ORIGINAL ARTICLE

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Infrared spectroscopic study of alkaline oxygen treatment of lignin with ATR technique in aqueous state 1: method for determining quantitative spectra of oxygen-degraded lignin

Abstract For the fundamental study of oxygen delignification of kraft pulp, structural changes of kraft lignin during alkaline oxygen treatment were investigated with the use of infrared measurement with attenuated total reflectance (ATR) technique. In the neutralized reaction mixture of alkaline oxygen-treated kraft lignin, there is a significant amount of NaCl, so that the spectral changes of water due to the coexistence of NaCl was investigated, and how to remove the huge absorption of NaCl solution is discussed. Sodium vanillate–NaCl solutions were employed as model solutions for the reaction mixture. Partial least square (PLS) regression was applied for the prediction of NaCl concentration, and the spectrum of NaCl solution was subtracted from the spectrum of sodium vanillate–NaCl solution as background measurement. This allowed us to obtain the vanillate spectra free from the absorption of NaCl solution. In addition, the mathematical method for reconstructing the spectrum of NaCl solution is discussed. The spectrum of NaCl solution is reconstructed as the linear combination of basic spectra calculated by singular value decomposition (SVD), and it was subtracted from that of the sodium vanillate–NaCl solution. By this procedure, the vanillate spectra were also obtained quantitatively, as has been demonstrated in PLS regression study. It was also confirmed that the quantitative spectra of high molecular weight fraction of alkaline oxygen-treated kraft lignin were obtained by the use of this reconstruction technique.

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Introduction

It is believed that delignification of kraft pulp during oxygen treatment under alkaline conditions is achieved by fragmentation of lignin and introduction of acidic groups (e.g., carboxyl, hydroxyl) with cleavage of the aromatic structure.[1] Many researchers have investigated the reaction mechanism with the use of lignin model compounds. It is, however, not clear what kinds of detailed structural changes occur in actual lignin. In particular, information about the structural changes in the high molecular weight fraction of lignin is lacking.

Many chemical degradation and spectroscopic methods [e.g., ultraviolet (UV), infrared (IR), Raman, and nuclear magnetic resonance (NMR) spectroscopy] have been applied in order to visualize the chemical structures of lignin. IR spectra of macromolecules including lignin give information about functional groups and intermolecular or intramolecular interaction. However, the analysis of these spectra is often difficult because of highly overlapped bands. Application of multivariate analysis to spectroscopy, for example, principal component analysis (PCA), generalized two-dimensional correlation spectroscopy,[2] and regression methods [partial least squares (PLS), principal component regression (PCR)],[3] may enable us to solve such difficulties, and hidden information about the chemical structures which cannot be drawn from the analysis of a single spectrum can be extracted by the analysis of many spectra with a certain perturbation. Actually, regression methods using diffuse reflectance infrared fourier transform (DRIFT) IR spectra have been applied to estimate the chemical composition of wood samples or pulp.[4,5] Recently, infrared attenuated total reflectance (ATR) spectra have also been applied to investigate the variation of lignin content in each pulp fiber in combination with PLS regression.[6]

IR spectra of lignin itself or lignin in pulp are generally collected in the solid state by the KBr disk method,[7] and by DRIFT.[8] Quantitative analysis is often quite difficult in the case of solid samples due to weighing of small sample amounts, influence of moisture, evaporation of volatile compounds, and so on. However, when the difficulties are overcome, the quantitative results enable us to easily use the mathematical analysis of spectra. On the other hand, IR measurement in the solution state is also applied when samples can be dissolved in some solvents. In this case, quantitative spectra are easy to obtain in comparison with measurement in the solid state because the sample is homogenous and the concentration is well controlled.

Among other solvents, IR measurement of aqueous solutions allows not only the determination of quantitative spectra, but also the detection of spectral changes with variation of pH.[9] This may provide more detailed information about dissociating groups in lignin than investigation by the use of $\Delta \varepsilon_i$ of UV spectroscopy. Because it is considered that the reaction of alkaline oxygen treatment initiates from the reaction between phenolate anion in lignin and molecular oxygen, the observation of dissociation of phenol may allow further investigation of this reaction.

The final object of this study is to understand the reactivity and structural changes of lignin, especially the high molecular weight fraction, during alkaline oxygen treatment. For this, softwood kraft lignin was employed as a sample for the fundamental study and was subjected to alkaline oxygen treatment. Infrared ATR measurement in the aqueous state was used to analyze the reaction mixture of alkaline oxygen treatment of kraft lignin without isolation.

