Comparison of the Vibration Mode of Metals in HNO₃ by a Partial Least-Squares Regression Analysis of Near-Infrared Spectra

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The near-infrared (NIR) spectra of such metals as Cu(II), Mn(II), Zn(II) and Fe(III) in HNO₃ in the 700–1860 nm region were subjected to a partial least-squares regression analysis and leave-out cross-validation to develop chemometric models. The models yielded a coefficient of determination in cross validation of 0.9744 [Cu(II)], 0.9631 [Mn(II)], 0.9154 [Zn(II)] and 0.741 [Fe(III)]. The regression coefficients for Cu(II), Mn(II) and Zn(II), but not for Fe(III), showed strong negative peaks at around 1050–1200 nm, a zone where spectral bands have been reported to decrease with increasing pH value. A positive peak at around 710–750 nm, which may have been due to water absorption, was observed in regression coefficients of Cu(II), Mn(II) and Zn(II) but not in Fe(III), while a negative peak was observed in that for Fe(III) at around 710–750 nm. These results indicate that the divalent cations [Cu(II), Mn(II) and Zn(II)] showed different absorption in the NIR region from the trivalent cation [Fe(III)], suggesting that the vibration mode of water, which mirrors the interaction between cations and water, may be influenced by valency.

Key words: near-infrared spectroscopy; cation; metal; partial least-squares regression; chemometrics

Materials and Methods

Sample preparation. To minimize metal contamination of the samples, all vessels used were immersed overnight in 0.1 M HNO₃ and then washed thoroughly with deionized water before use. Standard solutions (1,000,000 mg/l) for Cu(II) (Catalog No. 033-16201), Mn(II) (catalog no. 133-12131), Zn(II) (catalog no. 264-01421) and Fe(III) (catalog no. 094-03841) were weak overtones and combinations of the vibration bands of molecular bonds which are formed between hydrogen and such other atoms as nitrogen, oxygen and carbon.¹ NIRSpectroscopy has become a widely-used analytical method in the agricultural, pharmaceutical, chemical, medical and petrochemical industries in recent years.³–⁸ NIRSpectroscopy has been successfully applied to metal analysis in wines,⁹ legumes,¹⁰ forage,¹¹,¹² soil,¹³ sediment,¹⁴,¹⁵ grass and hay,¹⁶,¹⁷ where metals are buried in complex organic matrices. Although detectable, metals per se exhibit no absorption in the NIR region, since their vibrational modes are modulated by forming complexes with organic molecules containing C–H, N–H and O–H bonds.¹⁴ In fact, alterations in the vibrational mode are exploited in detecting metals in the foregoing agricultural materials. However, the effect of metals on absorption in the NIR region remains unknown when no organic matrices are present. The main purpose of this study is to examine whether different metals displayed different effects on the vibration mode of water in the absence of organic matrices. To clarify this issue, the vibrational modes of Cu(II), Mn(II), Zn(II) and Fe(III) in HNO₃ were compared by NIR spectroscopy.

Abbreviations: AAS, atomic absorption spectroscopy; GF-AAS, graphite furnace atomic absorption spectroscopy; ICP-AES, inductively coupled plasma atomic emission spectroscopy; NIR, near-infrared; PLS, partial least-squares regression; R²VAL, coefficient of determination in cross-validation; SECV, standard error of cross-validation; SD, standard deviation

