with an energy dispersive X-ray spectrometer (EDS), SEM enables qualitative/quantitative analysis of elements by detecting characteristic X-rays excited by the electron beam.[1-2-2-⑤] Although the detectable element range is different depending on window materials of the EDS detector, EDS usually can detect boron (B) to uranium (U). SEM/EDS enables point analysis with an electron beam fixed at a certain point, line analysis by linear electron-beam scanning, and two-dimensional distribution analysis of constituent elements (elemental mapping).
For polymer materials, SEM/EDS enables the fine morphology observation of the surface or cross section of the sample at the nanometer order, and allows for observation of distributions of additives and their elemental analysis, etc.

Application examples are listed below.

Application examples:

- Surface and cross-sectional observation of pores formed on a polymer material
- Observation of blended polymer by staining
- Observation and elemental mapping of polymer materials with multi-layered films
- Distribution measurement and elemental analysis of additives in polymer materials
- Analysis and location identification of inorganic foreign materials
- Observation of emulsion by a cryo system

References
1-2-2-① *NEW Scanning Electron Microscope* (in Japanese), Ed. Kanto Branch of Japanese Society of Microscopy, Kyoritsu Shuppan (2011), p3-5
1-2-2-② *NEW Scanning Electron Microscope* (in Japanese), Ed. Kanto Branch of Japanese Society of Microscopy, Kyoritsu Shuppan (2011), P27-34
1-2-2-③ *Application of Low-Vacuum Analysis SEM to Insulator Samples* (in Japanese), JEOL https://www.jeol.co.jp/applications/detail/885.html
1-2-2-④ *Overview of CRYO-SEM* (in Japanese), JEOL https://www.jeol.co.jp/applications/pdf/sem/sm_b004_00.pdf
1-2-2-⑤ *Basics of Elemental Analysis* (in Japanese), JEOL https://www.jeol.co.jp/words/semterms/a-z_12.pdf

1-2-3 X-ray Photoelectron Spectroscopy [1-2-3-①~④]

X-ray photoelectron spectroscopy (XPS) is an instrumental analysis method to measure a photoelectron energy spectrum emitted by X-ray irradiation and to obtain knowledge about different chemical states of elements (Li ~ U) in an analytical region within several nm of the surface.

In a photoelectron spectrum, the horizontal axis represents electron binding energy for elements, while the vertical axis represents the intensity of the emitted photoelectron. As the binding energy value depends on the element and electron states, a spectrum will enable the identification of elements in a sample and provide knowledge on their chemical bonding states. In other words, when an element combines with another element, the electron states change. The position of the photoelectron peak (binding energy value) changes accordingly.

These changes are called chemical shifts, which enable the identification of the chemical bonding states of an element.

Furthermore, a quantitative analysis is also possible using relative sensitivity factors specific to the instruments. The detection limit is 0.1 atomic percent on average. At this point, when the peak of interest overlaps with another element peak, another orbital peak is used, or the integrated intensity of each peak obtained by curve fitting is used.

Application examples of analysis of polymer materials are listed below.

Application examples:

- Study of molecular orientation
- Analysis of surface-treated materials
- Analysis of surface contamination and foreign materials
- Study of surface segregation of blended or copolymer materials
- Study of surface enrichment of additives
- Interface analysis
- Depth profiling

References
1-2-3-① Y. Takakuwa, *X-ray Photoelectron Spectroscopy* (in Japanese), issued by M. Watase, Kodansha Ltd. (2018).
1-2-3-② N.Ikeo, Y.Iijima, N.Niimura, M.Sigematsu, T.Tazawa, S.Matsumoto, K.Kojima, Y.Nagasawa, Handbook of X-ray Photoelectron Spectroscopy, JEOL Ltd., Tokyo, 1991.
1-2-3-③ N. Niimura, *Elucidation and Control of Tribology by Numerical Simulation and Surface Analysis* (Section 6. X-ray Photoelectron Spectroscopy, Part 2, Surface Analysis Methods in Chapter 10. Surface Analysis) (in Japanese), supervised by S. Sasaki, Techno Systems, Inc. p.1001-1004 (2018).
1-2-3-④ N. Niimura, *JAIMA Season,* 145, 5 (2016) (in Japanese).

1-2-4 Electron Spin Resonance Spectroscopy [1-2-4-①, ②]

Electron spin resonance spectroscopy (ESR) is a method to selectively observe electron spins in a substance.

Electron spins are characteristic of unpaired electrons and are called radicals when unpaired electrons reside in an organic substance.
The state of radicals can vary from very unstable to extremely stable. In the case of extremely unstable radicals, measurement is made by using a special system called time-resolved ESR, by forming a stable adduct with a stabilizer called a spin-trap reagent for measurement, or by stopping the annihilation reaction of radicals at low temperature. Relatively stable radicals can be directly placed into a dedicated sample tube for measurement.
Measurement is made by a sweeping an external magnetic field while irradiating microwave onto a sample. There are electron spins with two different energy levels: α-spins and β-spins. With a magnetic field satisfying the following equation, microwave at the frequency corresponding to an energy gap between electron spins are absorbed and generate signals.
$h\nu=g\beta_0 H$ (where, h: Planck constant; ν: microwave frequency; g: a specific value of radical; β_0: Bohr magneton; and H: magnetic field)

Signals in the ESR spectrum are shown in the form of a spectrum with the magnetic field axis.
Different ESR signals can be identified because magnetic fields that gives absorption vary depending on the radical type. However, even with the same sample, magnetic fields that give absorption vary depending on the cell form and the attachments used, so the g-value is used for identification. Typical g-values are shown in Table 1-2-4-①.

Table 1-2-4-① Typical g-values for respective radical species

Element	C-radical	CO-radical	OO-radical, N-radical	S-radical
g-value	2.003	2.004	2.005 ～ 2.006	2.011

As the measurement only requires irradiation of weak microwaves at mW scale, it is basically a non-destructive analysis and can be repeated on the same sample. Alternatively, other analyses can be performed on the sample after ESR measurement.

Application examples for the polymer materials are listed below.

Application examples:

- Measurement of reaction intermediates under radical polymerization
- Measurement of radical decomposition under photo degradation of polymer materials
- Measurement of radical decomposition under thermal degradation of polymer materials
- Measurement of residual polymerization catalyst and impurity metal ions

References
1-2-4- ① JEOL, *Electron Spin Resonance Spectrometer*, Introduction to JEOL Products (in Japanese) https://www.jeol.co.jp/science/esr.html
1-2-4- ② *Electron Spin Resonance: Micro Characterization of Materials* (in Japanese), Kodansha Scientific K.K.

1-2-5 Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance spectroscopy (NMR) is a method to analyze the molecular structures of a substance at the atomic scale by placing the atomic nucleus in a magnetic field and observing nuclear spin resonance.

An atomic nucleus with an odd mass number and/or an odd atomic number (e.g. ^{1}H, ^{13}C, and ^{15}N) has a magnetic moment. When such a nucleus is placed in an external magnetic field, the precession of the magnetic moment begins. The magnetic moment absorbs electromagnetic waves (radio waves) given at the same frequency as the precession. This is the mechanism of nuclear magnetic resonance. Therefore, NMR systems consist of a magnet that generates an external magnetic field, a spectrometer that generates radio waves to observe resonance phenomenon, and a control PC, as shown in Fig. 1-2-5-①.
While NMR is a non-destructive analysis method, NMR systems can be used for analysis of molecular structures and for quantitative analyses. The main information that can be obtained by NMR includes chemical shifts, spin coupling constant, peak area intensity, and relaxation time. As an example, a ^{1}H NMR spectrum of ethanol is shown in Fig. 1-2-5-②.

PC Spectrometer Magnet

Fig. 1-2-5-① NMR system（JNM-ECZ500R）

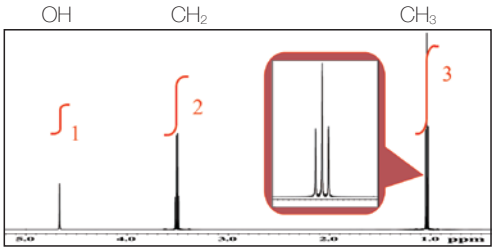


Fig. 1-2-5-② ^{1}HNMR spectrum of ethanol (CH_3 CH_2 OH)

(1) Chemical shifts are values plotted on the horizontal axis of the NMR spectrum, and represent differences in resonance frequency that are generated depending on the surrounding environment of the atomic nucleus. When environmental conditions such as functional groups vary, there will be changes in the magnetic shielding by electrons in proximity and the magnetic field sensed by the atomic nucleus; in other words, the resonance frequency value will be differently observed in proportion to the magnetic field. Therefore, the different frequency value can be used to analyze the surrounding environment of each atomic nucleus contained in a sample. In the example shown in Fig. 1-2-5-②, OH, CH_2, and CH_3 in ethanol are observed in chemical shifts corresponding to their respective ^{1}H environments.
(2) Spin coupling is a phenomenon of signal splitting caused by adjacent nuclei in a molecule observed as a peak shape. Adjacent atomic grouping can be identified based on the pattern and size of splitting. In the example shown in Fig. 1-2-5-②, the CH_3 peak is indicated by a triplet formed with the adjacent CH_2.
(3) The peak intensity of an NMR spectrum is represented by the peak area intensity. As the peak area intensity is proportional to the number of atomic nuclei in the environment, it can be used for quantification of atoms and molecules in a sample. In the example shown in Fig. 1-2-5-②, OH, CH_2, and CH_3 in ethanol are observed at intensity ratios corresponding to their numbers of respective ^{1}H.
(4) Relaxation time is a value to be reflected in peak width. It contains information about the kinetic properties of a sample.

Representative NMR analyses of polymer materials for physical properties are listed below.

Application examples:

(1) Structural analysis and identification of unknown chemical substances
(2) Mixture analysis
(3) Stereoregularity analysis
(4) Determination of crystallinity
(5) Molecular mobility and self-diffusion coefficient.

References
https://www.jeol.co.jp/science/nmr.html (in Japanese)

1-2-6 Mass Spectrometry 1-2-6-①~③)

Mass spectrometry (MS) is an analytical method to obtain mass information on chemical compounds by ionizing an inorganic or organic compound, separating and detecting resultant ions by *mass-to-charge ratio (m/z)*.

In an acquired mass spectrum, *m/z* and detected intensities of ions are plotted on horizontal and vertical axes, respectively. Resultant *m/z* of ions can be used for a qualitative analysis, while detected ion intensities enable quantitative analysis.
An MS system consists of three major parts: (1) an ion source, (2) a mass analyzer (separator), and (3) a detector. As neutral molecules cannot be mass-separated in the MS system, sample molecules must be ionized before being introduced to the mass spectrometer. An appropriate ionization method needs to be selected in consideration of the sample introduction method and sample properties. Typical ionization methods widely used today include electron ionization (EI), matrix-assisted laser desorption/ionization (MALDI), and electrospray ionization (ESI).
Ions generated in the ion source are successively carried to the mass analyzer for mass separation by electromagnetic interactions. Mass separation methods widely used today are listed in Table 1-2-6-①.

Table 1-2-6- ① Mass separation methods

Mass analyzer	Mass separation
Magnetic sector type	Mass (m) ∝ Magnetic field intensity (B^2)
Quadrupole type	Mass (m) ∝ High-frequency voltage (V)
Time-of-flight type	Mass (m) ∝ Time of flight (t^2)
Ion trap type	Mass (m) ∝ High-frequency voltage (V)
FT-ICR type	Mass (m) ∝ $-^1$ Frequency (f)

Mass-separated ions are then detected by the detector, and a mass spectrum is recorded based on the detected m/z and intensities of the ions.

Application examples of the polymer material analysis using MS are listed below.

Application examples:

- Qualitative analysis of the base polymer
- Analysis of additives in raw polymer materials (qualitative/quantitative)
- Investigation of cause of defective products
- Analysis of impurities in products
- Variance analysis between product lots
- Multi-variate analysis of characteristic components
- Analysis for compliance with laws and regulations (*RoHS*)

References
1-2-6-① *What is Mass Spectrometry?* (in Japanese), Mass Spectrometry Society of Japan
1-2-6-② *JEOL Mass Spectrometers: Ionization Guidebook* (in Japanese), JEOL Ltd., https://www.jeol.co.jp/applications/detail/1620.html
1-2-6-③ *JEOL Mass Spectrometers: Material Analysis Solutions* (in Japanese), JEOL Ltd., https://www.jeol.co.jp/applications/detail/1459.html

1-2-7 Fourier Transform Infrared Spectroscopy 1-2-7-①～③)

Fourier transform infrared spectroscopy (FT-IR) is a spectroscopic method to perform identification, chemical structure analysis, and physical property evaluation of chemical compounds by observing the infrared absorption spectrum characteristic of substances with high accuracy and high sensitivity.

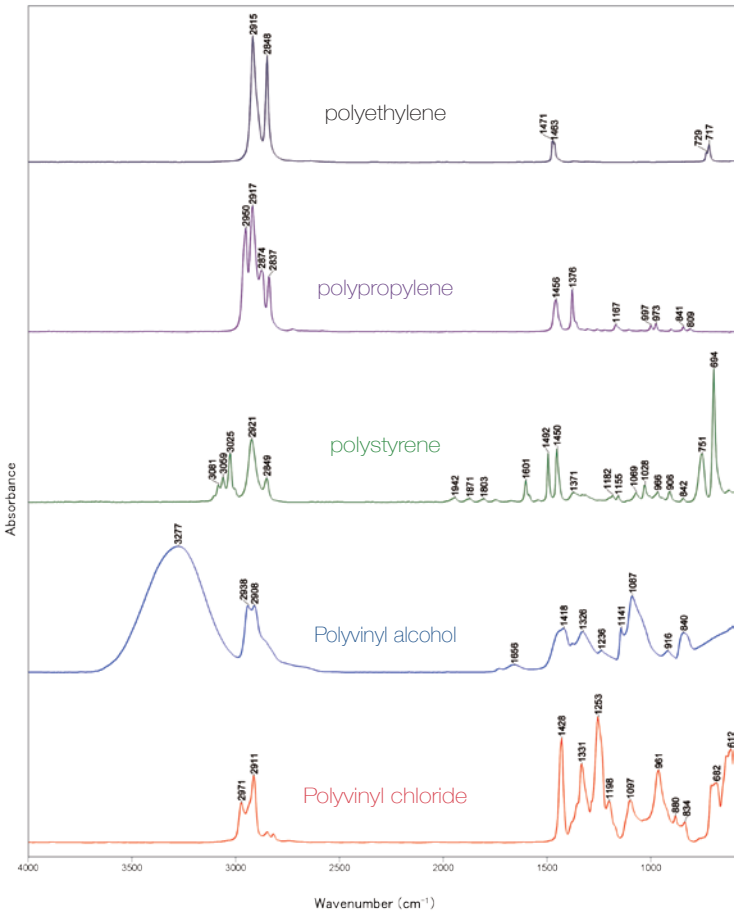
FT-IR is widely used as one of the most useful instrumental analysis methods in polymer material analysis. In ordinary FT-IR analysis, infrared light at wavelengths of 2.5 - 25 μm (wavenumber: 4000 - 400 cm^{-1}) called mid-infrared light (or normal-infrared light) is irradiated on a sample and transmissive light that passes through the sample (or light emitted from within a sample in the case of total reflection, etc.) is captured to observe the absorption spectrum. Energy carried by mid-infrared light is equivalent to an energy level that excites vibrations (spring-like stretching/bending vibrations in chemical bonding), particularly at sites of chemical bonding between relatively light atoms that consist of organic compounds. As natural vibration energy at a chemical bonding site is fixed depending on atomic mass and bonding patterns (single bonding, double bonding, etc.), a characteristic absorption band should appear on an absorption spectrum for each atomic group or functional group, as shown in Fig.1-2-7-①.

The center position, width, and intensity of each characteristic absorption peak change due to the influence of an adjacent group or a conformation. Therefore, detailed data analysis can provide various knowledge on molecular structure and chemical state.

Application examples of polymer materials are listed below.

Application examples:

- Resin type identification
- Functional group and partial structure analysis
- Evaluation of polymerization degrees
- Evaluation of branching degrees
- Evaluation of crystallinity
- Stereoregularity analysis
- Analysis of copolymer and polymer alloy proportions
- Additive and filler analysis
- Foreign material and bled matter analysis
- Analysis of alteration and deterioration caused by heat and ultraviolet rays, etc.
- Analysis of surface modification by plasma treatment, etc.
- Lamination condition analysis of laminated films, etc.
- Polymer orientation evaluation (by polarimetry)



Fig, 1-2-7-① Infrared absorption spectrum of vinyl resins

References

1-2-7-① H. Horiguchi, *Compilation of Infrared Light Absorption Illustrations: Basics and Reality of Organic Structure Chemistry* (in Japanese), Sankyo Shuppan Co., Ltd.

1-2-7-② M. Tasumi, *Basics and Reality of FT-IR* (in Japanese), Tokyo Kagaku Dojin

1-2-7-③ Polymer Analysis Research Council of Japan Society for Analytical Chemistry, New Edition: *Polymer Analysis Handbook* (in Japanese), Kinokuniya Company, Ltd.

1-3 “YOKOGUSHI” (Multifaceted) Analysis of Natural Lacquer

While the previous sections described features and application examples of various instrumental analysis methods, this section describes multifaceted applications of the analysis methods to natural lacquer. Figure 1-3-① illustrates an overview of “YOKOGUSHI” (multifaceted) analysis of natural lacquer.