When IR measurement is conducted in the aqueous state, the largest problem is the enormous absorption of water. In order to acquire the spectrum of the solute itself, this enormous absorption of water should be properly subtracted from the spectrum of the aqueous solution. Moreover, when some salts are dissolved in an aqueous solution like the reaction mixture of alkaline oxygen treatment, the subtraction of the water spectrum may become more difficult. For example, although the ions of alkaline halides or alkaline-earth halide salts themselves do not absorb in the infrared region, it is well known that the shapes of water spectra are changed depending on the types or concentrations of the salts.[10] Therefore, it is necessary to investigate the shape of the water spectrum or spectral changes due to the effect of salts to obtain quantitative spectra of solute.

The IR spectrum of liquid water is composed of four bands around 200 (v_T), 700 (v_L), 1630 (v_2), and 3430 cm^{-1} (v_S). In addition, there is a broad band around 2125 cm^{-1}, a so-called associational band, for which it is proposed that the wavenumber is expressed as $v_2 + v_L - v_T$.[11] Organic compounds composed of carbon, hydrogen, and oxygen generally have almost no absorption in the IR region around the associational band of liquid water, so that the band can be assumed to be caused only by liquid water when an aqueous solution is subjected to IR measurement. Because this band contains information about three bands below 2000 cm^{-1}, the observation conditions or spectrum

shape of water in the aqueous solution may be obtained by analyzing the associational band in the spectrum of an aqueous solution.

In this article, we discuss how to eliminate the strong influence of water from the infrared ATR spectrum of an aqueous solution, and examine the method for determining the quantitative spectra of solute. We focus on the associational band of liquid water. Two model solutions, sodium vanillate NaCl solution and the aqueous solution of high molecular weight fraction of alkaline oxygen-treated kraft lignin, were used in order to confirm the applicability of the discussed method for the reaction mixture of alkaline oxygen treatment of lignin.

Materials and methods

Sample preparations

Aqueous sodium chloride solution

Aqueous sodium chloride solutions of 21 different concentrations between 0.0 and 2.0 M at 0.1-M intervals were prepared.

Sodium vanillate and sodium chloride aqueous solution

Sodium vanillate and sodium chloride (VA-NaCl) mixture solutions of five different concentrations were prepared. The concentrations of sodium vanillate were 10, 20, 30, 40, and 50 mM. For each vanillate concentration level, the sodium chloride concentrations were 0.50, 1.30, 0.20, 1.70, and 0.80 M, respectively.

Aqueous solution of high molecular weight fraction of alkaline oxygen-treated kraft lignin

Purified softwood kraft lignin (2.5 g) and 20 g of sodium hydroxide were dissolved in deionized water, and the volume was adjusted to 500 ml. The mixture was subjected to alkaline oxygen treatment at 70°C for 60 min under 0.3 MPa pressurized oxygen. The beginning of the reaction period (0 min) was defined as the time when the reaction temperature reached 70°C.

The reaction mixture was acidified to pH 3 with aqueous HCl. The precipitate formed by addition of NaCl to saturation, was collected by centrifugation and washed with saturated aqueous NaCl solution that was adjusted to pH 3 with aqueous HCl. This centrifugation and washing procedure was repeated twice. The washed precipitate was dissolved in dilute aqueous NaOH solution, and five different diluted solutions were prepared with deionized water. The concentrations of the diluted solutions were 5.0, 7.5, 10.0, 12.5, 15.0 g/20 ml. The pH values of all solutions were 8.0–8.5.
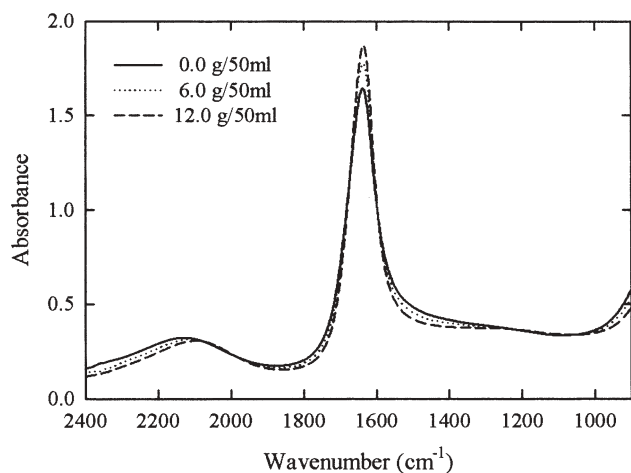
Fig. 1. Spectral changes of water depending on NaCl concentration