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 Although atomic absorption spectroscopy (AAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) are usually used for the quantitative determination of metals, applicable samples are mainly restricted to the liquid form.¹¹ Near-infrared (NIR) spectroscopy is a fast multicomponent assay using NIR radiation.²,³ The NIR spectral region is dominated by weak overtones and combinations of the vibration bands of molecular bonds which are formed between hydrogen and such other atoms as nitrogen, oxygen and carbon.⁴ NIRSpectroscopy has become a widely-used analytical method in the agricultural, pharmaceutical, chemical, medical and petrochemical industries in recent years.³–⁸ NIRSpectroscopy has been successfully applied to metal analysis in wines,⁹ legumes,¹⁰ forage,¹¹,¹² soil,¹³ sediment,¹⁴,¹⁵ grass and hay,¹⁶,¹⁷ where metals are buried in complex organic matrices. Although detectable, metals per se exhibit no absorption in the NIR region, since their vibrational modes are modulated by forming complexes with organic molecules containing C–H, N–H and O–H bonds.¹⁴ In fact, alterations in the vibrational mode are exploited in detecting metals in the foregoing agricultural materials. However, the effect of metals on absorption in the NIR region remains unknown when no organic matrices are present. The main purpose of this study is to examine whether different metals displayed different effects on the vibration mode of water in the absence of organic matrices. To clarify this issue, the vibrational modes of Cu(II), Mn(II), Zn(II) and Fe(III) in HNO₃ were compared by NIR spectroscopy.

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purchased from Wako (Tokyo). Working stock solutions containing each metal at a concentration of 10,000 μg/l were prepared by diluting the standard solution with 0.1 M HNO₃ which had been prepared by diluting concentrated HNO₃ (Wako catalog no. 140-04016) with milliQ water (Millipore, Molsheim, Germany). The concentration of each sample solution of Cu(II), Mn(II), Zn(II) or Fe(III) varied from 0.03815 μg/l to 10,000 μg/l, these samples being prepared by 2-fold serial dilution of the 10,000 μg/l stock solution with 0.1 M HNO₃.

NIR instruments. The NIR spectra were recorded in the wavelength range of 400–2500 nm at 2-nm intervals by spectrophotometry (6500 instrument; NIRSystems, Silver Spring, MD, USA). The spectral data were collected as the absorbance value \[ \log(1/T) \], where \[ T = \text{transmittance} \]. A quartz cuvette cell with a 1-mm optical length was used for measurement. The cell was positioned in a cell holder in conjunction with a temperature bath to maintain it at 37 °C. Triplicate NIR spectra were taken from each sample.

Data processing. Pirouette software (ver. 3.11; Infometrics, Woodinville, WA, USA) was employed for data processing. To minimize the baseline shift and noise prior to calibration, the spectral data were mean-centered and transformed by smoothing based on the Savitsky–Golay algorithm. The mathematical formu-
calculated intercept was used as an estimate whether a sample was positive for metal detection. The accuracy of the model was evaluated by SECV, the standard deviation (SD) divided by SECV and the coefficient of determination in cross-validation \( (R^2_{\text{VAL}}) \).

AAS. The concentrations of Cu(II), Mn(II), Zn(II) and Fe(III) were determined by graphite furnace atomic absorption spectroscopy (GF-AAS) with polarized Zee-man-effect background correction (Z-9000 atomic absorption spectrophotometer, Hitachi, Japan). The analysis lines used were 324.8, 279.5, 213.9 and 248.3 nm for Cu(II), Mn(II), Zn(II) and Fe(III), respectively. The following thermal treatment program was used: drying from 80 °C to 120 °C for 30 s, ashing from 300 °C to 600 °C for 30 s, holding at 600 °C for 10 s, atomization at 2700 °C for 10 s, and cleaning at 3000 °C for 3 s. Argon gas was used for purging at a flow rate of 200 ml/min during the drying and ashing cycles and at 30 ml/min during atomization. Each sample was determined in triplicate, and the mean value was derived accordingly. The metal values are presented as the absorbance.

ICP-AES. Emission intensity were measured with an ICP-AES spectrometer (SPS1200 VR; Seiko, Tokyo, Japan) as previously reported. The emission lines used were 324.754 nm for Cu(II), 257.610 nm for Mn(II), 213.856 nm for Zn(II), and 259.940 nm for Fe(III). The Ar plasma was maintained with an incident power of 1.3 kW.