[1] For the analysis of monomer components isolated from raw natural lacquer, the following three analyses were conducted: (1) identification of compounds using NMR, (2) functional group analysis using FT-IR, and (3) fragment ion analysis using MS.

[2] For the analysis of the hardening process in film formation, time-course analysis was conducted using XPS.

[3] For a comparison of natural lacquer films formed under different forming conditions (comparative analysis), the following seven analyses were conducted: (1) observation of surface colors and wrinkles using light microscopy (LM) or optical microscopy (OM), (2) more detailed observation of wrinkles using white light interferometry, (3) hardness measurement using indentation hardness testing, (4) fine morphological observation using SEM, (5) confirmation of C-O radical amount difference using ESR, (6) confirmation of differences among functional groups using FT-IR, and (7) confirmation of chemical shift differences in ^{13}C using NMR.

[4] For thermal degradation analysis, the following five analyses were conducted: (1) morphological change observation using SEM, (2) analysis of thermogravimetric changes and evolved gas analysis using thermogravimetry/mass spectrometry (TG/MS), (3) observation of radical amount changes using ESR, (4) identification of pyrolysis products using pyrolysis gas chromatography/mass spectrometry (PyGC/MS), and (5) detection of the trace products using PyGCxGC/MS.

[5] For trace components analysis, the following three analyses were conducted: (1) observation of the local state of trace components (polysaccharide: plant gum) using TEM, (2) detection of pyrolysis products using PyMS, and (3) detection of specific peaks using NMR.

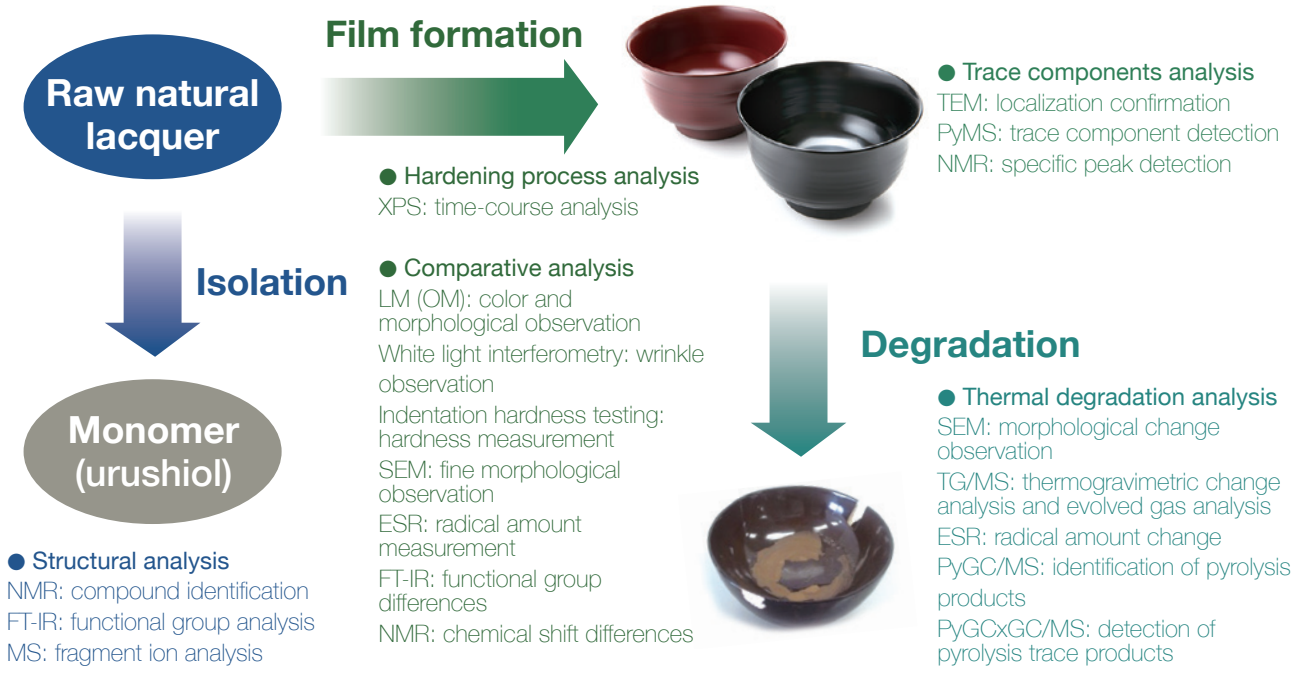


Fig. 1-3-① Overview of “YOKOGUSHI” (multifaceted) analysis of natural lacquer

2 Analysis of Urushiol Components: “Monomer Analysis”

Urushiol is monomer components of natural lacquer. In this chapter, 3-pentadecylcatechol, which is a saturated component of urushiol, was analyzed using NMR, FT-IR and MS. And the effectiveness of respective methods is explained.

2-1 Analysis by NMR

This section presents NMR spectra of urushiol, the primary component of natural lacquer, and examples of various NMR analysis techniques. (The molecular formula, i.e. $C_{21}H_{36}O_2$, and the aromatic ring have already been confirmed by MS and FT-IR.)
For this measurement, a sample was dissolved into deuterated acetone and JNM-ECZ400R spectrometer and 5 mm ROYALPROBE™ were used.

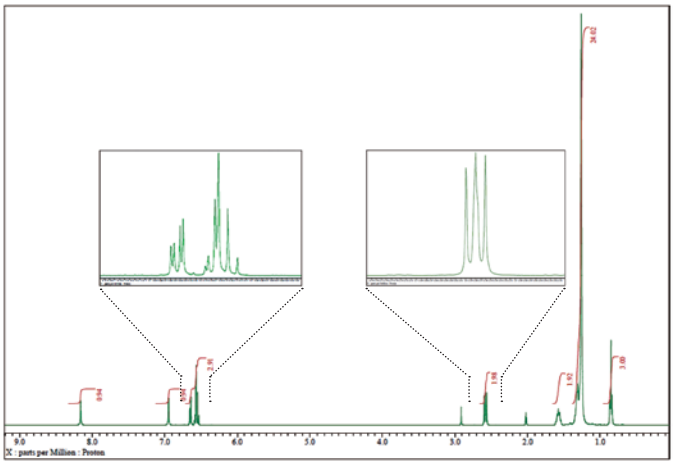


Fig. 2-1-① ^{1}H NMR spectrum of urushiol

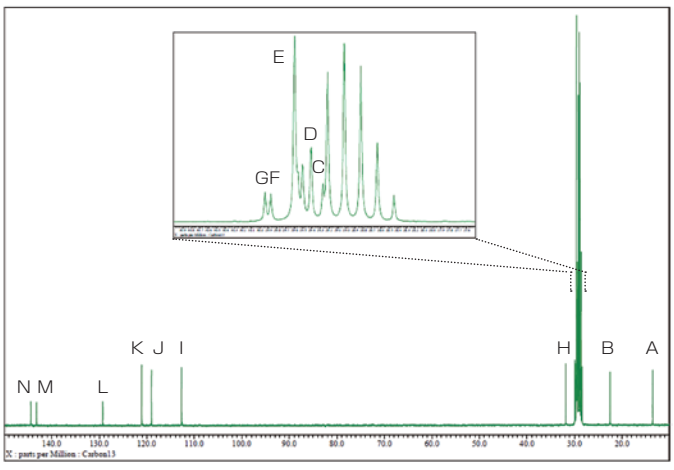


Fig. 2-1-② ^{13}C NMR spectrum of urushiol

Number of accumulations: 8. Measurement time: About 1 min.
Peaks without integration are derived from signals of water and solvent (deuterated acetone).

Figure 2-1-① shows a ^{1}H NMR spectrum of urushiol. As ^{1}H signals trigger peak splitting due to neighboring ^{1}H interactions, analysis has to be conducted with this splitting. Each peak area is proportional to the abundance of ^{1}H. Normalizing the integrated intensity by taking the intensity of the highest magnetic field peak (right side of the spectrum) as 3 resulted in an intensity-area ratio of 1:1:3:2:2:24:3 starting from the lower magnetic field side (left side of the spectrum). Then, a ^{13}C NMR measurement followed.

Number of accumulations: 1,024. Measurement time: About 52 min.
For the analysis, labels were assigned from the side of the high magnetic field. Peaks with no label are of solvent (deuterated acetone) signals (septet).

Figure 2-1-② shows a ^{13}C NMR spectrum of urushiol. In the ^{13}C NMR measurement, observation is made for atomic nuclei ^{13}C (carbon of mass number 13) with a natural abundance of 1.1% and thus, the intensity is low. Therefore in general, spectral acquisition is focused on sensitivity in exchange for quantitative performance. The resultant spectrum of this measurement is easy to analyze because a ^{13}C NMR spectrum has superior separation over a ^{1}H NMR spectrum, and observation while irradiating ^{1}H results in a spectrum without peak splitting derived from ^{1}H. Labels are then assigned to peaks for further analysis starting with the higher magnetic field side of the ^{13}C spectrum.
A DEPT 135 measurement was then conducted to determine functional group of each peak of the ^{13}C spectrum. The result is shown in Fig. 2-1-③.

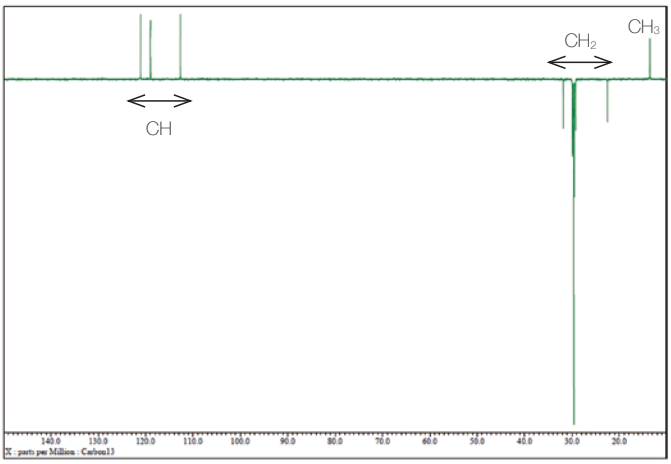


Fig. 2-1-③ DEPT 135 spectrum of urushiol

Number of accumulations: 512. Measurement time: About 26 min.

The DEPT method is to determine ^{13}C functional group. DEPT 135 indicates CH_3, CH with upward peaks and CH_2 with downward peaks, with no observation of quaternary carbon. Using this result combined with the acquired ^{13}C spectrum, it can be determined that label A corresponds to the methyl group, labels B to H correspond to the methylene group, labels I, J, and K correspond to the methine group, and labels L, M, and N correspond to quaternary carbon. Furthermore, with the chemical shift values also taken into account, labels I, J, and K are estimated to be peaks of aromatic rings. Next, a heteronuclear multiple-quantum coherence (HMQC) spectrum was measured to associate ^{1}H and ^{13}C peaks. The result is shown in Fig. 2-1-④.

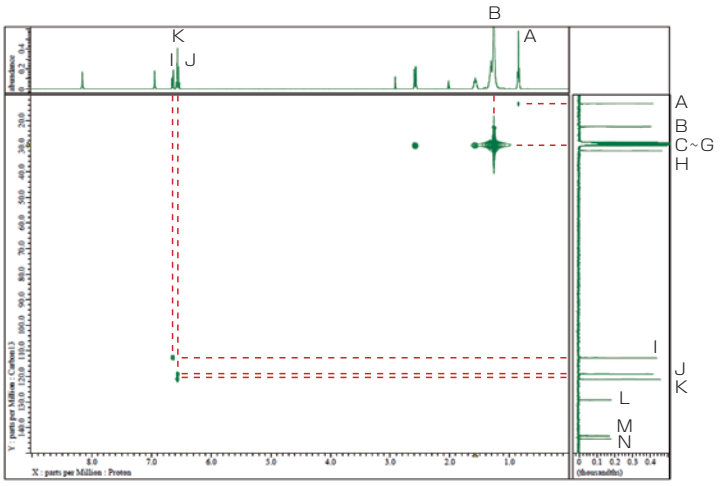


Fig. 2-1-④ HMQC spectrum of urushiol

Number of accumulations: 8. Measurement time: About 1 h.
^{1}H-^{13}C are indicated by auxiliary dash lines in red.

The HMQC method is to observe a correlation peak between the directly bonded ^{1}H and ^{13}C. As a result, ^{1}H labeling was performed in accordance with the bonded ^{13}C. As the labeling of peaks from C to H is difficult because of small peak splitting due to chemical shifts, the heteronuclear single-quantum coherence (HSQC) method was used as it provides better separation performance while offering ^{1}H-^{13}C bonding information as with HMQC. The result is shown in Fig. 2-1-⑤.

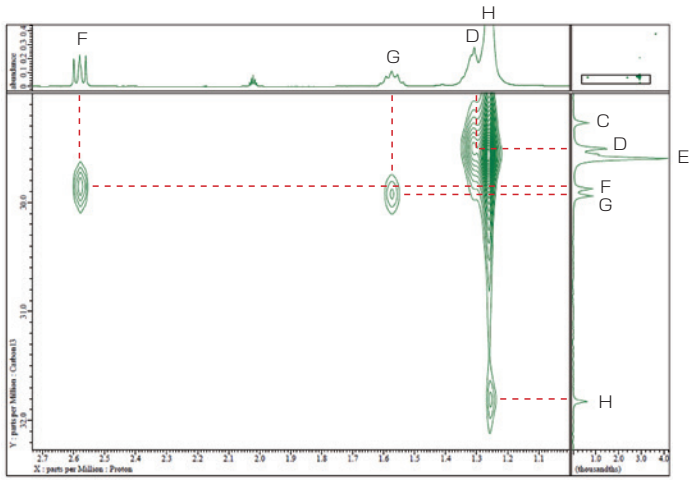


Fig. 2-1-⑤ HSQC spectrum of urushiol

Number of accumulations: 4. Measurement time: About 3 h. With a narrow ^{13}C range (vertical axis) and an increased number of measurement points, a spectrum with high resolution (high separation) was acquired. For the vertical axis of the ^{13}C spectrum, in order to facilitate spectral analysis, a DEPT45 spectrum with no observation of quaternary carbon (solvent) was displayed with auxiliary dash lines in red.

D, F, G, and H peaks were labeled based on the measurement result of the HSQC spectrum.
Next, ^{1}H-^{1}H correlation measurement called COSY (COrrelation SpectroscopY) was conducted.

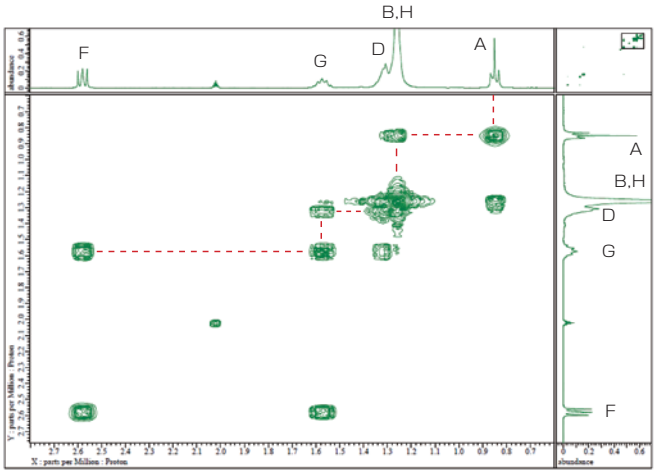


Fig. 2-1-⑥ COSY spectrum of urushiol

Number of accumulations: 2. Measurement time: 16 min. The higher magnetic field side was enlarged and auxiliary dash lines in red were added.

The COSY method is to observe a correlation peak between ^{1}H of neighboring functional groups. Based on the measurement result, an estimation was made of the bonding of A-(the peak at 1.26 ppm: including B and H)-D-G-F starting from the methyl group A. Based on the integral values and functional group information, this signifies a CH_3-$(CH_2)_{14}$ structure. This was followed by a heteronuclear multiple bond coherence (HMBC) measurement.

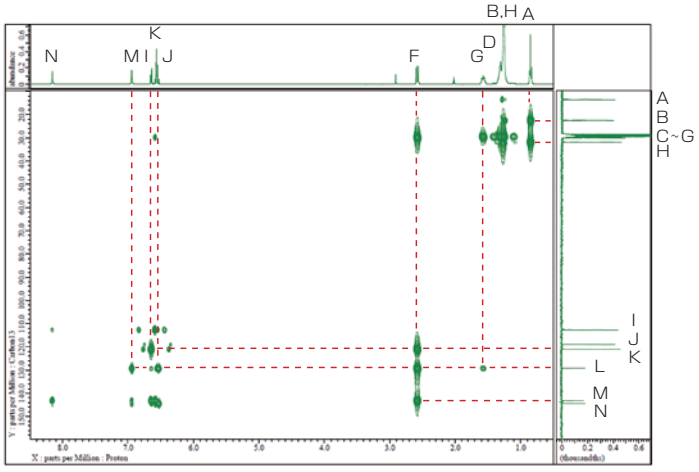


Fig. 2-1-⑦ HMBC spectrum of urushiol

Number of accumulations: 4. Measurement time: About 32 min. Auxiliary dash lines in red were added.

The HMBC method is to observe a correlation peak between ^{1}H and ^{13}C in a two- or three-bonding state. From the result of this measurement combined with the aforementioned COSY result, an estimation was made of the bonding of A-(the peak at 1.26 ppm: including B and H)-D-G-F-L-(K,M). FT-IR analysis result suggests that the sample contains aromatic ring structures, and the chemical shift values of the ^{13}C spectrum also show a probability that labels I to N are aromatic rings. Only three bonding of HMBC correlation peaks of aromatic rings are observed while no two- or four-bonding are observed. Taking this result into account, the structure was estimated from the molecular formula as shown in Fig. 2-1-⑧. Where, B and H were determined based on the HMBC measurement result and the ^{13}C peak assignment of the general alkyl group.