Fig. 2. Infrared attenuated total reflectance (ATR) spectra of sodium vanillate in NaCl coexisting aqueous solution. Spectrum *b* is an example when proper subtraction is carried out. Spectra *a* and *c* are examples of improper subtraction

Analysis

All spectra were recorded on a Jasco FT/IR-615 equipped with Spectra-Tech baseline horizontal ATR kit (ATR-8100H). The ATR plate was made of ZnSe crystal. Using the empty plate as the background, spectra of each liquid sample were recorded under dry nitrogen. Each spectrum was based on 256 averaged interferograms that were collected at $4 \mathrm{cm}^{-1}$ spectral resolution. All spectra were even $0.964233 \mathrm{cm}^{-1}$ distributed 1661 points data from 899.6298 to $2500.2574 \mathrm{cm}^{-1}$. All spectra were smoothed with the use of the Savitzky-Golay algorithm with 13 points, and were set against the straight baseline with absorbance at $1929.4312 \mathrm{cm}^{-1}$ (1069th point) and $2220.6297 \mathrm{cm}^{-1}$ (1371th point) being zero.

PLS analysis was performed according to the previously described algorithm.[12] Mathematical operations were performed using GNU Octave 2.1.36 (University of Wisconsin, Department of Chemical Engineering, Madison, WI, USA).

Results and discussions

Prediction of NaCl concentration by PLS analysis

In the neutralized reaction mixture of alkaline oxygen treated kraft lignin to be analyzed, there is not only oxygen-degraded kraft lignin but also plenty of NaCl. Sodium chloride is the one of the compounds that has a strong effect on water spectra as shown in Fig. 1. Therefore, in order to obtain the spectrum of oxygen-degraded kraft lignin, it should be reasonable that the spectrum of NaCl solution of the exactly same concentration as that of the reaction mixture is subtracted from the spectrum of the reaction mixture. However, NaOH, which finally generates NaCl after by neutralization with HCl, will be consumed by the generation of acidic groups or carbonate during alkaline oxygen treatment, so that the concentration of NaCl dissolved in the neutralized reaction mixture may be unknown.
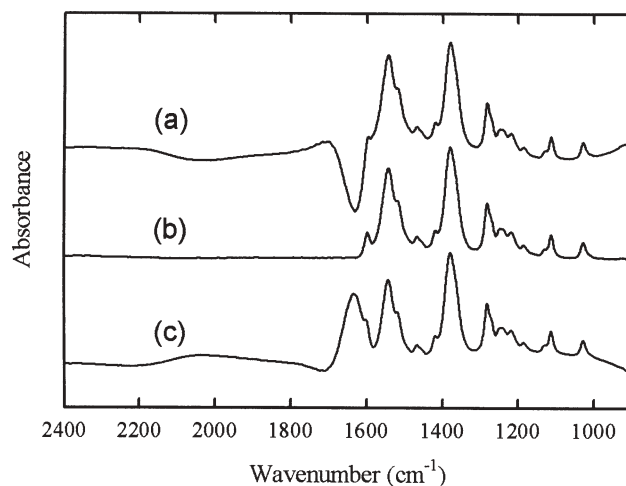
Figure 2 shows the examples of proper and incorrect subtraction from the spectra of VA-NaCl solution. Spectrum b was obtained by subtraction of a spectrum of NaCl solution of the same NaCl concentration. When the subtraction was carried out using an unsuitable NaCl concentration, distortion of the baseline and/or appearance of artificial peaks took place. Spectrum a is the case of the NaCl concentration being too high, and spectrum c is the case of the concentration being too low. Therefore, it is desirable to predict or determine the NaCl concentration of the neutralized reaction mixture in advance, e.g., by some regression methods.[13]

In order to predict the NaCl concentration, PLS regression was applied, and a prediction model which regresses the NaCl concentration from the IR spectrum of the neutralized reaction mixture was constructed by analysis of various concentrations of NaCl solution. To avoid the disturbance of prediction from the peaks of oxygen-degraded lignin, the IR region around the associational band which was considered to be due only to water (or NaCl solution) was applied for PLS prediction. Assuming application of NaCl solution for subtraction and its concentration are suitable, the spectra of oxygen-degraded lignin should be obtained quantitatively by measuring the neutralized reaction mixture with a predicted NaCl solution background.