Calculating the limit of detection (LD) for the metal concentration. LD is defined here as the analyte concentration to yield a signal intensity three-fold the SD of a blank solution, apart from the mean intensity of the blank solution. According to Miller’s study, LD (\( y_B \)) is calculated from the mean \( \bar{y}_B \) and SD \( S_B \) of the signal for the blank control (the signal for 0.1 m HNO₃ without a metal), as shown in Equation 1, to define whether a sample was positive for metal detection. The value of the calculated intercept was used as an estimate of \( y_B \), the blank signal per se. To estimate \( S_B \), the statistic \( S_{y/B} \), estimating the random error in the y-direction, as expressed in Equation 2, was used in place of \( S_B \). The \( \bar{y}_r \) value is the point on the calculated regression line corresponding to the individual x-value.

\[
y = y_B + 3S_B. \quad (1)
\]

\[
S_{y/B} = \left( \frac{\sum(y_i - \bar{y}_r)^2}{n - 2} \right)^{1/2}. \quad (2)
\]

Results

NIR spectra of the samples containing Cu(II), Mn(II), Zn(II) and Fe(III) were measured in the range of 0–10,000 μg/l. In the present study, the mass concentration was used for indicating the metal concentration, because the usefulness of an analytical method to measure a metal is often assessed by LD, which is usually compared according to the mass concentration. Based on the spectral data pooled in the wavelength range of 400–2500 nm, the spectra within the 700- to 1860-nm region were more favourable for constructing NIR models representing the Cu(II), Mn(II), Zn(II) and Fe(III) concentrations than those in the regions of 400–700, 700–1100, 1100–1800, 1800–2500 and 400–2500 nm (data not shown). Note that no apparent spectral differences were apparent in the wavelength region of 700–1860 nm among the spectra for Cu(II), Mn(II), Zn(II) and Fe(III) in 0.1 M HNO₃ (data not shown). Similar spectra were obtained for water and 0.1 M HNO₃ (data not shown). There were also no apparent changes among the second derivative spectra of Cu(II), Mn(II), Zn(II) and Fe(III) in 0.1 M HNO₃ solution (data not shown).

Two pre-processing procedures, mean-centering and smoothing, were undertaken. The process emphasizes the change in the spectra, since a subtle change in the intensity can be effectively detected in a mean-centered
spectrum, and the noise intensity can be minimized in the smoothed spectrum. The processed spectra were then subjected to a PLS analysis prior to determining the predominant components in the spectra. The full cross-validation was applied to appropriate the PLS calibration models, where SECV attached a minimum value at factors of 6 for Cu(II), 12 for Mn(II), 14 for Zn(II) and 9 for Fe(III). Thus, these values provide the optimal number of PLS factors for deriving the metal concentration (Table 1).

The metal concentrations predicted from the constructed PLS models agree well with the actual metal concentrations for Cu(II), Mn(II), Zn(II) and Fe(III) in the concentration range of 0–10,000 mg/l (Fig. 1).

Moreover, the $R^2_{\text{VAL}}$ values registered 0.974, 0.963, 0.915 and 0.741 for Cu(II), Mn(II), Zn(II) and Fe(III), respectively (Table 1), thus satisfying the criteria for establishing the NIR model where $R^2_{\text{VAL}} > 0.70$.\(^{13}\) It has been reported that the reliability of the analysis depended on the SD/SECV ratio; i.e., poor, acceptable and high reliability was obtained with SD/SECV < 1.6, 1.6–2.0 and >2.0, respectively.\(^{13}\) Using SECV calculated from the linear regression lines (Table 1), the SD/SECV values for Cu(II), Mn(II) and Fe(III) were 6.37, 4.88, 3.36 and 2.00, respectively. Therefore, the present NIR model consisted of spectral data from the 700–1860 nm region, making it possible to precisely predict the concentration of a tested metal within the 1- to 10,000-µg/l range.