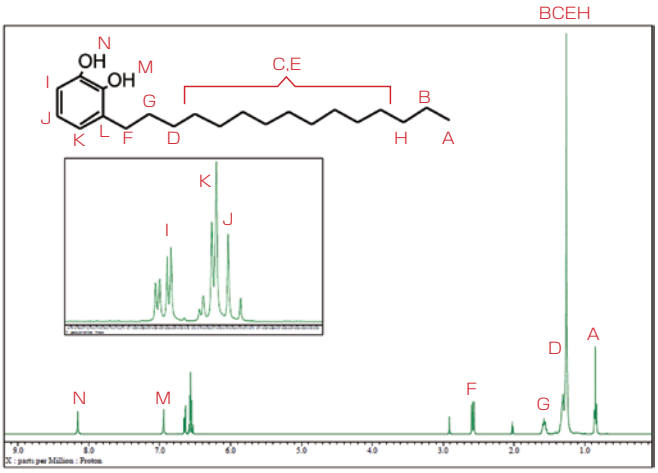


Fig. 2-1-⑧ ^{1}H NMR spectrum and estimated structure of urushiol

Finally, an enlarged view of peaks I, J, and K of the ^{1}H spectrum is shown in Fig. 2-1-⑨.

Peaks are largely split at the three-bonding sites $^{3}J_{IJ}$ and $^{3}J_{JK}$, and modestly split at the four-bonding site $^{4}J_{IK}$. The reason for the asymmetric display of the peaks here is the roof effect caused by small differences of chemical shifts in the bonded peaks.

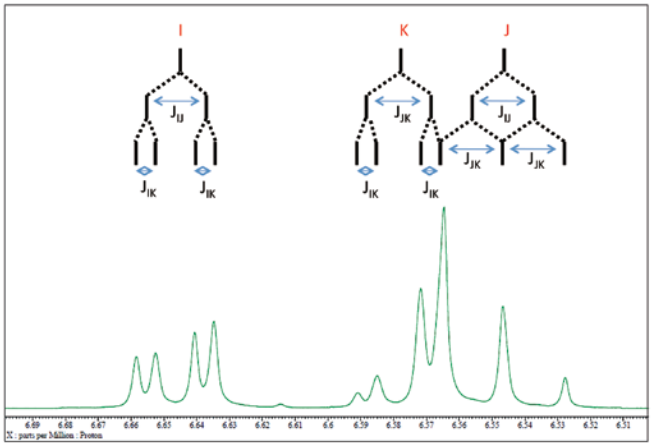


Fig. 2-1-⑨ Enlarged view of peaks I, J, and K of the ^{1}H spectrum

2-2 Analysis by FT-IR

FT-IR measurement was conducted to identify atomic group (functional group) of the molecules from a vibrational spectrum. The results of the FT-IR analysis are shown in Figure 2-2-①.

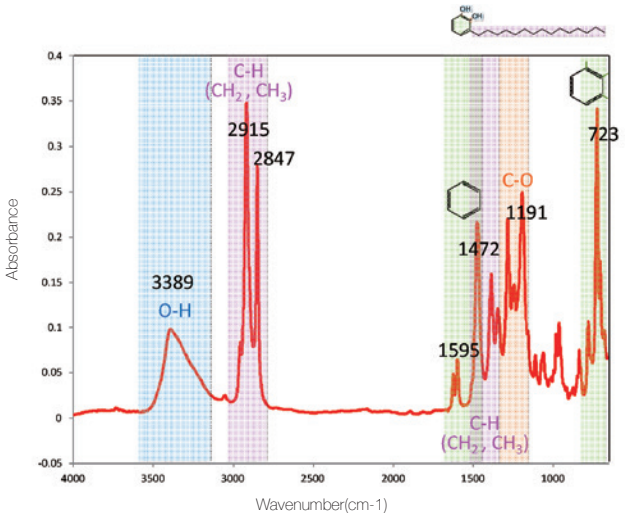


Fig. 2-2-① FT-IR analysis result

Absorption peaks were detected in the proximity of 3389 cm^{-1} for O-H stretching vibration of catechol rings, in the proximity of 2915, 2847, and 1472 cm^{-1} for C-H stretching vibration and bending vibration of the alkyl side-chain, and at 1595 and 723 cm^{-1} for skeletal vibration including C=C stretching vibration of aromatic rings and for =C-H out-of-plane bending vibration. Thus, absorption peaks of respective functional groups specific to 3-pentadecylcatechol were successfully observed by FT-IR.

2-3 Analysis by MS

A mass spectrum provides an understanding of the molecular mass (molecular weight) from molecular ions detected on the highest *m/z* side (may not be detected) and the molecular structure from fragment ions. Figure 2-3-① shows the mass spectrum obtained by electron ionization (EI).

The molecular ions of 3-pentadecylcatechol were detected at *m/z* 320, and fragment ions characteristic of alkylcatechol were also detected at *m/z* 123. Through electron ionization, thermoelectrons cause electron desorption in molecules and fragmentation is promoted by the simple cleavage triggered by the generated unpaired electrons. At this time, the tendency of electron desorption is the highest for non-bonding electrons (n-electrons) of unshared electron pairs (lone pairs), followed by π(pi)-electrons. The lowest is σ (sigma)-electrons. Figure 2-3-② illustrates the fragmentation process, in which π-electrons of the catechol rings desorb and then β (beta)-cleavage triggered by unpaired electrons generates 2,3-dihydroxybenzyl cation (*m/z* 123). [2-3-①]
Thus, the molecular mass and structure of 3-pentadecylcatechol were successfully confirmed by using MS .

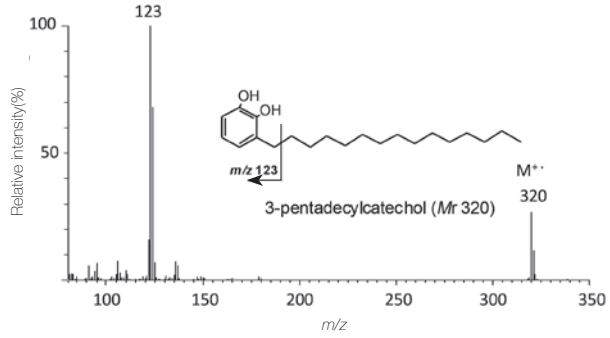
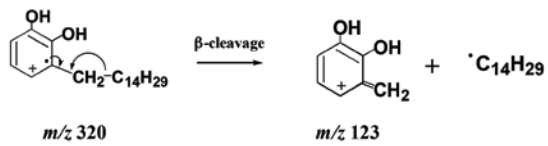


Fig. 2-3-① Mass spectrum obtained by electron ionization (EI)



① Electron deposition tendency: n-electrons of unshared electron pairs (lone pairs) > π-electrons >> σ -electrons
② Simple cleavage caused by unpaired electrons

Fig. 2-3-② Fragmentation process due to electron ionization (EI)

References

2-3-① N. Niimura, *Int. J. Polym. Anal. Charact.*, **17**, 540 (2012).

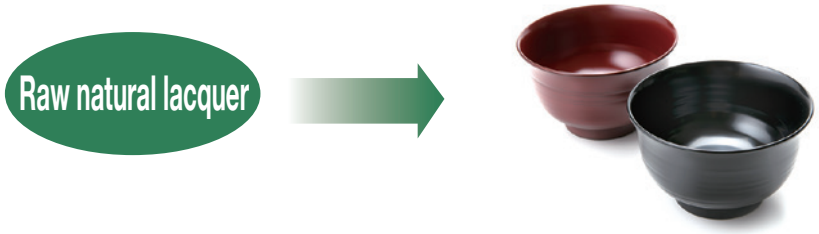
3 Analysis of Natural Lacquer during Hardening: “Time-Course Analysis during Film Formation” 3-①)

After natural lacquer was applied to a glass plate, this glass plate was dried in a humidity-controlled chamber with relative humidity of 70 % at 25°C. Then, the hardening process was observed using pencil hardness testing, which shows film hardness in pencil hardness, and XPS.

3-1 Hardness Measurement by Pencil Hardness Testing

The results of pencil hardness testing are shown in Table 3-1-①.
It was observed to be the softest with a pencil hardness of 4B on day 1, hardened to HB on day 7, and the hardest with 7H on day 50.

Table 3-1-① Results of pencil hardness testing



Drying time (days)	1	2	3	7	10	15	22	30	40	50
Pencil hardness	4B	3B	2B	HB	HB	F	H	2H	4H	7H

3-2 Analysis by XPS

3-2-1 Composition Analysis

XPS is capable of analyzing the composition of materials based on the binding energy value on a wide spectrum. In addition, the integrated intensity of respective peaks is calculated, and quantitative analysis can be performed by the relative sensitivity factor method.

Figure 3-2-1-① shows a wide spectrum after drying for 49 days. Auger and photoelectron peaks for oxygen, nitrogen and carbon were detected. The photoelectron peak intensity of oxygen and carbon was integrated and the ratio of oxygen to carbon was calculated. Figure 3-2-1-② shows the time course of the ratio of oxygen to carbon (O/C atomic ratio), which indicates that the ratio increases along with hardening as the film dries. As mentioned before, natural lacquer films were reported to be hardened through oxidative polymerization. Therefore, the increase of the ratio of oxygen to carbon is regarded to be caused by oxidative polymerization. These results exemplify that oxidative polymerization proceeds at a relatively high pace in the first 25 days of drying and the pace gradually decreases thereafter.

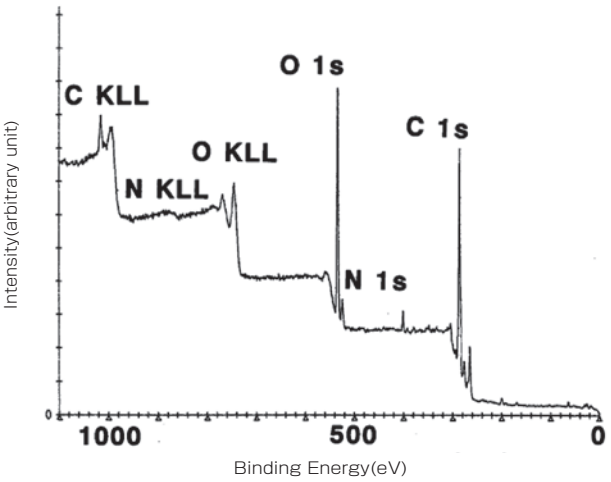


Fig. 3-2-1-① Wide spectrum after drying for 49 days

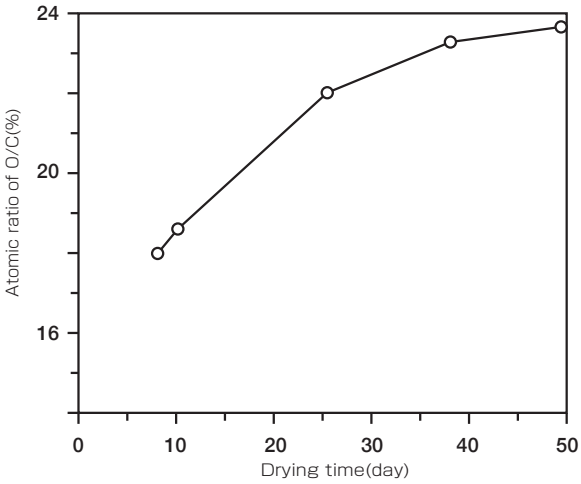


Fig. 3-2-1-②Time course of the ratio of oxygen to carbon

3-2-2 Chemical Bonding State Analysis

Chemical shift values were confirmed from the narrow spectrum of XPS, and chemical bonding states were analyzed.

As a result, in addition to C-C and C=C bonding, C-OH and C-O-C chemical bonding states were observed from the C1s spectrum.[3-②,③] Figure 3-2-2-① exemplifies C1s spectra (narrow spectra) after drying for 8 days (blue line) and 49 days (red line). In this example, it was confirmed that C-OH and C-O-C chemical bonding states after 49 days were larger than those after 8 days, which exemplifies that the oxidative polymerization shown in Figs. 1-② and 1-③ proceed considerably in 41 days.

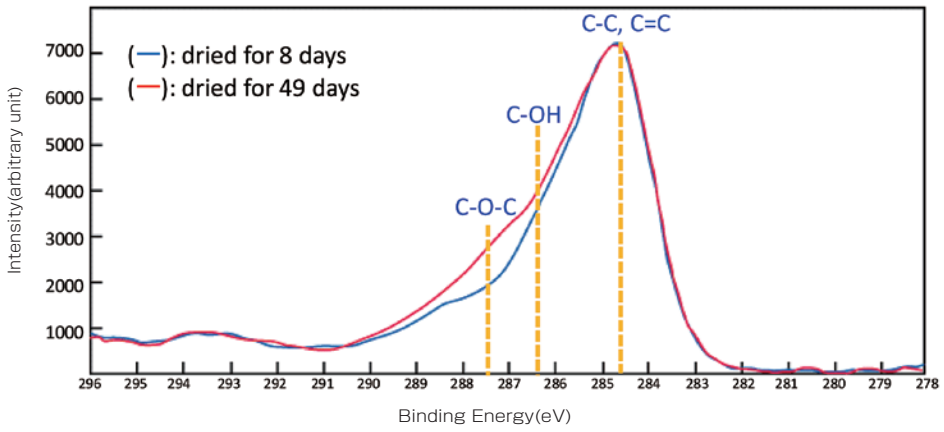


Fig. 3-2-2-① C1s spectra (narrow spectra)

References
3-① N. Niimura, Y. Iijima and T. Miyakoshi, *Surf. Interface Anal.*, 24, 237 (1996).
3-② N. Niimura, *The Industrial Coating* (in Japanese), 185, 64 (2003).
3-③ N. Niimura, *The Industrial Coating* (in Japanese), 217, 53 (2009).

4 Comparison of Natural Lacquer Films Formed under Different Conditions: “Comparative Analysis”

As natural lacquer dries through oxidative polymerization by enzyme, moderate temperature (20 - 25°C) and humidity (70 - 80 %) are required. Oxygen is supplied to the natural lacquer film from the surface. Thus, when the film is thick, the oxygen that reaches inside is insufficient, and only the surface is dried resulting in surface wrinkles and poor finishing. Lacquer workers paint as thinly as possible and repeatedly coat to avoid such wrinkles.
In this chapter, three types of natural lacquer films formed under different coating and drying conditions (see Table 4-①) are analyzed and compared.

Table 4- ① Film forming conditions of Raw natural lacquer ① - ③

Name	Conditions		Film thickness (μm)	Drying conditions	
	Wrinkle	Discoloration *		Temperature (°C)	Humidity (% RH)
Raw natural lacquer ①			30	25	80
Raw natural lacquer ②	○		75	25	80
Raw natural lacquer ③	○	○	80	25	55 ↓ 65
				Finally heated at 160°C	

Discoloration*: The surface has become slightly discolored

4-1 Surface Observation

4-1-1 Optical Microscopy, Surface Shape Measurement and Hardness Measurement

Fig. 4-1-① External appearance of Raw natural lacquer ①

Figure 4-1-① shows the external appearance of Raw natural lacquer ①.

Images of three natural lacquer surface morphologies taken by a digital microscope are shown in Fig. 4-1-②.
Wrinkles were observed on Raw natural lacquers ② and ③ with their patterns being different.

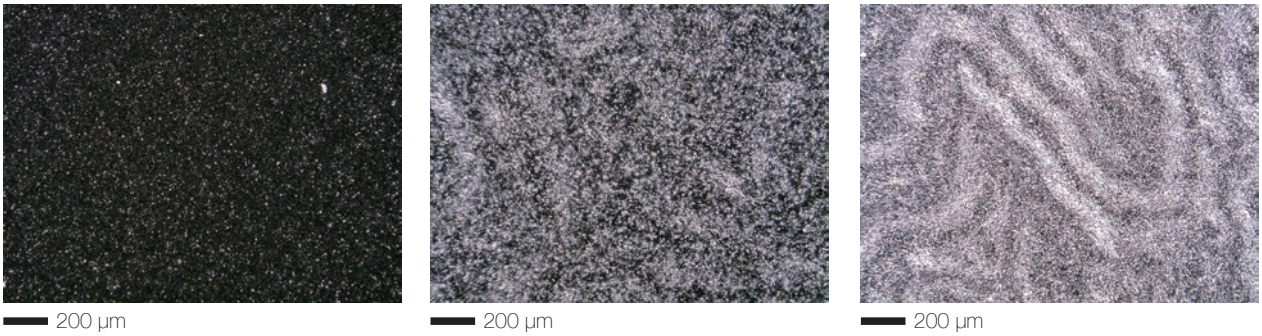


Fig. 4-1-② Digital microscope images (Raw natural lacquers ①, ②, and ③ from the left)

Figure 4-1-③ shows the results of the calculation of the arithmetic average roughness (Ra) based on surface shape measurement using a scanning white-light interferometer. Ra is obtained by extracting the standard length from the roughness curve in the direction of its average line, adding the absolute deviation values from the average line to the measurement curve, and averaging the sum. It is found that the drying conditions of Raw natural lacquer ① are appropriate because Ra is 0.135 μm and there are no wrinkles. Meanwhile, Raw natural lacquer ② and Raw natural lacquer ③ exhibited an Ra value of 9.621 μm and 4.636 μm, respectively. These results indicate that the coating and drying conditions of the lacquers are inappropriate.