PLS analysis between spectra of 21 NaCl solutions (absorbance values around 1929.4312–$2220.6297 \mathrm{cm}^{-1}$; independent variables, 303 points) and corresponding NaCl concentrations (dependent variable) was carried out. The PLS prediction model for regressing NaCl concentration was constructed with the first three components, because prediction residual error sum of squares (PRESS) indicated the first minimum value when the PLS prediction model was constructed with the first three components during cross validation with leave-one-out strategy (Fig. 3).

When the spectra of VA-NaCl solutions, which were applied as model solutions of the neutralized reaction mix-

Table 1. Comparison of prepared NaCl concentration of sodium vanillate and NaCl mixture solution with the predicted value obtained from partial least squares analysis of spectra

Prepared NaCl concentration (mol/l)	0.50	1.30	0.20	1.70	0.80
Predicted NaCl concentration (mol/l)	0.53	1.31	0.22	1.70	0.79
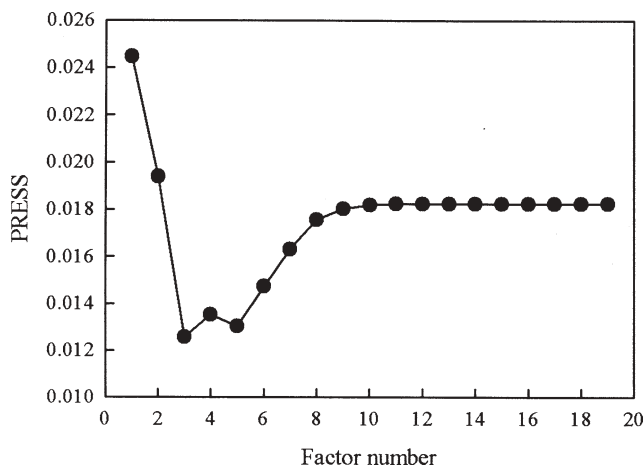
Fig. 3. Prediction residual error sum of squares (*PRESS*) from leave-one-out cross validation versus number of partial least squares (PLS) factors
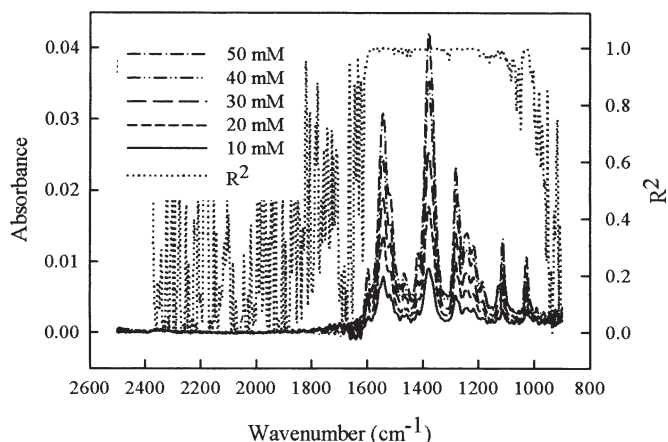
Fig. 4. Infrared ATR spectra of various concentrations of sodium vanillate obtained by measuring with optimum NaCl solution background. *R*, correlation coefficient between vanillate concentration and absorbance at each wavenumber

ture, were subjected to PLS prediction, the predicted NaCl concentrations were close to the prepared concentrations (Table 1). After preparing NaCl solutions with concentrations adjusted to the predicted values, VA-NaCl solutions were subjected to infrared ATR measurement using these NaCl solutions as background. Then the vanillate spectra set with variation of concentration was obtained (Fig. 4). Correlation coefficient (R) between the vanillate concentration and the absorbance of spectra at each wavenumber was calculated. The value of R^2 at each wavenumber was close to 1.00 in the region where peaks appeared. This result

indicated that quantitative spectra were obtained and the spectra of water (or NaCl solution) were almost completely cancelled.

In this session, only the NaCl concentration, which was predicted from the PLS analysis of the associational band of VA-NaCl solution, was used to achieve proper subtraction. Although actually prepared NaCl solution was subtracted as background measurement, the huge absorption of NaCl solution in VA-NaCl solution was cancelled successfully. Most notably, the intensities of all vanillate spectra in the region where the associational band of water exists were almost zero (Fig. 4). It is reasonable to say that the number of optimum PLS factors should be three, because the wavenumber of the associational band was thought that it is expressed as a linear combination of the wavenumbers of three bands. These results indicate that the spectral shape of NaCl solution could be investigated by analyzing only its associational band, and implied that mathematical reconstruction of the NaCl solution spectrum in the range of 900–2500 cm^{-1} is possible from the information of the associational band of the reaction mixture. Assuming that the reconstruction could be carried out successfully, the spectrum of oxygen-degraded lignin should be obtained directly from the spectrum of the reaction mixture. This makes it unnecessary to prepare the NaCl solution for background measurement.