The dynamic range of the metal determination by AAS was much smaller than that evaluated by NIR spectroscopy, irrespective of the sensitivity. As tabulated in Table 2, the determination by AAS was limited to concentrations up to 312, 10,000, 0.61 and 156 mg/l for Cu(II), Mn(II), Zn(II) and Fe(III), respectively. In contrast, a wider dynamic range was derived by ICP-AES, which demonstrated a good linear correlation between the emission intensity and metal concentration of, at least, up to 10,000 mg/l.

Based on Equation 1, the LD values for the metals were calculated for the three methods (Table 2). The LD value by NIR spectroscopy

<table>
<thead>
<tr>
<th>$R^2_{\text{VAL}}$</th>
<th>SECV [µg/l]</th>
<th>SD/SECV</th>
<th>Factors used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II)</td>
<td>0.974</td>
<td>383.4</td>
<td>6.37</td>
</tr>
<tr>
<td>Mn(II)</td>
<td>0.963</td>
<td>501.0</td>
<td>4.88</td>
</tr>
<tr>
<td>Zn(II)</td>
<td>0.915</td>
<td>726.6</td>
<td>3.36</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>0.741</td>
<td>1223.3</td>
<td>2.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Method</th>
<th>Cu(II) Range</th>
<th>Mn(II) Range</th>
<th>Zn(II) Range</th>
<th>Fe(III) Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIR spectroscopy</td>
<td>0–10,000</td>
<td>0–10,000</td>
<td>0–10,000</td>
<td>0–10,000</td>
</tr>
<tr>
<td>LD</td>
<td>1156.9</td>
<td>1398.8</td>
<td>2171.0</td>
<td>4222.6</td>
</tr>
<tr>
<td>AAS</td>
<td>0–312.5</td>
<td>0–10,000</td>
<td>0–0.6104</td>
<td>0–156.25</td>
</tr>
<tr>
<td>LD</td>
<td>10.2</td>
<td>4.8</td>
<td>0.4</td>
<td>77.5</td>
</tr>
<tr>
<td>ICP-AES</td>
<td>0–10,000</td>
<td>0–10,000</td>
<td>0–10,000</td>
<td>0–10,000</td>
</tr>
<tr>
<td>LD</td>
<td>53.1</td>
<td>47.7</td>
<td>58.8</td>
<td>71.4</td>
</tr>
</tbody>
</table>

\[\text{Equation 1}\]

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was less sensitive than those by AAS and ICP-AES. These results suggest that NIR spectroscopy is not a better method than ICP-AES or AAS in the terms of sensitivity. ICP-AES appeared to be appropriate for analyzing metals in HNO\textsubscript{3} because of the wide dynamic range and excellent LD values for most of the elements tested.

The regression coefficient incorporates the model coefficients. A line-plot of this object reveals the independent variables (wavelength) necessary for modeling the dependent variable, i.e. metal concentration (Fig. 2). The regression coefficient in this model registered positive and negative peaks for Cu(II), Mn(II) and Zn(II) at around 710–750 nm, while only one negative peak was portrayed for Fe(III) in the region (Fig. 3A). A negative peak (ca. 1050–1200 nm) was observed in loading of the regression coefficients of Cu(II), Mn(II) and Zn(II), but not in that of Fe(III) (Fig. 3B).