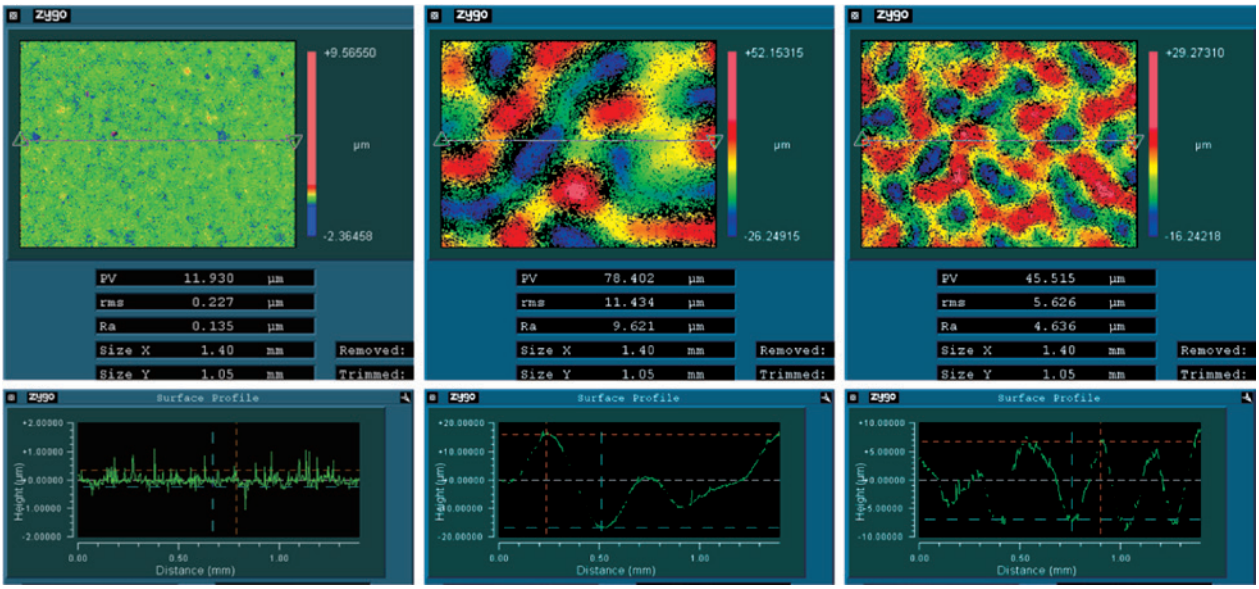


Fig. 4-1-③ Result of surface shape measurement (Raw natural lacquers ①, ②, and ③ from the left)

Figure 4-1-④ and Table 4-1-② also present optical microscope images and hardness measurement results by ultra-micro indentation hardness testing, respectively. Based on the H_{IT} (indentation hardness) figures in the table, the hardest was Raw natural lacquer ③, followed by Raw natural lacquer ② and Raw natural lacquer ①. H_{IT} standard deviation was also larger with Raw natural lacquer ③ than the others, which is regarded to be caused by a thermal drying process that was added only to Raw natural lacquer ③ due to its being insufficiently hardened through polymerization and oxidation, resulting in different degrees of drying among micro areas.

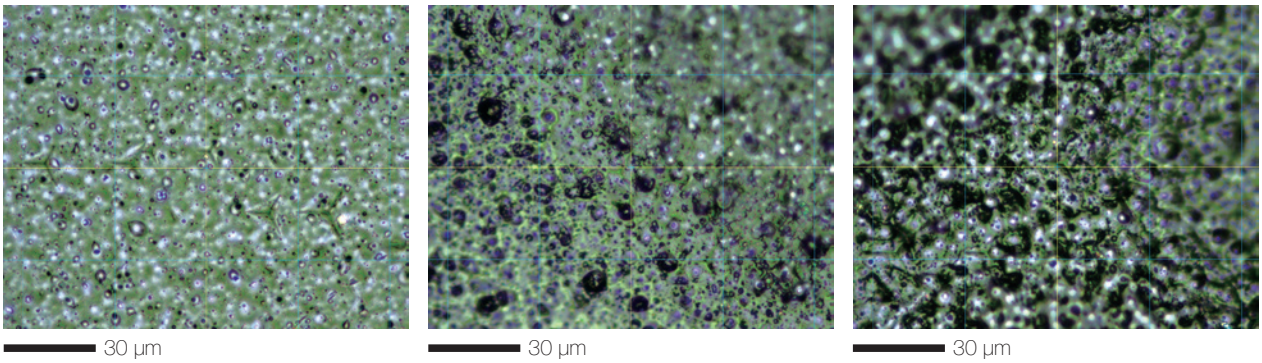


Fig. 4-1-④ Optical microscope images (Raw natural lacquers ①, ②, and ③ from the left)

Table 4-1- ② Hardness measurement results

Samples	Average		H_{IT} SD	H_{IT} MAX	H_{IT} MIN
	h_max [nm]	H_{IT} [N/mm^2]			
Raw natural lacquer ①	2373.1	211.9	23.9	250.0	184.0
Raw natural lacquer ②	2208.6	258.5	13.2	275.3	245.9
Raw natural lacquer ③	2140.3	276.5	69.8	403.4	179.1

4-1-2 SEM Observation

Natural lacquer, subjected to coating is generally left in a humid atmosphere for drying, and it is also known that the coated surface shape and physical properties largely vary depending on the coating and drying conditions.[4-1-2-①)]
In the previous section, the relevant process has been examined through optical microscopy, surface shape measurement and hardness measurement.
It is also beneficial to make further investigation of the surface topography using SEM. Due to the nature of the signals acquired by SEM, it is possible to get a detailed representation of the specimen surface in three dimensions. Observation of natural lacquer specimens in field emission SEM (FE-SEM) requires use of low accelerating voltage and/or low vacuum conditions to overcome possible specimen charging and minimize electron beam damage.
In this section, we discuss observation of Raw natural lacquer ① - ③ using this SEM methodology.

4-1-2-1 Experimental

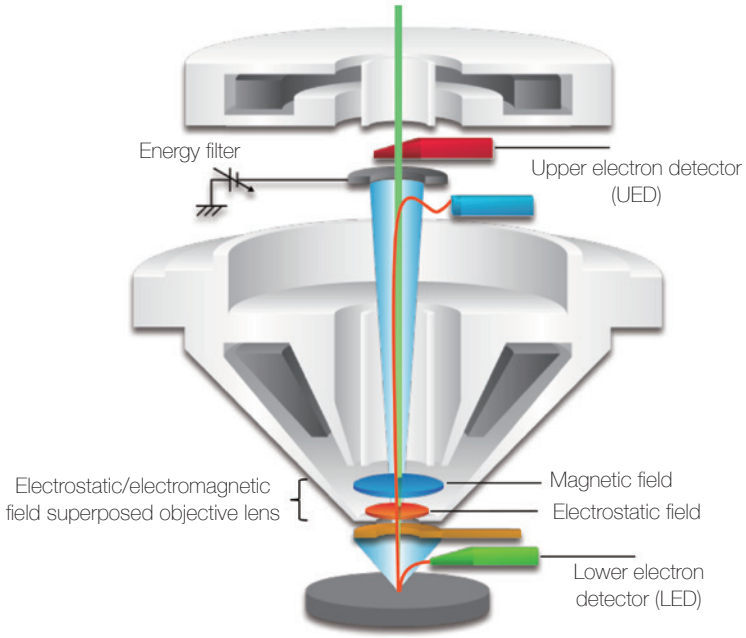


Fig. 4-1-2-① Schematic of objective lens and detectors

The FE-SEM adopts an electrostatic/electromagnetic field superposed objective lens. This lens is designed to reduce chromatic aberration of the incident electron beam by generating an electrostatic field beneath the magnetic field. In particular, it has an excellent spatial resolution, especially at low accelerating voltage. [4-1-2-②)] Further, as illustrated in Fig. 4-1-2-①, an energy filter placed just below the upper electron detector (UED) enables energy selection of electrons emitted from a sample. With this filter, UED can detect high-energy electrons (backscattered electrons) only, which allows the observation of the surface composition of natural lacquer. Located next to a sample is the lower electron detector both (LED), which provides a better three dimensional view of the specimen due to its relative position with respect to the sample. Since these detectors both can be used at low accelerating voltage, measurement (observation) can be performed with suppressed charging and without conductive coating.

The FE-SEM has a low vacuum function that allows observation of non-conductive specimens without any need for coating.This technique is effective in reducing charging on insulating materials as with the aforementioned low accelerating voltage method. Also, this technique can reduce charging at high accelerating voltage, enabling not only observation but also elemental analysis requiring high accelerating voltage, without conductive coating. [4-1-2-③)]

4-1-2-2 Results and Discussion

Figure 4-1-2-② shows the results of morphological observations of Raw natural lacquers ① - ③ at a low accelerating voltage.

Observation conditions — Accelerating voltage: 1 kV, Detector: LED

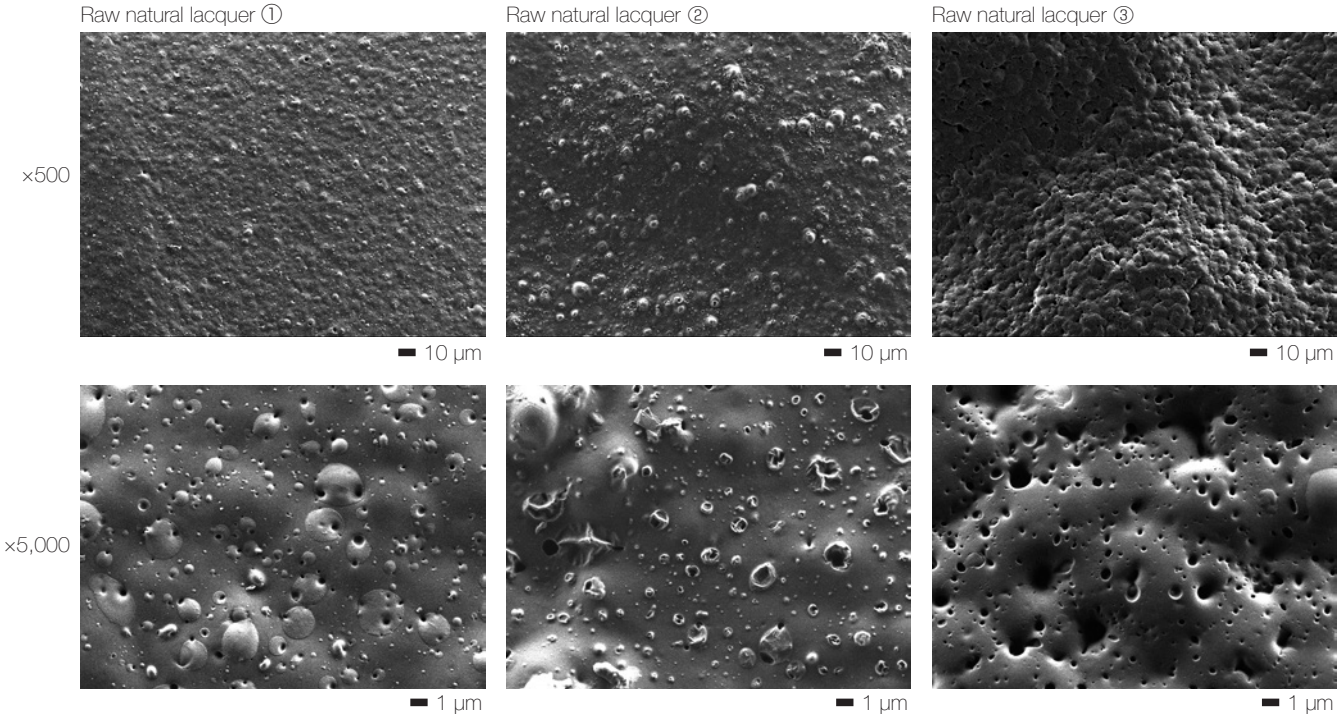


Figure 4-1-2-② shows the results of morphological observations of Raw natural lacquers ① - ③ at a low accelerating voltage.

The SEM observation results indicate that the number of micropores on the sample surface is the smallest with Raw natural lacquer ①, followed by Raw natural lacquer ② and Raw natural lacquer ③. This is explained as follows: Raw natural lacquers ② and ③ contain more water droplets due to their thicker film. Since micropores are generated due to the evaporation of water droplets during the drying process, the number of micropores on Raw natural lacquer ② and ③ is larger than that on Raw natural lacquer ①. Furthermore, Raw natural lacquer ③ was heated at the final step of drying. Therefore, the number of micropores caused by water evaporation is larger than that on Raw natural lacquer ②.

References
4-1-2-① T. Miyakoshi, Lacquer Studies (in Japanese), Maruzen Publishing (2016).
4-1-2-② M. Suga, *et. al., Progress in solid state chemistry,* **42**, 7, (2014).
4-1-2-③ Y. Hasebe, et. al., JEOL SEM Users' Meeting (in Japanese) (2016).

4-2 Cross-Sectional Observation 4-2-①～③)

4-2-1 Experimental

Raw natural lacquers ①-③ were first embedded into epoxy resin and then their cross-section preparation was performed by an ultra-microtome. A low-vacuum method was applied to perform cross-sectional observation and elemental analysis without a conductive coating. The low-vacuum method is a technique of reducing the degree of vacuum to a desired level by introducing N_2 gas into a SEM sample chamber in order to reduce charging. When N_2 gas is introduced to an evacuated SEM chamber, N_2 molecules are accumulated in the chamber. When an electron beam collides with the N_2 molecules, N^+ ions are generated. Such ions will then neutralize a negatively-charged sample surface and reduce charging.

4-2-2 Measurement Results

SEM images of cross sections of the three raw natural lacquers, sectioned by an ultramicrotome, acquired by using a low-vacuum backscattered electron detector with no conductive coating, are shown in Fig. 4-2-①.

In the low-magnification images on the upper part, more micropores are seen on the back side of Raw natural lacquer ②, whereas micropores are dispersed over the entire cross section of Raw natural lacquer ③. The high-magnification images on the lower part show that micropores become less circular and more random-shaped along with changes in drying conditions in Raw natural lacquer ① to Raw natural lacquer ③.

-Observation conditions-
Accelerating voltage: 5 kV, Low-vacuum: 100 Pa, Detector: Low-vacuum backscattered electron detector

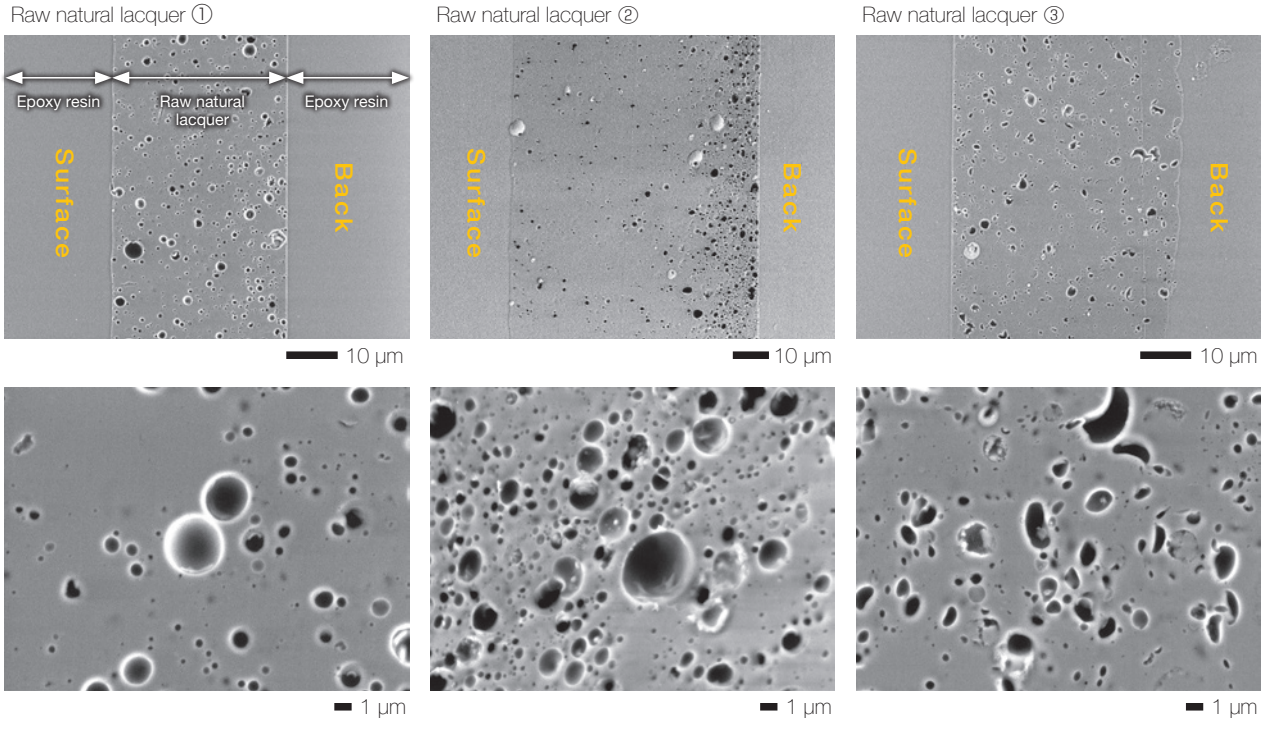


Fig. 4-2-① Low-vacuum backscattered electron images of cross sections (milled by ultramicrotome) of raw natural lacquers using SEM

Elemental analysis was conducted for the pores in raw natural lacquers observed above. The results are shown in Fig. 4-2-②.
For the analysis, the JEOL Dry SD™ EDS detector "JED-2300" was used.
The result shows that, for observation of Raw natural lacquer ①, gray micropores were seen more than black micropores. More K and Ca were detected in gray micropores, which were greater in number than black micropores. On the other hand, in Raw natural lacquers ② and ③, black micropores were greater in number and K and Ca volumes were smaller than in gray micropores. These K and Ca components are absorbed from soil along with water and are concentrated in the micropores as the water content evaporates.