Reconstruction of the spectral shape of aqueous NaCl

In order to reconstruct the spectral shape of NaCl solution in the reaction mixture, we assumed the spectrum of NaCl solution in reaction mixture was expressed as a linear combination of some basic spectra. In this study, the average spectrum of 21 NaCl solutions and the vectors calculated using singular value decomposition (SVD) were used as basic spectra.

Figure 5 shows an example of this procedure. Spectrum a is the difference spectrum between the spectrum of the solution of alkaline oxygen-treated lignin and the average spectrum of 21 NaCl solutions (x_{ave}). Spectra b, c, and d were created by multiplying three basic spectra (w_1, w_2, and w_3) by certain constant coefficients, respectively. By subtracting spectra b, c, and d from spectrum a, the spectrum of alkaline oxygen-treated lignin (spectrum e) was obtained. Namely, the spectrum of NaCl solution in aqueous solution was successfully reconstructed as a linear combination of four basic spectra [$(-0.1945)w_1 + (-0.1498)w_2 + (-0.2332)w_3 + x_{ave}$] in this example.

Thus, in a manner to be described, first, some basic spectra were calculated, and then, corresponding constant coefficients were found.
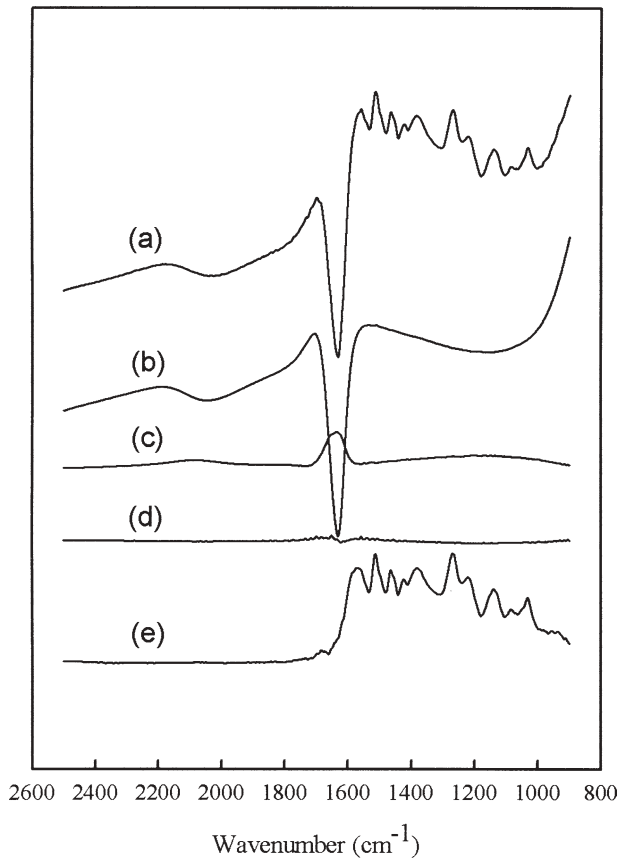
where s_k is the factor kth singular value corresponding to the factor kth pair of eigenvectors $\boldsymbol{v}_k$ (1661 × 1) and $\boldsymbol{u}_k$ (21 × 1). Singular value s_1 has the biggest value and s_2 has second biggest, and so on. In case of this study, each eigenvector $\boldsymbol{v}_k$ gives a spectral component, and $\boldsymbol{u}_k$ will give the component that relates to the variation of NaCl concentrations. Singular value s_k is the value that express how much $\boldsymbol{v}_k$ and $\boldsymbol{u}_k$ contribute to matrix $\boldsymbol{X}$.