**Discussion**

PLS modeling not only facilitates the development of a quantitative model, but also elucidates the spectroscopic characterization of a sample. Several studies have been reported on the spectroscopic analysis of metals in forage, legume, hay and grass in the NIR region.\textsuperscript{10-12,16,17} Spectroscopic characterization was established from data on the regression coefficients of Cu(II), Mn(II), Zn(II) and Fe(III) in HNO\textsubscript{3} with the present PLS model; viz., positive and negative peaks for Cu(II), Mn(II) and Zn(II) at around 710–750 nm, while only one negative peak was observed for Fe(III) in this region. Those peaks were close to the water band (the third overtone of the O-H-stretching mode of water has been assigned to around the 760-nm peak),\textsuperscript{3} and the peaks around 710–750 nm may have been due to absorbed water. A negative peak (ca. 1050–1200 nm) was observed in loading of the regression coefficients of Cu(II), Mn(II) and Zn(II), although the same peak was not observed with Fe(III). Therefore, valency may have affected the negative peak (ca. 1050–1200 nm) which interestingly has been documented to decrease with increasing NaOH concentration.\textsuperscript{21} These observations are consistent with the idea that the model can successfully quantify Cu(II), Mn(II), Zn(II) and Fe(III), because the metals are dissolved in 0.1 M HNO\textsubscript{3} which contains water and shows a low pH value. On the other hand, it needs to be elucidated whether the difference in regression coefficient between divalent cations [Cu(II), Mn(II) and Zn(II)] and a trivalent cation [Fe(III)] is only attributable to the valency; for example, the ion radius, hydration number and charge population might also affect the regression coefficient. Additionally, it is of interest that other peaks for the regression coefficients were not identical to those obtained by other authors using agricultural crops.\textsuperscript{10-12,16,17} This discrepancy may be due to the indirect measurement of metals in agricultural crops by the absorbance in the NIR region. The peaks in the NIR region may be mainly attributable to the absorbance of complexes formed between metals and organic functional groups or water.\textsuperscript{11}

The methods for measuring metal concentration have been used mainly AAS and ICP-AES.\textsuperscript{13} The sensitivity of NIR spectroscopy was found to be low compared with AAS or ICP-AES. However, AAS is applicable within only a narrow range of metal concentration,\textsuperscript{1} although both AAS and ICP-AES are comparably metal-specific.
Fig. 3. Plot with Loading of Regression Coefficients for the Partial Least-Squares Regression (PLS) Models Based on NIR Spectra in the 710–750 nm (A) and 1050–1200 nm (B) Region for Metals in Aqueous HNO₃ (refer to Fig. 1).
and species-sensitive. Different from application-restricted AAS and ICP-AES, NIR spectroscopy could be used as a large-scale routine method for measuring metal concentration in the order of 1,000 μg/l. However, if an exact measurement of a metal at a low concentration is required, NIR spectroscopy will not provide a better method than ICP-AES or AAS. To improve the sensitivity and specificity of the NIR analysis of a metal better method than ICP-AES or AAS. To improve the if an exact measurement of a metal at a low concentration is required, NIR spectroscopy will not provide a better method than ICP-AES or AAS. To improve the sensitivity and specificity of the NIR analysis of a metal better method than ICP-AES or AAS. To improve the sensitivity and specificity of the NIR analysis of a metal better method than ICP-AES or AAS. To improve the sensitivity and specificity of the NIR analysis of a metal better method than ICP-AES or AAS. To improve the sensitivity and specificity of the NIR analysis of a metal better method than ICP-AES or AAS. To improve the sensitivity and specificity of the NIR analysis of a metal better method than ICP-AES or AAS. To improve the sensitivity and specificity of the NIR analysis of a metal better method than ICP-AES or AAS. To improve the sensitivity and specificity of the NIR analysis of a metal better method than ICP-AES or AAS. To improve the sensitivity and specificity of the NIR analysis of a metal better method than ICP-AES or AAS. To improve the sensitivity and specificity of the NIR analysis of a metal better method than ICP-AES or AAS. To improve the sensitivity and specificity of the NIR analysis of a metal better method than ICP-AES or AAS. To improve the sensitivity and specificity of the NIR analysis of a metal better method than ICP-AES or AAS. To improve the sensitivity and specificity of the NIR analysis of a metal better method than ICP-AES or AAS. To improve the sensitivity and specificity of the NIR analysis of a metal better method than ICP-AES or AAS. In conclusion, the potential of NIR spectroscopy was investigated for classification and character quantification of a metal in aqueous HNO₃. The results show that an NIR spectral analysis by our PLS model provided a powerful tool for investigating the vibration and interaction of a metal with water. Knowledge of the wavelengths of different metal species with different factors influencing the PLS model would facilitate a better understanding and proper monitoring of certain metals in the field of applied chemistry.

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