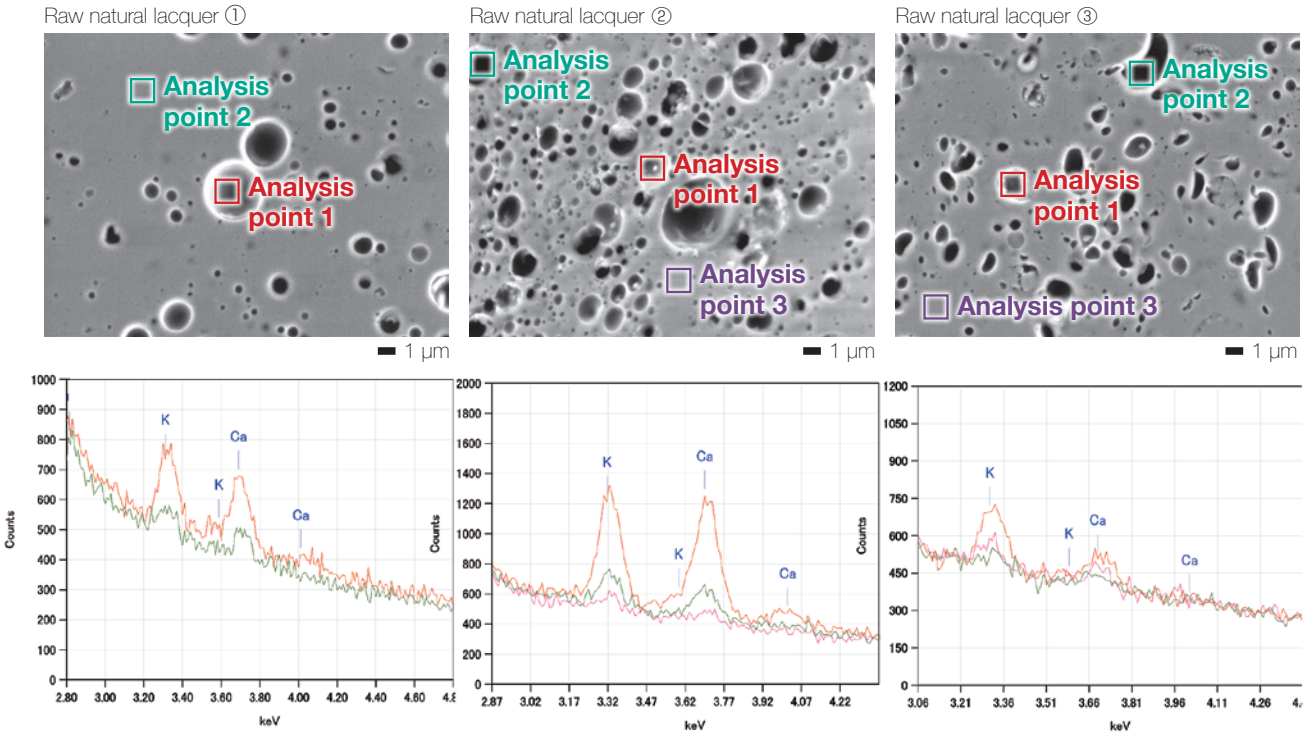


Fig. 4-2-② EDS point analysis result of cross sections (prepared by ultramicrotome) of raw natural lacquers

As there were large differences in the number of pores and their shapes depending on the drying conditions, particle analysis was also conducted for quantitative analysis (Fig. 4-2-③). For the particle analysis, the capabilities of the EDS detector (JED-2300) were utilized for calculation with threshold values set for the micropore contrast in the low-vacuum backscattered electron images. Points marked in yellow are micropores detected by the particle analysis. The sizes and degree of circularity decreased in the order of Raw natural lacquer ①, Raw natural lacquer ②, and Raw natural lacquer ③.

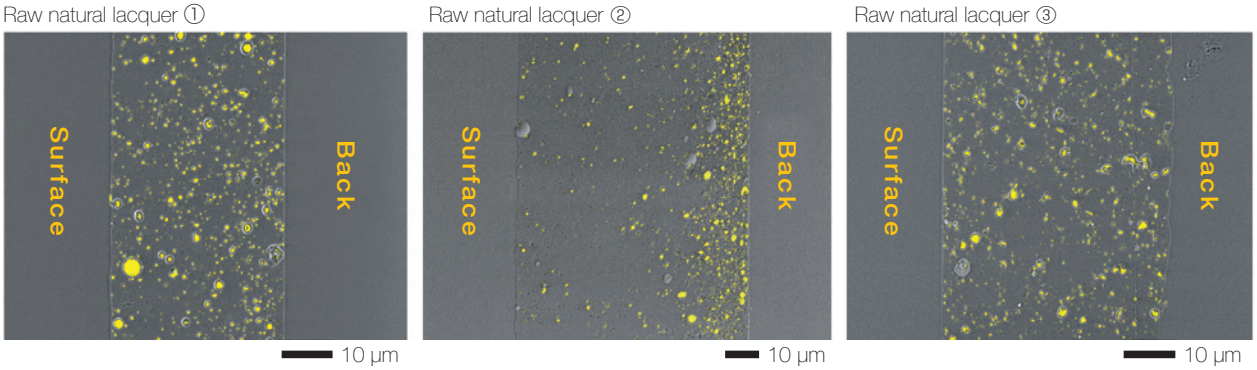


Fig. 4-2- ③ Micropore dispersion in Raw natural lacquer ① - ③ detected by particle analysis

Table 4-2- ① Micropore shape evaluation (average area and circularity)

	Area (Pixel)	Circularity
Raw natural lacquer ①	26.27	0.456
Raw natural lacquer ②	20.01	0.391
Raw natural lacquer ③	19.97	0.357

References
4-2-① M. Suga, *et. al., Progress in solid state chemistry,* 42, 7, (2014).
4-2-② Y. Hasebe, et. al., JEOL SEM Users' Meeting (in Japanese) (2016).
4-2-③ Y. Sakuda, *et.al., Zeolite,* 34, 24, (2017).

4-3 Radical Amount Measurement

ESR measurement was performed to identify the correlation between film forming conditions and radical amount.

4-3-1 Measurement Conditions

Microwave frequency: 9439 MHz
Microwave Power: 1 mW
Sweep magnetic field: 336±10 mT
Magnetic field modulation width: 0.25 mT
Sweep time: 1 min
Time constant: 0.1 s
Amplitude gain: 100
Temperature: room temperature

4-3-2 Sampling

Raw natural lacquer ① 13.5 mg
Raw natural lacquer ② 13.2 mg
Raw natural lacquer ③ 11.0 mg
Each sample was scraped off from a glass plate using a spatula and placed into an ESR sample tube for measurement.

4-3-3 Measurement Results

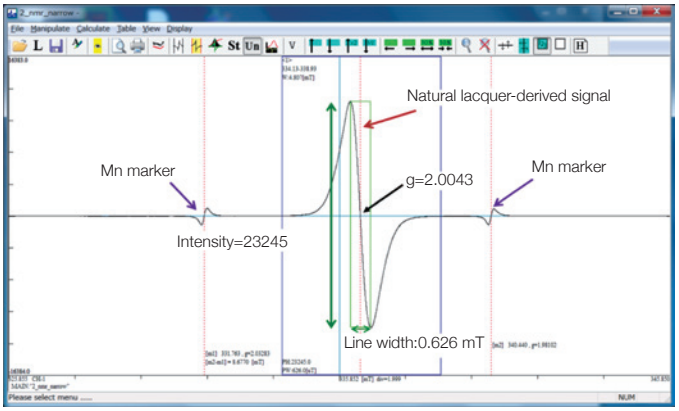


Fig. 4-3-① ESR spectrum of Raw natural lacquer ②

An ESR spectrum is shown in the form of differential waveforms. The JEOL ESR system features simultaneous measurement of the Mn marker (Mn^{2+}) incorporated in the system. Since this measurement enables the correction of the magnetic field, an accurate g value can be obtained for identification of radical types. For this sample, the result of g=2.0043 indicates that it contains phenoxyl radical.
In order to examine whether any other radicals are contained in the sample, a supplementary measurement was conducted with a broader sweep magnetic field width. The result is shown in Fig. 4-3-②.

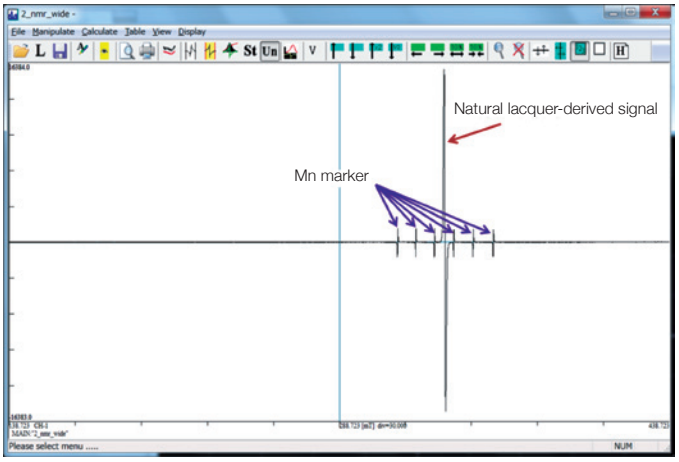


Fig. 4-3-②
ESR spectrum acquired from Raw natural lacquer ② using a wide magnetic field

As shown in Fig. 4-3-②, six signals from the Mn^{2+} marker and natural lacquer-derived signals detected in Fig. 4-3-① were observed. No other significant radical signals were detected.

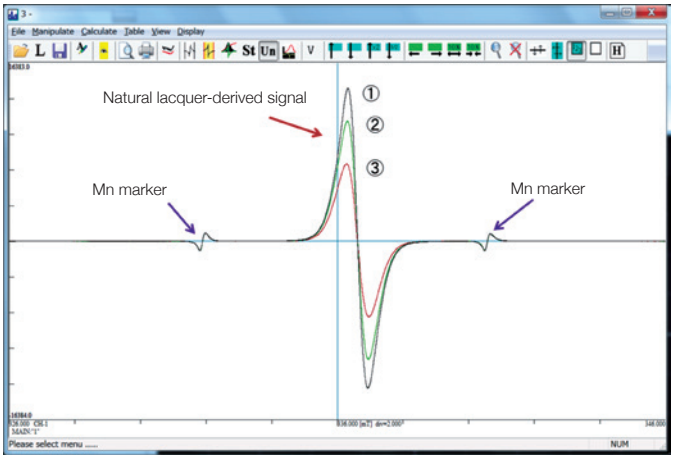


Fig. 4-3-③ Comparison of ESR spectra of Raw natural lacquers ①-③

Next, the other samples were measured with the same measurement conditions as Fig. 4-3-①. The results are shown in Fig. 4-3-③. Radicals of the same g value were detected from any sample. However, there were differences in signal line width (ΔH), as well as apparent differences in the radical amount of the samples. ΔH and radical amount of each sample were converted into density based on sample quantities as shown in Table 4-3-①. Generally, one radical molecule is counted as one spin; radical amount is displayed as spin amount. Therefore, the unit of Spins/mg is used here.

Table 4-3- ① Comparison of ΔH and spin density obtained from ESR spectra of Raw natural lacquers ① - ③

No.	ΔH (mT)	Spins/mg
①	0.615	1.8×10^{14}
②	0.645	1.5×10^{14}
③	0.685	1.3×10^{14}

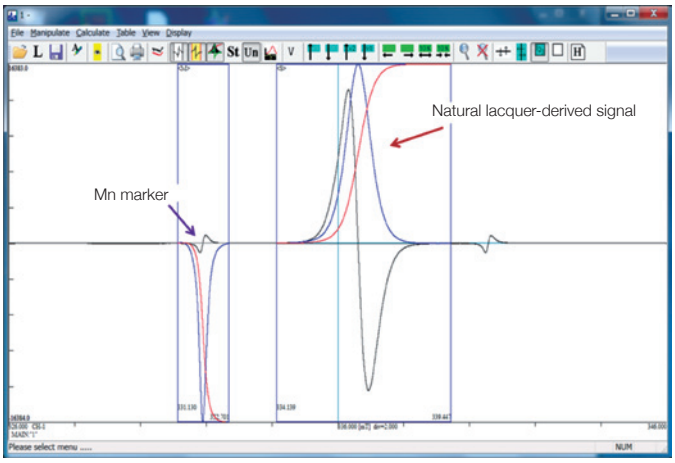


Fig. 4-3-④ Example of peak area quantification by double integrations

For quantitative evaluation, each spectrum was integrated twice to obtain its peak area, as shown in Fig. 4-3-④. At this time, the simultaneous measurement of a certain amont of Mn marker enabled correction and improved the quantification accuracy. In order to obtain the absolute quantity of radicals, coal, whose spin number is known, was used as a standard sample to determine each sample's spin number.

4-3-4 Summary

It was revealed that natural lacquer films still contain radicals even after about one year from film formation. It was regarded that, from the measured g-value, that the radicals are urushiol radicals generated from urushiol. Radical amount was the largest in Raw natural lacquer ① and the smallest in Raw natural lacquer ③. As it is already known that polymerization of natural lacquer films takes several years,[4-3-①,②], these measurement results suggest that oxidative polymerization of Raw natural lacquer ① has been more advanced.
ΔH value was the largest in Raw natural lacquer ③ and the smallest in Raw natural lacquer ①. This order correlates with the results of hardness testing by an ultra-micro indentation hardness tester in which the hardness was highest with Raw natural lacquer ③, followed by Raw natural lacquer ② and Raw natural lacquer ① as shown in Table 4-1-②; because the ΔH value reflects the kinetic properties of the radical.

References

4-3-① J. Kumanotani, Natural Lacquer - Next Generation Paint - Lecture Complication (in Japanese), Sasaki Printing & Publishing (1983).
4-3-② What is Urushi?, A. Yamagishi (in Japanese) http://urushi.com/yamagishi/urushinohanashi.htm

4-4 Comparison of Functional Groups

4-4-1 FT-IR

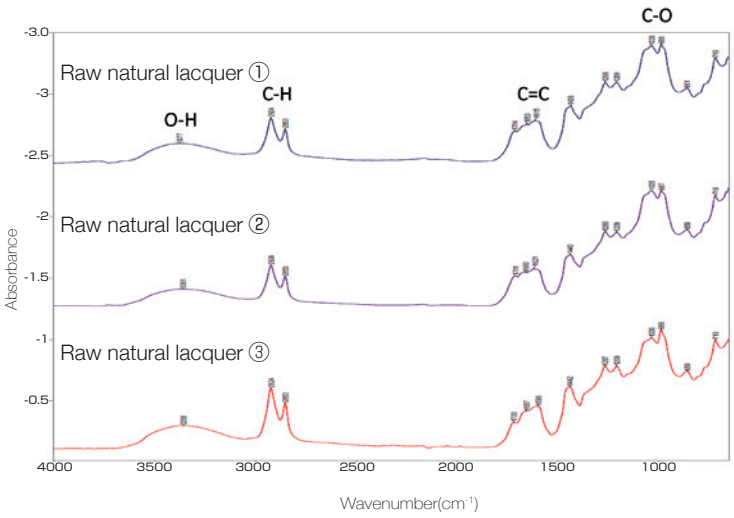


Fig. 4-4-1-① Result of FT-IR analysis

Figure 4-4-1-① shows the result of FT-IR analysis. Absorption peaks of the functional groups (O-H, C-H, C=C, and C-O) characteristic of natural lacquer films were detected from the respective spectra. Although almost no difference was observed in each spectrum among the three samples, the C-O absorption peak (at near 989 cm^{-1}) detected from Raw natural lacquer ③ exhibited slightly higher intensity.

4-4-2 NMR

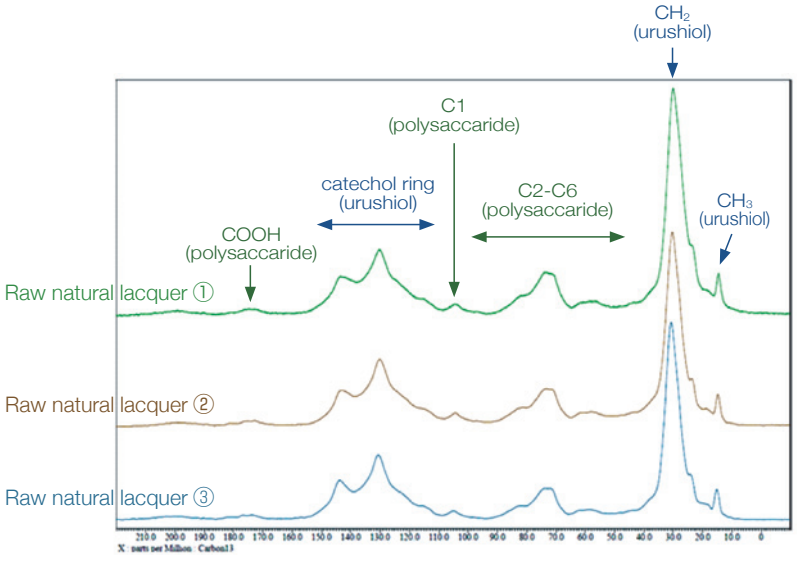


Fig. 4-4-2-① Solid-state ^{13}C NMR spectra of Raw natural lacquers ①-③

Figure 4-4-2-① shows solid-state ^{13}C NMR spectra acquired from Raw natural lacquers①-③. While peaks derived from urushiol and polysaccharide components were observed in each spectrum, no significant differences were found among the spectra.
(Each natural lacquer sample was peeled off from a glass plate, put into a solid-state NMR sample tube, and measured by the CPMAS method with high-speed spinning. NMR systems used were the JNM-ECZ600R spectrometer and 3.2 mm HXMAS probe.)