Because matrix $\boldsymbol{X}$ is given as Eq. 1, it is approximated by the components having large singular values. Singular values after the fourth component were judged as small enough to ignore, and eigenvectors $\boldsymbol{v}_k$ corresponding to these singular values contain much undesirable information about vapor and noise. Thus, $\boldsymbol{X}$ was approximated with just the first three components (when just these three components were applied, the total variance accounted for 90.4%), and then $\boldsymbol{y}_k$ was expressed as follows:

$$
\boldsymbol{X} \approx \begin{pmatrix} \boldsymbol{u}_1 & \boldsymbol{u}_2 & \boldsymbol{u}_3 \end{pmatrix} \begin{pmatrix} s_1 & & 0 \\ & s_2 & \\ 0 & & s_3 \end{pmatrix} \begin{pmatrix} \boldsymbol{v}_1^{\mathrm{T}} \\ \boldsymbol{v}_2^{\mathrm{T}} \\ \boldsymbol{v}_3^{\mathrm{T}} \end{pmatrix}
$$

$$
= \begin{pmatrix} u_{11} & u_{21} & u_{31} \\ u_{12} & u_{22} & u_{23} \\ \vdots & \vdots & \vdots \\ u_{1i} & u_{2i} & u_{3i} \\ \vdots & \vdots & \vdots \\ u_{121} & u_{221} & u_{321} \end{pmatrix} \begin{pmatrix} s_1 \boldsymbol{v}_1^{\mathrm{T}} \\ s_2 \boldsymbol{v}_2^{\mathrm{T}} \\ s_3 \boldsymbol{v}_3^{\mathrm{T}} \end{pmatrix} \tag{2}
$$

$$
\begin{aligned}
\boldsymbol{y}_i &\approx u_{1i} s_1 \boldsymbol{v}_1 + u_{2i} s_2 \boldsymbol{v}_2 + u_{3i} s_3 \boldsymbol{v}_3 + \boldsymbol{x}_{ave} \\
&= u_{1i} \boldsymbol{w}_1 + u_{2i} \boldsymbol{w}_2 + u_{3i} \boldsymbol{w}_3 + \boldsymbol{x}_{ave}
\end{aligned} \tag{3}
$$

where u_{k1}–u_{k21} are the 21 elements of vector $\boldsymbol{u}_k$, and $\boldsymbol{w}_k$ is equal to $s_k \boldsymbol{v}_k$.

In Eq. 3, each spectrum of the 21 NaCl solutions was approximated by the linear combination of basic spectra $\boldsymbol{w}_1$, $\boldsymbol{w}_2$, $\boldsymbol{w}_3$, and $\boldsymbol{x}_{ave}$, by the use of four constant coefficients (in the case of $\boldsymbol{y}_i$, the coefficients are u_{1i}, u_{2i}, u_{3i}, and 1). We decided to apply $\boldsymbol{w}_1$, $\boldsymbol{w}_2$, $\boldsymbol{w}_3$, and $\boldsymbol{x}_{ave}$ as basic spectra.

Spectra of other NaCl solutions except for the 21 spectra will also be approximated by the linear combination of the same four basic spectra with four different coefficients. Therefore, the spectra of NaCl solution in reaction mixture $\boldsymbol{y}_{NaCl}$ (1661 × 1) can be expressed by the use of coefficients b_1, b_2, and b_3, instead of u_{1i}, u_{2i} and u_{3i}.

$$
\boldsymbol{y}_{NaCl} \approx b_1 \boldsymbol{w}_1 + b_2 \boldsymbol{w}_2 + b_3 \boldsymbol{w}_3 + \boldsymbol{x}_{ave} = \boldsymbol{Wb} + \boldsymbol{x}_{ave} \tag{4}
$$

where $\boldsymbol{w}_1$, $\boldsymbol{w}_2$, and $\boldsymbol{w}_3$ constitute matrix $\boldsymbol{W}$ (1661 × 3), and coefficients b_1, b_2, and b_3 constitute coefficient vector $\boldsymbol{b}$ (3 × 1).

Two associational bands (around 2125 cm^{-1}) of a reaction mixture and NaCl solution in the corresponding reaction mixture should be the same, because the associational band is considered as being derived from only water (or NaCl solution). Therefore, using same coefficient $\boldsymbol{b}$ in Eq. 4, the partial spectrum around 1929–2221 cm^{-1} ($\boldsymbol{y}_P$; 303 × 1) in

Fig. 5. Explanation of direct determination method. Spectrum *a* is the difference spectrum between the solution of alkaline oxygen-treated lignin and the averaged spectrum ($\boldsymbol{x}_{ave}$). Spectra *b*, *c*, and *d* were made from multiplying $\boldsymbol{w}_1$, $\boldsymbol{w}_2$, and $\boldsymbol{w}_3$ by coefficients −0.1945, −0.1498, and −0.2332, respectively. Spectrum *e* is the spectrum of alkaline oxygen-treated lignin

To make basic spectra, the average spectrum of 21 NaCl solutions ($\boldsymbol{x}_{ave}$; 1661 × 1) was subtracted from each spectrum of NaCl solution ($\boldsymbol{y}_i$; 1661 × 1), and using these 21 average-subtracted spectra, matrix $\boldsymbol{X}$ (21 × 1661) was constructed. Because the rank of matrix $\boldsymbol{X}$ is 20, $\boldsymbol{X}$ was expressed as follows after SVD of $\boldsymbol{X}$:

$$
\boldsymbol{X} = \begin{pmatrix} \boldsymbol{y}_1^{\mathrm{T}} - \boldsymbol{x}_{ave}^{\mathrm{T}} \\ \boldsymbol{y}_2^{\mathrm{T}} - \boldsymbol{x}_{ave}^{\mathrm{T}} \\ \vdots \\ \boldsymbol{y}_i^{\mathrm{T}} - \boldsymbol{x}_{ave}^{\mathrm{T}} \\ \vdots \\ \boldsymbol{y}_{21}^{\mathrm{T}} - \boldsymbol{x}_{ave}^{\mathrm{T}} \end{pmatrix}
$$

$$
= \begin{pmatrix} \boldsymbol{u}_1 & \boldsymbol{u}_2 \ldots \boldsymbol{u}_k \ldots \boldsymbol{u}_{20} \end{pmatrix} \begin{pmatrix} s_1 & & & & & 0 \\ & s_2 & & & & \\ & & \ddots & & & \\ & & & s_k & & \\ & & & & \ddots & \\ 0 & & & & & s_{20} \end{pmatrix} \begin{pmatrix} \boldsymbol{v}_1^{\mathrm{T}} \\ \boldsymbol{v}_2^{\mathrm{T}} \\ \vdots \\ \boldsymbol{v}_k^{\mathrm{T}} \\ \vdots \\ \boldsymbol{v}_{20}^{\mathrm{T}} \end{pmatrix} \tag{1}
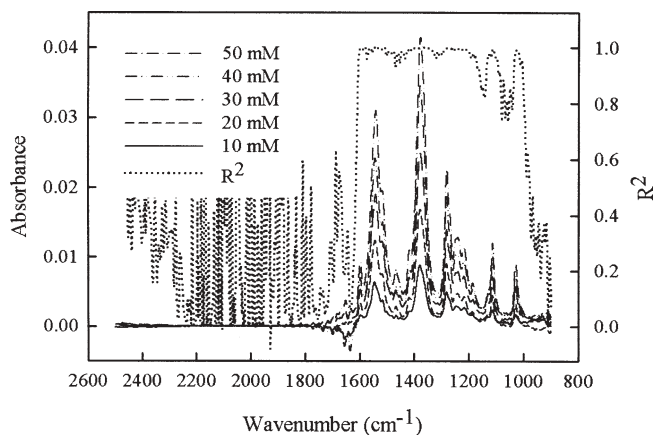$$

Fig. 6. Infrared ATR spectra of vanillate obtained by subtracton of reconstructed spectra of NaCl solution from the spectra of sodium vanillate–NaCl solution

Fig. 7. Infrared ATR spectra of alkaline oxygen-treated lignin obtained by subtraction of reconstructed spectra of NaCl solution from the spectra of its aqueous solution

the spectrum of the reaction mixture (y; 1661×1) can be expressed as the linear combination of partial basic spectra (w_{1P}, w_{2P}, w_{3P}, and x_{aveP}; 303×1). These partial basic spectra are defined as the values around 1929–$2221\,cm^{-1}$ within basic spectra w_1, w_2, w_3, and x_{ave}, respectively (Eq. 5).

$$b_1 w_{1P} + b_2 w_{2P} + b_3 w_{3P} + x_{aveP} = W_p b + x_{aveP} \approx y_P \quad (5)$$

Therefore, by calculation of the coefficient vector b from Eq. 5, it is possible to approximate and reconstruct y_{NaCl} from Eq. 4. To calculate one of least-square solutions of b from Eq. 5, pseudo inverse W_P^+ (3×303) was used (Then, the solution $b = W_P^+ (y_P - x_{aveP})$ has the least norm in many least-square solutions). Assuming the least-square solution b is suitable for reconstructing the spectrum of NaCl solution in the reaction mixture, the spectrum of oxygen-degraded lignin y_{sol} (1661×1) is defined as the difference spectrum between y and the spectrum of reconstructed NaCl solution y_{NaCl} (Eq. 6).