5 Heat Resistance Assessment of a Natural Lacquer Film: ”Thermal Degradation Analysis”

A natural lacquer film is not only elegant in appearance but also excellent in heat resistance. However, as shown in Fig. 5-①, when the lacquer film was heated to 300°C, it was observed by SEM that many pores were formed on the surface. In this chapter, the behaviors of a natural lacquer film with increased temperature are discussed by using various analysis methods.

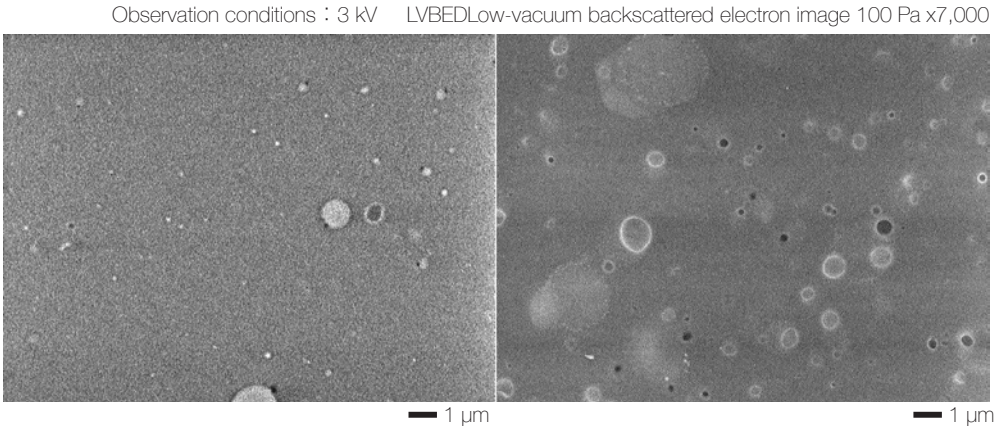


Fig. 5-① Surface change of a natural lacquer film before heating (left) and after heating at 300°C (right)

5-1 Thermal Analysis by TG/MS

TG/MS is a method to simultaneously perform analysis of physical property change and evolved gas analysis (EGA) of a sample under heating using a complex instrument system consisting of a mass spectrometer connected to a thermogravimeter.

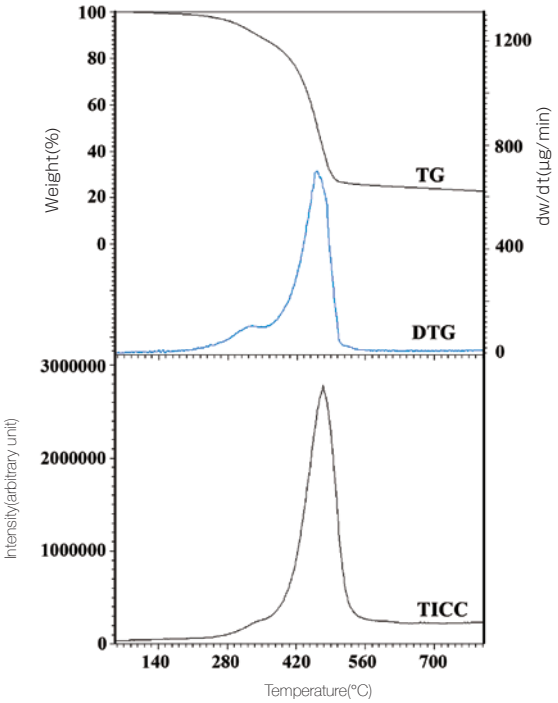


Fig. 5-1-① Result of TG/MS analysis

Figure 5-1-① shows the analysis result of the natural lacquer film obtained by using TG/MS. From the thermogravimetry (TG) curve and differential thermogravimetry (DTG) curve, it was found that the weight of the natural lacquer film gradually decreases between 200°C and 400°C, and there is a sharp decrease in the weight between 400°C and 500°C. Additionally, the total ion current chromatogram (TICC) exhibited peaks in two steps, which correlates with the TG and DTG curves. From 200°C to 400°C, the signal intensity is relatively low due to the small number of molecular ions and fragment ions generated from the evolved gas components. However, from 400°C to 500°C, the signal intensity increases sharply due to the rapid increase in evolved gas components. [5-1-①)]

References
5-1-① N. Niimura, *The Industrial Coating* (in Japanese), **194**, 51 (2005).

5-2 Evaluation of Temperature Dependence of Radicals by ESR

ESR is capable of making measurements while imposing a thermal load on a sample with use of a variable temperature controller incorporated. When using the ES-13070VT400 variable temperature controller for high temperatures, a sample can be heated up to a desired temperature level between 50°C and 400°C. The temperature dependence of radicals in the natural lacquer film was evaluated using this system.

5-2-1 Acquisition Conditions

Microwave frequency: 9056 MHz
Microwave power: 1 mW
Sweep magnetic field: 322.56±7.5 mT
Magnetic field modulation width: 0.25 mT
Sweep time: 1 min
Time constant: 0.1 s
Amplitude gain: 100
Initial temperature: Room temperature
Heating temperature: Between 50 and 300°C each 50°C at intervals of 10°C

5-2-2 Sample

Raw natural lacquer ① was used as a sample.

5-2-3 Measurement Result

Figure 5-2-① shows an ESR spectrum acquired from the natural lacquer film heated in cell from 50 to 300°C.

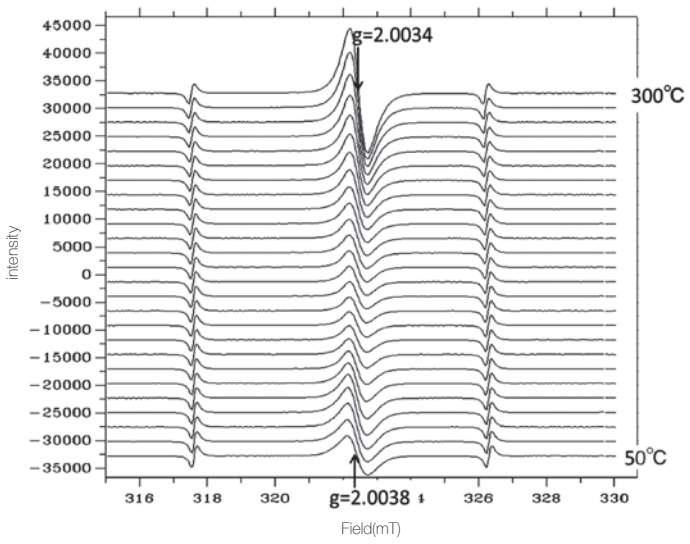


Fig. 5-2- ① ESR spectrum acquired from the natural lacquer film heated in cell

At room temperature, the g-value was g=2.0043, which has already changed to 2.0038 at 50°C. As the temperature continued to increase, the g-value decreased to reach 2.0034 at 300°C. This suggests that the primary radicals altered from phenolic radicals to carbon radicals.
The peak area of each spectrum shown in Fig. 5-2-① was obtained through double integration and the spin number of each sample was determined using coal, whose spin number is known, as a standard sample.

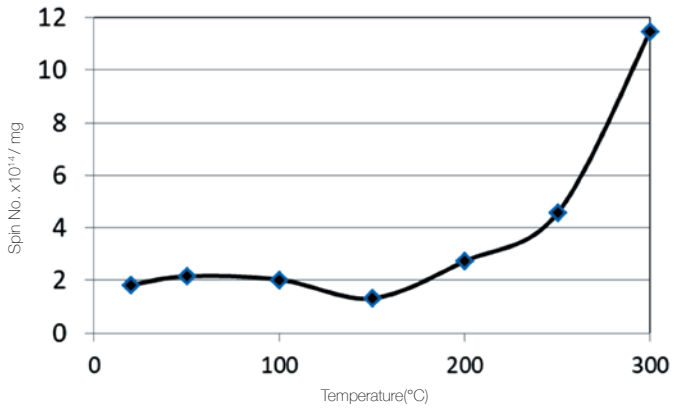


Fig. 5-2-② Temperature dependence of spin number for the natural lacquer film

The spin number at typical temperature level is shown in Fig. 5-2-②. As shown in Fig. 5-2-②, no significant changes in radical amount were observed up to around 200°C, but radical amount gradually increased at temperature higher than 200°C. However, the g-value of signals obtained at 200°C was approximately 2.003.
This result indicates that natural lacquer films are pyrolyzed at 200°C.

5-2-4 Summary

It was found that the property of the natural lacquer film is changed with heating at temperatures as low as 50°C. However, lacquerworks used as dishware can be exposed to temperatures of 80 to 90°C, reversibility against heating at such a relatively low temperature range is drawing interest.
While denaturation of natural lacquer was observed at low-temperature heating, the increase of radicals was moderate, whereas radicals at g=2.003, which is a sign of main-chain scission, increased at temperature higher than 200°C. These findings suggest that pyrolysis of the natural lacquer film takes place at around 200°C.

5-3 Analytical Pyrolysis by PyGC/MS

PyGC/MS is a method to analyze the chemical structure of the original polymer by instantaneously heating a polymer sample in a pyrolyzer and identifying the pyrolysis products using GC/MS. This method allows the measurement of micro samples that are insoluble in solvents, such as natural lacquer films, at a quantity of about 0.01-1 mg without special pretreatment.

5-3-1 Analysis of Pyrolysis Products at Different Pyrolysis Temperatures

The total ion current chromatograms (TICC) of the natural lacquer film obtained by pyrolysis at 200°C, 300°C, and 400°C are shown in Fig. 5-3-①.

From TICC at 200°C, pyrolysis products of glycoprotein such as butanoic acid (peak n-1) and hexanoic acid (peak n-2) were detected. Urushiol components such as 3-pentadecylcatechol (peak u-1) and 3-pentadecenylcatechol (peak 5) were detected to a small extent in TICC at 300°C and to a greater extent in TICC at 400°C. As these are saturated or monoenyl components, they are terminal groups of urushiol polymer.[5-3-①] Therefore, they were detected as pyrolysis products at a relatively low temperature range below 500°C, i.e. 300-400°C.

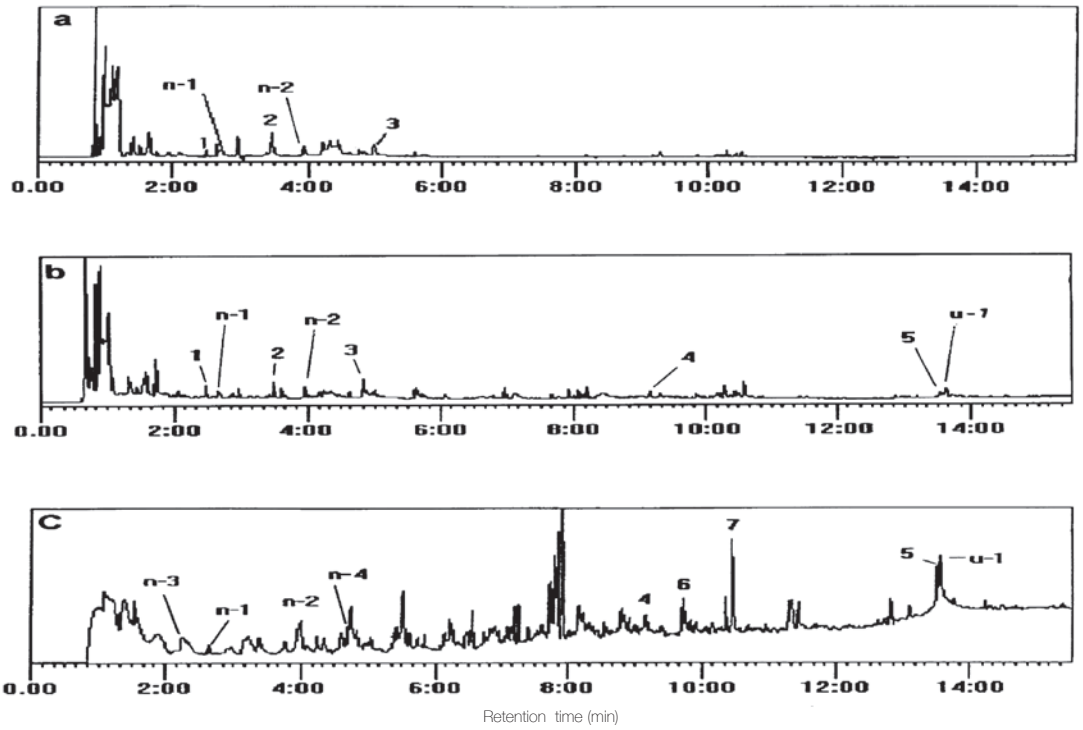


Fig. 5-3-① TICCs at different pyrolysis temperatures

a) Py temp.: 200°C
b) Py temp.: 300°C
c) Py temp.: 400°C
u-1: 3-pentadecylcatechol n-1: butanoic acid n-2: hexanoic acid n-3:toluene n-4: 4-methylphenol
1: 3-methyl-2-hexanol 2: 2-methoxy-1-phenylethanone 3: 1-isopropoxybutane 4: 3-hexylcatechol
5: 3-pentadecenylcatechol 6: 3-heptylcatechol 7: hexadecanoic acid

5-3-2 Separation of Pyrolysis Products by Two-stage Prylolysis

Figure 5-3-②-a shows TICC at 500°C. In the TICC, pyrolysis products of urushiol polymer skeleton were detected as being mixed with pyrolysis products of glycoproteins, saturated urushiol components and monoene components, which were detected at 200-400°C.

Therefore, the chromatogram is very complex and is difficult to analyze. In such a case, a two-stage pyrolysis method is useful. Pyrolysis at 500°C following the first pyrolysis at 400°C results in TICC with no indication of pyrolysis products generated below 400°C. Figure 5-3-②-b shows TICC obtained by the two-stage method, which is much simpler than that obtained by the single-stage method (conventional method) shown in Fig. 5-3-②-a. From the TICC, alkene and alkane from C5 to C19 were detected, and these were found to be pyrolysis products of polymer components coupled with urushiol via side chains.[5-3-②] Thus, the two-stage approach can make the analysis easier by simplifying complex TICC.

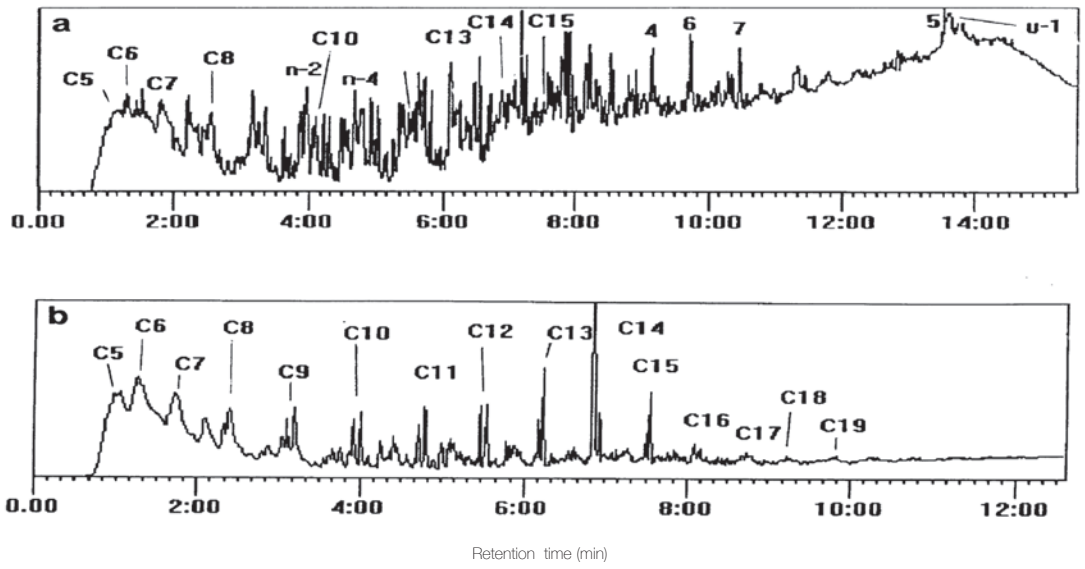


Fig. 5-3-② TICCs obtained by single-stage pyrolysis and two-stage pyrolysis (Py temp.: 500°C)

a) Single-stage pyrolysis (conventional method)
b) Two-stage pyrolysis (after heating at 400°C for 30 min)
u-1: 3-pentadecylcatechol n-2: hexanoic acid n-4: 4-methylphenol
4: 3-hexylcatechol 5: 3-(8-pentadecenyl)catechol 6: 3-heptylcatechol 7: hexadecanoic acid
C5: pentane C6: 1-hexene C7: heptane C8: 1-octene, octane C9: 1-nonene, nonane C10: 1-decene, decane
C11: 1-undecene, undecane C12: 1-dodecene, dodecane C13: 1-tridecene, tridecane C14: 1-tetradecene, tetradecane
C15: 1-pentadecene, pentadecane C16: 1-hexadecene, hexadecane C17: 1-heptadecene, heptadecane
C18: 1-octadecene, octadecane C19: 1-nonadecene, nonadecane

5-3-3 Separation of Pyrolysis Products by Extracted Ion Chromatogram (EIC)

On an EIC extracted with the fragment ions characteristic of specific compounds (e.g. the fragment ion detected at *m/z* 108 is a characteristic of alkylphenol), it is easy to detect a target compound because compounds with no such ions are not detected. An EIC (*m/z* 108) obtained by the two-stage method is shown in Fig. 5-3-③-a, in which a series of alkylphenol components was detected, which is difficult to detect in TICC.

This separation technique using EIC can also be applied to more complex TICC obtained by the single-stage method. An EIC (*m/z* 108) obtained by the single-stage method is shown in Fig. 5-3-③-b, in which a series of alkylphenol components was detected as well as the EIC obtained by the two-stage method. Thus, pyrolysis products that are difficult to detect in TICC can be detected in EIC. The series of alkylphenol components is generated by ether-bond and side-chain cleavage of nucleus-side chain C-O coupling urushiol polymers shown in Figure 1-②.[5-3-③]

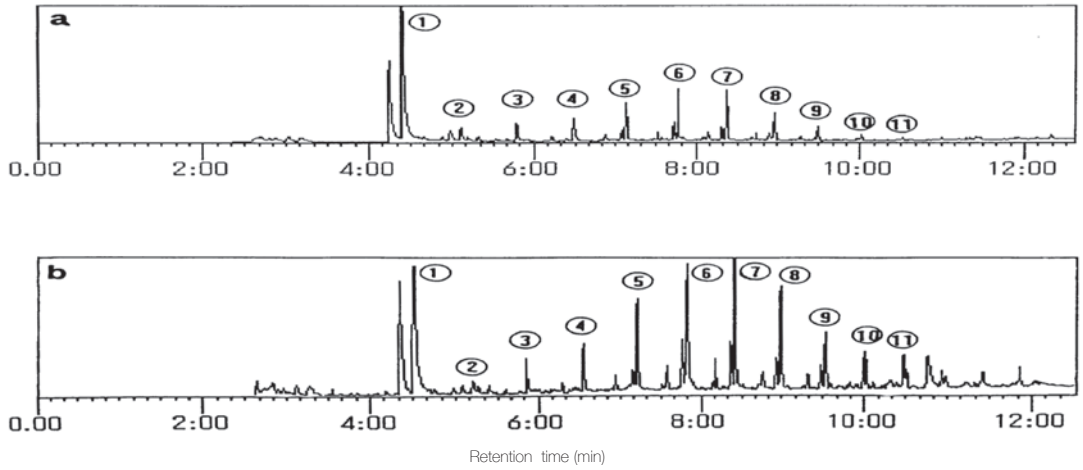


Fig. 5-3-③ EICs obtained by the two analysis methods (*m/z* 108, Py temp.: 500°C)

a) By two-stage method (after heating at 400°C for 30 min.) b) By single-stage method
① 2-methylphenol ② 2-ethylphenol ③ 2-propylphenol ④ 2-butylphenol
⑤ 2-pentyphenol ⑥ 2-hexylphenol ⑦ 2-heptylphenol ⑧ 2-octylphenol
⑨ 2-nonylphenol ⑩ 2-decylphenol ⑪ 2-undecylphenol

References
5-3-① N. Niimura and T. Miyakoshi, *Coating Technology* (in Japanese), **33**, 166 (1998).
5-3-② N. Niimura and T. Miyakoshi, *Coating Technology* (in Japanese), **33**, 204 (1998).
5-3-③ N. Niimura and T.Miyakoshi, *J. Mass Spectrom Soc. Jpn.*, **51**, 439 (2003).

5-4 Analytical Pyrolysis by PyGC × GC/MS

In recent years, due to the advancement of gas chromatograph (GC) technologies, especially those associated with capillary columns, columns with a wide variety of separation abilities have been developed. Also, the comprehensive two dimensional GC system (GC x GC) with advanced capillary column technologies has been developed for separation analysis of chemical substance groups with more complex compositions.[5-4-①,②]

A GCxGC consists of two tandem-connected different capillary columns of different separation modes and a cryo trap system (modulator) between the first and second columns. This system configuration allows for separation analysis in two different separation modes at one time. Components separated and eluted at the first column are trapped by the modulator for a certain time (normally about 5 to 10 seconds) and immediately introduced to the second column. The second column is a short column with a small internal diameter and separation takes place in a very short period in which components eluted from the first column are trapped (for 5 to 10 seconds). That is, substances eluted from the first column are trapped by segments of 5 to 10 seconds and then separated through the second column. Unlike normal chromatograms, the resultant chromatogram of this process is two-dimensional with two temporal (elution time) axes.

While the most commonly-used MS for general GC-MS is quadrupole MS (QMS), QMS cannot be regarded as optimal MS for GC x GC. For a GC x GC system, the second column is short with a small inner diameter and separation takes place rapidly within 5 to 10 seconds in the second column, so the peak width of the resultant chromatogram should become narrower. In order to obtain a sufficient number of data points for a narrow chromatogram peak, a detector with a high-speed data reading capacity is needed. However, there is a limit to the data reading speed of QMS. And while the use of QMS as a detector for GC x GC is not impractical, it would pose the issue of a limited number of data points in the second dimension of the chromatogram. Therefore, QMS is not an optimal MS for GC x GC.

Accordingly, JEOL developed a GCxGC-TOFMS system which combines a GC x GC system with a gas chromatograph time-of-flight mass spectrometer (GC-TOFMS) that enables fast acquisition of mass spectra with high mass resolution. Since 2004, the GC x GC-TOFMS system has been used for diverse complex sample analyses.[5-4-③~⑦] This section introduces application examples of natural lacquer film analysis using a system that combines JEOL's latest GC-TOFMS "JMS-T200GC AccuTOF™ GCx-plus" with the optional ion source "photo-ionization ion source" and GC x GC.

5-4-1 GC x GC-PI-TOFMS System

5-4-1-1 GC x GC System

A modulator is located at the connection section of the two columns as illustrated in Fig. 5-4-①. After components eluted from the first column are temporarily (5 to 10 seconds) cryo-trapped at one part of the connection section of the columns by "Cold jet gas" from the modulator, the components are instantaneously heated and introduced to the second column by "Hot jet gas". These operations are continuously repeated at certain intervals (5 to 10 seconds) for two-dimensional chromatogram separation as shown in Fig. 5-4-②.

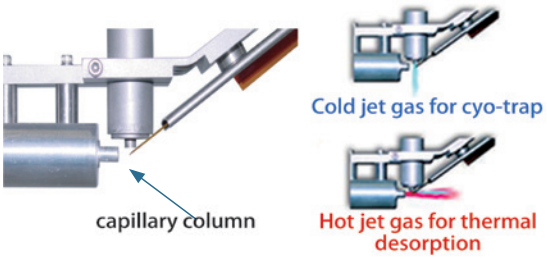


Fig. 5-4-① Schematic diagram of GC x GC modulator of Zoex ZX2 (Zoex)

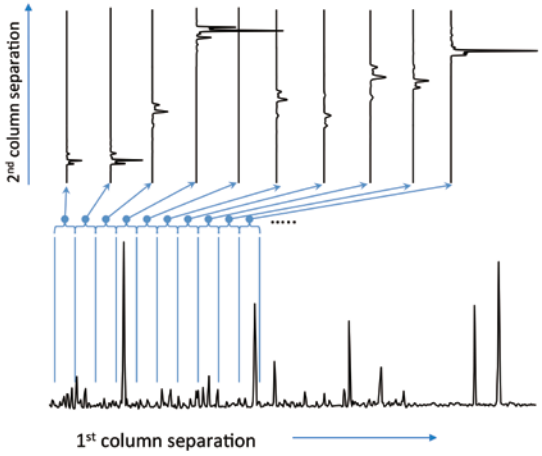


Fig. 5-4-② Conceptual diagram of two-dimensional chromatogram acquired by GC x GC

5-4-1-2 Photoionization (PI) Ion Source

The most common ionization method for GC / MS is electron ionization (EI), which produces a large number of fragment ions due to the high ionization energy. Fragment ion information is useful for such purposes as structural analysis of target compounds and identification of compounds from mass spectral patterns by library search. However, in some cases, molecular ions cannot be obtained. In such a case, it becomes difficult to obtain molecular weight information and element composition information of the compound.
The ionization method with high ionization energy is called hard ionization, and the ionization method with low energy is called soft ionization. Soft ionization is an effective method for obtaining molecular ions. Besides chemical ionization (CI), which is commonly used as a soft ionization method in the GC/MS field, JEOL developed and offers the "photoionization (PI) ion source" that ionizes target substances using vacuum ultraviolet (VUV) rays (Fig. 5-4-③) as an optional ion source for GC-TOFMS (JMS-T200GC) and GC-QMS (JMS-Q1500GC).

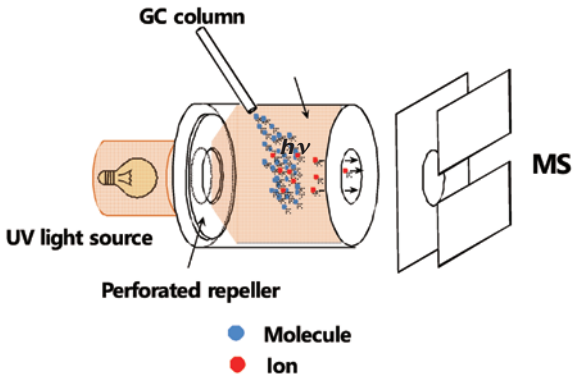


Fig. 5-4-③ Schematic drawing of the PI ion source

For the PI ion source, a deuterium lamp with light wavelength 115 - 400 nm is used as a light source. The energy of the light source for this lamp with a low wavelength of 115 nm is 10.8 eV, and the primary ionization energy for general organic compounds is around 10 eV. PI is very soft (low energy) compared with EI.

5-4-2 Application of GC x GC-TOFMS

As a natural polymer material, natural lacquer has been used as a coating and adhesive material since ancient times. In recent years, "lacquerwork," which is a traditional craftwork made by repeatedly coating base materials such as paper and wood with natural lacquer, has been appreciated as art work and is used in various ways for souvenirs and daily necessaries. Natural lacquer is water-in-oil emulsion, and PyGC/MS has been used to analyze hardened natural lacquer films and to elucidate the mechanisms of oxidative reaction. However, a normal GC separation ability is not sufficient, when an excessive volume of pyrolysis products is generated. [5-4-⑧~⑫]
To overcome this limitation, GC x GC was used for the analysis of natural lacquer films due to its greater separation ability than the normal GC method.[5-4-⑬] For ionization, EI (hard ionization) and PI (soft ionization) were used and a comparison was made of the mass spectra obtained by using these ionization methods.

5-4-2-1 Measurement Conditions

Table 5-4- ① Measurement conditions

System	JMS-T200GC(JEOL Ltd.)
Pyrolysis temp.	500°C
Ionization mode	EI+: 70 eV, 300 μA PI+: D_2 LAMP: 115-400 nm (10.8 eV @115 nm)
GC columun	1st: BPX5(SGE), 30 m×0.25 mm, 0.25 μm 2nd: BPX50(SGE), 3 m×0.1 mm, 0.1 μm
Modulation period	8 sec
Oven temp.	50°C(1min)->3°C/min->300°C(6min)
Inlet temp.	300°C
Inlet mode	Split 50:1
He flow	1.5 mL/min(Constant Flow)
m/z range	*m/z* 35-650
Recording interval	EI: 50 Hz, PI: 25 Hz

Detailed measurement conditions are summarized in Table 5-4-①. The GC x GC system used was Zoex ZX2 (Zoex). For the first column, a micropolar column BPX5 (SGE Analytical Science, length: 30 m, I.D.: 0.25 mm, film thickness: 0.25 μm) was used. For the second column, a polar column BPX50 (SGE Analytical Science, length: 3 m, I.D.: 0.1 mm, film thickness: 0.1 μm) was used.

5-4-2-2 Measurement Results

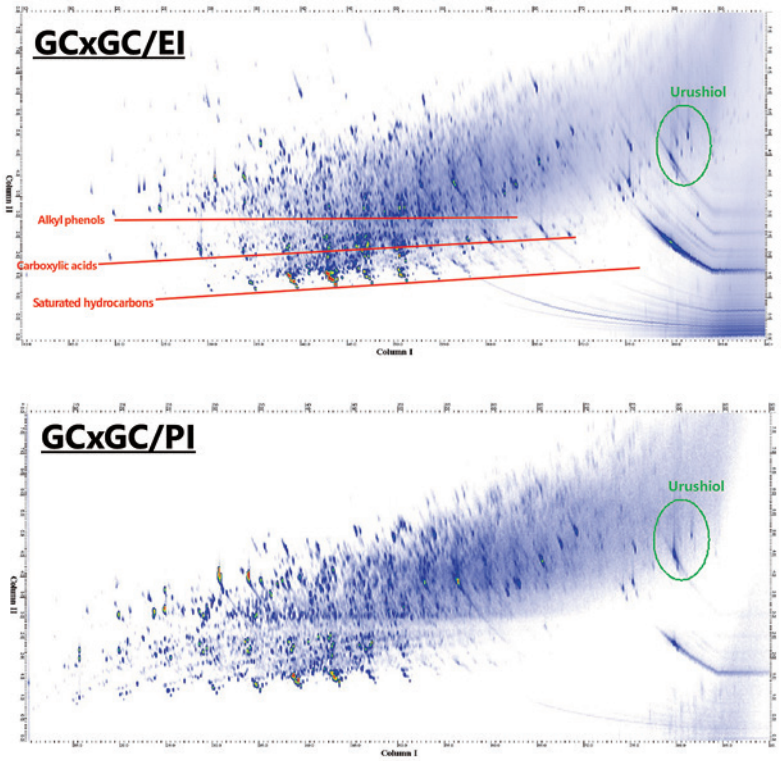


Fig. 5-4-④ TICCs of GC x GC/EI and GC x GC/PI

Figure 5-4-④ shows TICC of GC x GC/EI and GC x GC/PI.
On both TICC, urushiol, i.e. the primary component, was observed. Also, groups of compounds, such as hydrocarbons, carboxylic acids, aromatic ketones and alkylphenols, which are pyrolysis products of the natural lacquer film, were successfully separated and detected. Over 1000 components were detected through automatic peak detection using the GC x GC/EI data.

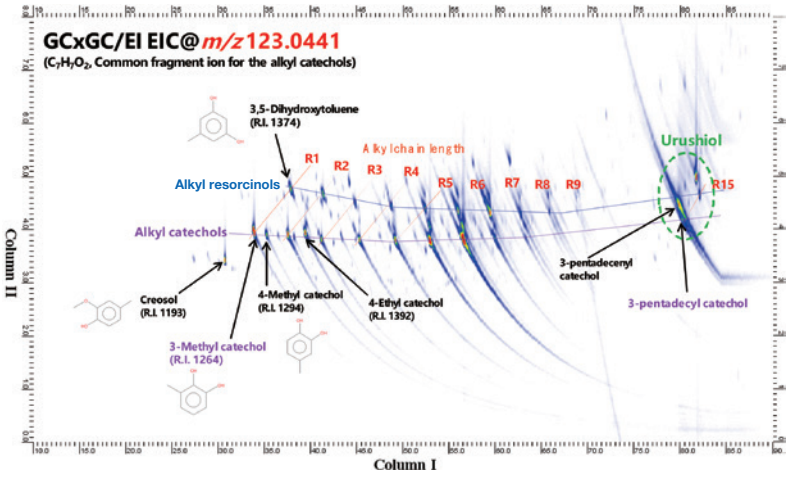


Fig. 5-4-⑤ EIC (*m/z* 123.0441 ($C_7H_7O_2$) ±0.01) of GC x GC/EI

Next, an EIC extracted with the fragment ion at *m/z* 123.044 characteristic of alkylcatechol is shown in Fig. 5-4-⑤.

Alkylcatechols were detected at the center of the EIC at around the retention time of 4 sec. in the second column. Compounds of alkyl group with chain lengths from R1 to R9 were observed as being prominent and R15 urushiol was also observed.

Also, alkylresorcinols, which are alkylcatechol isomers, were observed at around the retention time of 5 sec. in the second column. Alkylresorcinols are minor components and have previously received less attention in the analysis of natural lacquer films using PyGC/MS. In ordinary GC measurement, separation of those components is insufficient and therefore, the alkylresorcinols components were coeluted with the primary component of alkylcatechols. This might result in low attention in the analysis of those components. However, a GC x GC system can clearly separate and detect even such minor and coeluting isomer compounds for its superior separation ability.

EI/PI mass spectra of 3-pentadecylcatechol and 3-pentadeceenylcatechol are shown in Fig. 5-4-⑥. The molecular ions were detected in the EI/PI mass spectra, and the measured accurate mass of the molecular ions was matched with the calculated exact mass obtained by the molecular composition formula for the above compounds at high mass accuracy. With PI, in particular, the mass spectrum is so simple, as it contains no fragment ions other than molecular ions, and it is useful in the analysis of specific target compounds.