$$\begin{aligned} y_{sol} &\approx y - y_{NaCl} = y - (Wb + x_{ave}) = (y - x_{aveP}) \\ &- W\left[W_P^+ \left(y_p - x_{aveP}\right)\right] \end{aligned} \quad (6)$$

According to this procedure, the spectra of sodium vanillate were calculated from the VA–NaCl solution (Fig. 6). The correlation coefficient (R) between sodium vanillate concentration and the intensity at each wavenumber was also calculated. R^2 was near 1.00 in the region where intensity was relatively high, as same as when we measured with the optimum NaCl solution background. Although there was slight trouble with subtraction at around $1630\,cm^{-1}$, the result of R^2 showed that quantitative vanillate spectra were obtained and the spectrum of water was cancelled successfully by this procedure.

To determine whether the method is also applicable for reaction mixtures that we want to analyze, the aqueous solution of high molecular weight fraction of alkaline oxygen-treated kraft lignin was also subjected to this proce-
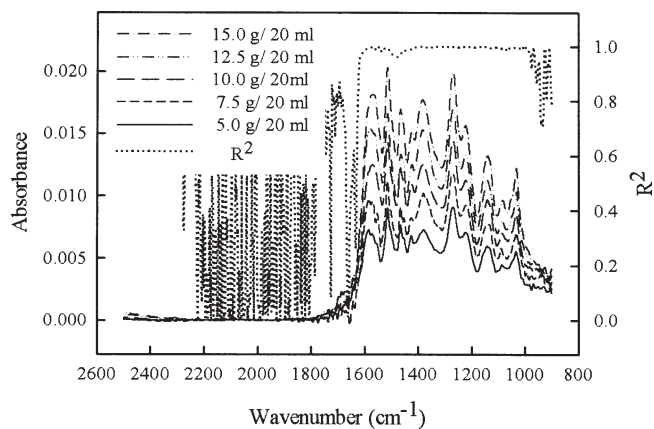
dure (Fig. 7). The results showed the spectra of alkaline oxygen-treated lignin were obtained quantitatively from a viewpoint of R^2, and the water spectra were cancelled effectively, as in the case of sodium vanillate.

Conclusions

In order to obtain the infrared ATR spectrum of oxygen-degraded lignin as a difference spectrum between a neutralized reaction mixture of alkaline oxygen treatment of lignin and NaCl solution, methods for determining a suitable concentration and spectrum shape of the NaCl solution for subtraction were discussed. This is because there is a significant amount of NaCl in the neutralized reaction mixture.

Sodium chloride concentration was predicted by PLS analysis of an associational band (1929–$2221\,cm^{-1}$) on the infrared ATR spectrum of sodium vanillate–NaCl solution. By subtracting the spectrum of actual NaCl solution whose concentration was predicted by the PLS analysis, the vanillate spectra were obtained quantitatively. The result showed that it should be possible to mathematically reconstruct the spectrum of NaCl solution in a reaction mixture with the use of the associational band in the spectra of reaction mixture.

The spectrum of NaCl solution in vanillate and NaCl mixture solution was reconstructed as the linear combination of four basic spectra calculated by SVD of the spectra set of NaCl solution. By subtracting the mathematically reconstructed spectra, quantitative spectra of vanillate were obtained as the difference spectra. Using the same procedure, spectra of the high molecular weight fraction of alkaline oxygen-treated kraft lignin in aqueous solution were also obtained quantitatively.

In this report, the method to obtain the spectrum of a compound of interest, in an aqueous solution with a significant amount of NaCl and small amount of the compound

was discussed. Large amounts of NaCl affects the shape of the water spectrum significantly, but the effect of the compound of interest was ignored. For example, significant amounts of sugars like glucose, if it is present, may affect the shape of water spectra, because of the formation of hydrogen bond between the water and hydroxyl groups. Thus, in the case of an aqueous solution containing significant amounts of compounds like sugar, further examination of the effect of the compound of interest may be necessary.

Nonetheless, in this study, the discussed methods allowed us to obtain spectra in which the huge absorption of water or NaCl solution was almost completely eliminated. This included both cases of the aqueous solution of vanillate and high molecular weight fraction of oxygen-degraded kraft lignin. Therefore, the methods will allow us to analyze the neutralized reaction mixture of alkaline oxygen-treatment of lignin quantitatively, and to monitor the reaction progress.

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