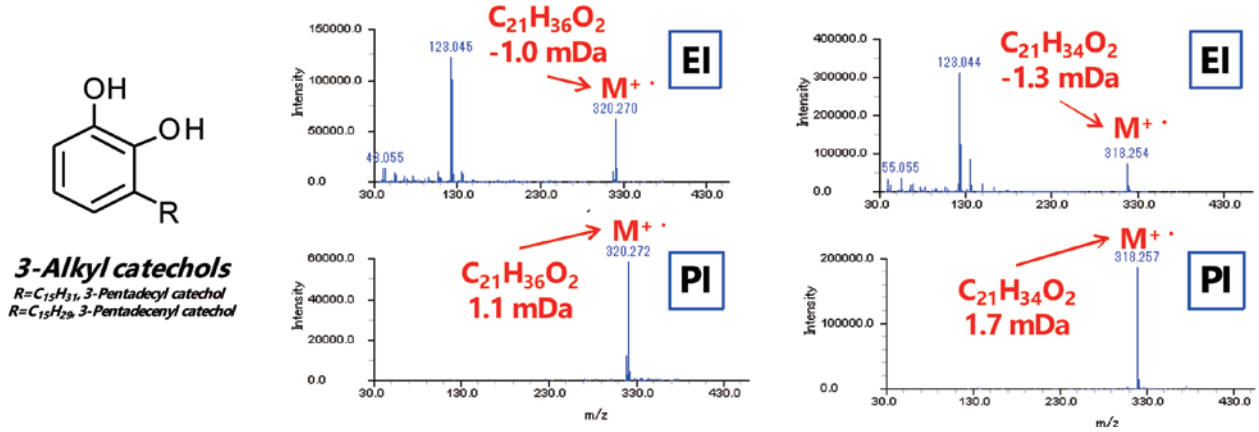


Fig. 5-4-⑥ Mass spectra of 3-pentadecylcatechol (left) and 3-pentadecenylcatechol (right)

5-4-3 Summary

GC x GC is an advanced GC technique and its chromatogram separation ability is much greater than conventional GC separation. However, in order to maximize the analysis capability of GC × GC; the application of TOFMS, which can collect mass spectra at high speed with high mass resolution, and multifaceted analysis using hard ionization and soft ionization are required. A GC × GC-PI/EI-TOFMS system that meets these requirements is a very powerful tool for analyzing complex mixtures, such as the pyrolysis products of lacquer films.

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6 Detection of Polysaccharide (Plant Gum): “Trace Components or Additives Analysis”

In the field of archeology, the analysis of coatings of historical craftworks and artworks provides important information for the identification of not only coating material but also their place of origin and estimate age. It is also an effective way to study cultures, standards of living, and trade routes, etc. in the respective era. It also plays an important role in restoration and repair work for cultural properties in the field of conservation science.

The natural lacquer films have been identified by detecting the pyrolysis products of urushiol, which is the main components of natural lacquer, by PyGC/MS or TG/MS using “the *B/E = const*. linked scan”, and “the B^2/E = *const*. linked scan”, respectively with a sector mass spectrometer. [6-①~⑫] However, it has been reported that components similar to the pyrolysis products of urushiol are detected in some natural resins [6-⑬~⑱] and this makes it difficult to identify natural lacquer films. In response, we considered detecting polysaccharides, which are trace components of natural lacquer. Any detection of trace components characteristic of natural lacquer along with the pyrolysis products of urushiol would be valuable information. As mentioned before, polysaccharides are contained in natural lacquer sap at a rate of 5 - 7%, but no pyrolysis products of polysaccharides are detected in the analysis of the natural lacquer film using PyGC/MS.[6-⑲] Therefore, the detection of polysaccharides was studied using TEM, Py/MS and NMR. This chapter also introduces the detection of polysaccharides in the natural lacquer film to explain analysis method for trace component or additives in polymer.

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6-1 Morphological Observation by TEM

In order to observe ultrastructure, the natural lacquer film was embedded in epoxy resin, cut into an ultra-thin section (thickness: 60 nm) using an ultramicrotome Leica EM UC7, and observed by TEM. For the TEM observation, JEM-1400Flash (JEOL) was used at an accelerating voltage of 100 kV.

6-1-1 Results and Discussion

Figure 6-1-① shows the TEM image of the natural lacquer thin section. Precipitates of polysaccharides (plant gum) in micropores were observed. As described before, natural lacquer is a water-in-oil emulsion. Therefore, this is because the natural lacquer dries and the water droplets evaporate to form micropores, and the polysaccharide dissolved in the water droplets precipitates out.

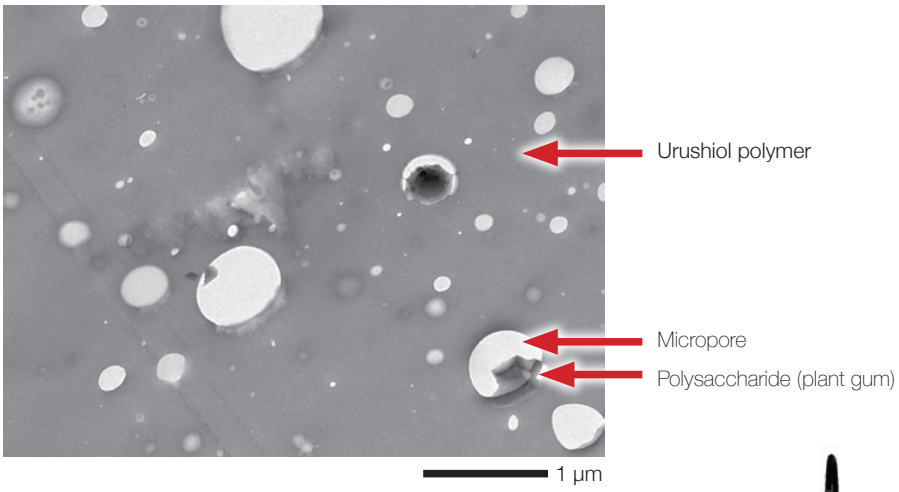

Fig. 6-1-① TEM image of the natural lacquer thin section

6-2 Analysis of pyrolysis products by Py/MS

Py/MS is a method to analyze pyrolysis products without using chromatography such as GC by using a multifunctional device in which a pyrolyzer and a mass spectrometer are directly connected. The absence of chromatography shortens the measurement time and minimizes the loss of pyrolysis products introduced into the mass spectrometer. However, the mass spectra tend to be complicated because they are not chromatographically separated.

6-2-1 Application of Pyrolysis/Electron Ionization Mass Spectrometry (Py/EIMS)

Among ionization methods used in mass spectrometry, electron ionization is the most common method applied to gas-phase samples. It ionizes the samples using thermoelectrons generally accelerated to 70 eV. In addition to molecular ions, which may not be detected in some cases, it produces a large number of fragment ions.

Figure 6-2-1-① shows the mass spectrum of the natural lacquer film pyrolyzed at 320°C. Molecular ions of 3-pentadecylcatechol, i.e. a saturated component of urushiol, were detected at *m/z* 320, and fragment ions characteristic of alkylcatechol at *m/z* 123. From these findings, it was confirmed that pyrolysis products at 320°C include 3-pentadecylcatechol (*Mr* 320). However, no pyrolysis products of polysaccharides were detected.

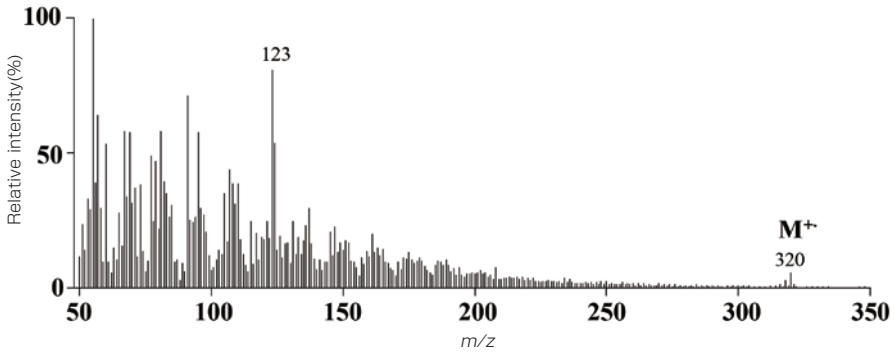

Fig. 6-2-1-① EI mass spectrum of natural lacquer film pyrolyzed at 320°C

6-2-2 Application of Pyrolysis/Chemical Ionization Mass Spectrometry (Py/CIMS)

Electron ionization, which produces a large number of fragment ions, is classified into hard ionization, whereas soft ionization refers to ionization methods that produce fewer fragment ions. Soft ionization methods applied to gas-phase samples include chemical ionization and field ionization.

As mentioned above, mass spectra tend to be complex in Py/MS, because Py/MS is performed without chromatographic intervention. Therefore, soft ionization producing fewer fragment ions is very useful for Py/MS. This section explains application results of chemical ionization. Chemical ionization reacts sample molecules with reactive ions such as $[R+H]^+$ generated from reagent gases (R). These reactions include the addition and desorption of protons, the desorption of hydrides, the transfer of electrons, and the addition of ions.

Figure 6-2-2-① shows the CI mass spectrum of the natural lacquer film pyrolyzed at 320°C. Protonated molecule ions of hydroxymethylfurfural (HMF, *Mr* 126) were detected at *m/z* 127. In addition, protonated molecule ions and fragment ions of anhydrosugars were detected at *m/z* 163 and 145, respectively. They are

pyrolysis products of polysaccharides: galactose, which is the primary constituent sugar of polysaccharides, are pyrolyzed to generate anhydrosugars such as HMF and 1, 6-anhydrogalactopyranose (*Mr* 162). Thus, Py/MS using the chemical ionization method enabled the identification of polysaccharide components in the natural lacquer film. Meanwhile, 3-pentadecylcatechol, which is detected in the EI mass spectrum, was not detected, because 3-pentadecylcatechol has less proton affinity.

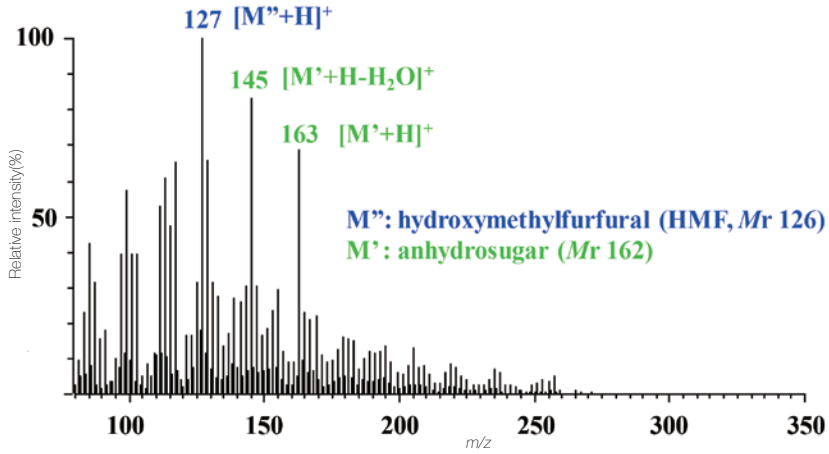

Fig. 6-2-2-① CI mass spectrum of the natural lacquer film pyrolyzed at 320°C (reagent gas: isobutane)

6-2-3 Application of Pyrolysis/Field Ionization Mass Spectrometry (Py/FIMS)

Another soft ionization method commonly applied to gas-phase samples is field ionization, which is an ionization method that detach electrons from a sample by interaction with a high electric field (–10 kV). With FI, nonpolar and micropolar compounds produce molecular ions, while polar compounds produce protonated molecular ions.

Figure 6-2-3-① shows the FI mass spectrum of the natural lacquer film, in which the molecular ions of HMF and the protonated molecular ions of anhydrosugars were detected at *m/z* 126 and *m/z* 163, respectively. Moreover, the molecular ions of 3-pentadecylcatechol were detected at *m/z* 320.[6-2-①]

Thus, Py/MS using the field ionization method enables the identification of urushiol components and polysaccharide components in the natural lacquer film.

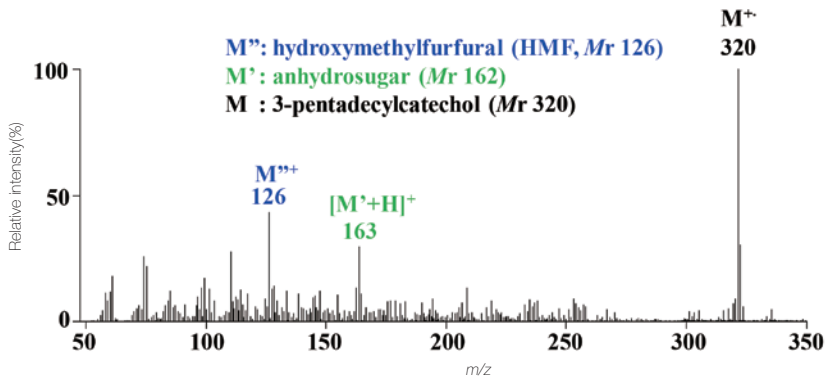

Fig. 6-2-3-① FI mass spectrum of the natural lacquer film pyrolyzed at 320°C

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6-3 Detection of Specific Peaks by Solid-State NMR

Solid-state NMR is a non-destructive analytical method and enables comprehensive acquisition of signals from the target nuclides in a sample. Thus, the solid-state NMR is effective to analyze impurities as well.
Solid-state NMR also allows for non-destructive analysis of samples insoluble in a solvent.

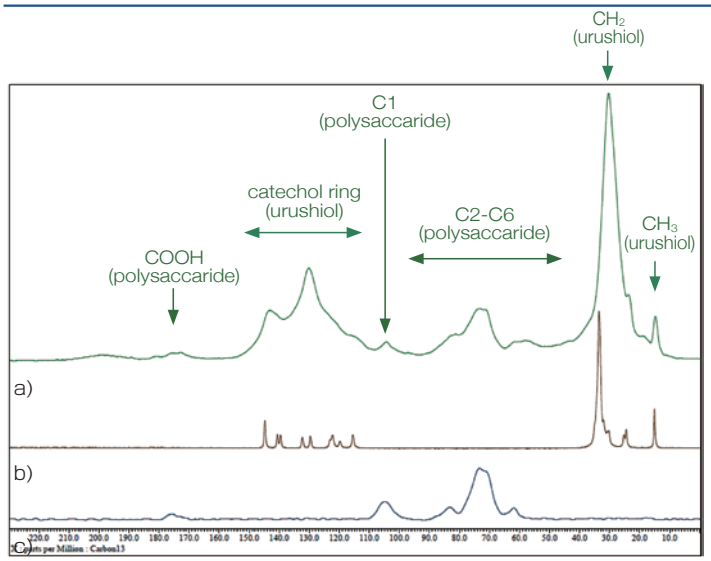

Fig. 6-3-① Solid-state ^{13}C NMR spectrum

a) Natural lacquer film, b) Urushiol, c) Polysaccharide
(Measurement time: a) 24 h, b) 3 h, c) 11 h)

Figure 6-3-① shows solid-state NMR spectra of the natural lacquer film, urushiol and polysaccharide components. On the NMR spectrum of the natural lacquer film, in addition to urushiol, peaks of COOH derived from polysaccharide components and anomeric carbons (C1-C6) were detected. Thus, trace components in the natural lacquer film can be identified using solid-state NMR in a non-destructive way.[6-3-①]
(A JNM-ECZ400R equipped with a 4 mm HXMAS probe for solid-state NMR was used as a spectrometer, and measurement was performed using the CPMAS method.)

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7 Conclusion

In this Urushi Note, we have explained the instrumental analyses of polymer materials, exemplifying the applications to natural lacquer films. In Chapter 1, the composition and polymerization mechanism of natural lacquer have been summarized. In addition, the features of various analytical instruments have been presented along with application examples of polymer materials. In Chapter 2, the structures of urushiol, which is the monomer components of the natural lacquer, were actually analyzed using NMR, FT-IR, and MS. Additinally, the analytical methods by the respective instruments have been explained. In Chapter 3, the hardening process of the natural lacquer was studied using the pencil hardness testing and XPS. The considerable progress of oxidative polymerization has been confirmed. In Chapter 4, the natural lacquer films formed under different conditions were analyzed using LM, SEM, ESR, IR, and NMR. The correlation between these results and their surface states has been discussed. In Chapter 5, the thermal degradation of the natural lacquer film was analyzed using SEM, TG/MS, ESR, PyGC/MS and PyGCxGC/MS. The degradation process has been discussed. In Chapter 6, polysaccharide components in the natural lacquer film were analyzed using TEM, Py/MS and solid-state NMR. The effectiveness of these analytical methods in detecting trace components has been described. In the future, such multifaceted analytical methods will be important tools for analyzing increasingly complicated polymer materials. It would be a great pleasure if this Urushi Note could help in the development of polymer materials.

Acknowledgment
We would like to express our sincere appreciation to Professor Tetsuo Miyakoshi of Meiji University, and Dr. Yoshimi Kamiya of Kanazawa University.

List of Instruments Applied to Respective Analysis Items

In this Urushi Note, evaluation methods of polymer materials have been described with examples of application to natural lacquer. The list of JEOL instruments applied to respective items is shown below.

Analysis items \ Applied Instruments	NMR	FT-IR	MS	XPS	SEM	ESR	TEM
Monomer analysis (example: analysis of Urushiol components)	○	○	○				
Time-course analysis during film formation (example: analysis of the hardening process of natural lacquer)				○			
Comparative analysis (example: comparison of natural lacquer films formed under different conditions)	○	○			○	○	
Thermal degradation analysis (example: heat resistance assessment of a natural lacquer film)			○		○	○	
Trace components or additives analysis (example: detection of polysaccharide)	○		○				○

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