APPLICATIONS OF FT RAMAN SPECTROSCOPY FOR THE CHARACTERIZATION OF CELLULOSE

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FT Raman spectroscopy was used for the investigation of cellulose and cellulose derivates. Lattice structures of cellulose, polymorphic modifications I and II, as well as amorphous structure were clearly identified by means of FT Raman vibrational spectra. Chemometric models were developed utilizing univariate calibration as well as methods of multivariate data analyses of the FT Raman spectral data. Cellulose properties like the degree of crystallinity XcRaman and the degree of substitution DSCMC as well as DSAC were determined.

Keywords: FT Raman spectroscopy, cellulose; cellulose derivatives

Introduction

Vibrational spectroscopy, especially Raman spectroscopy, has played an important role in the investigation of cellulose structures. The fundamental studies of Atalla and co-workers confirmed the advantage of this analytical method over IR and NMR spectroscopy, for determining the molecular conformations and hydrogen bonding patterns of cellulose and cellulosic biomaterials.[1-9] The main reason for the trend to Raman spectroscopy was the development of effective FT Raman spectrometers using NIR or red excitation lasers which avoid the fluorescence of the samples which normally blank the Raman signals. The development of high sensitive detectors in conjunction with the coupling of optical fibres and microscopes enhanced the capacity of Raman spectroscopy for analytical analysis and online process control. FT Raman microscopes equipped with mapping units became very powerful tools especially for in situ investigations of biomaterials.[10-12] We began a still ongoing examination of cellulose structures using methods of deconvolution of the FT Raman spectra, utilizing all the advantages of new spectrometers, computational technologies as well as methods for analysing the Raman vibrational spectra. We developed methods for quantification and fast prediction of cellulose properties using methods of spectra fine resolution in combination with multivariate analyses of the spectral data.

In this paper we report on new results and give a summary of past results of our FT Raman spectroscopic investigations on cellulose, which clearly demonstrate the capability and convenience of this analytical method.

Investigations on cellulose structures

Cellulose modifications I and II

FT Raman spectra of the polymorphic modifications cellulose I and II reflect the conformational differences of both lattice types most clearly in the low frequency
range of the spectra, see Figure 1 (on top). Obviously, cellulose modification I and II differ in the conformational arrangements of the side chains of the anhydroglucopyranose residues. Our findings by FT Raman experiments which were reported earlier\[13\], confirmed findings by Wiley & Atalla\[5, 6\] using “classical” Raman spectroscopy with visible laser excitation. The FT Raman spectra of cellulose modification I indicated the simultaneous presence of two stereo chemically non-equivalent CH$_2$OH groups resulting from the rotation of the side chains about the C(5)–C(6) atoms. In cellulose II, only one type of the CH$_2$OH groups was present. This causes the two scissoring vibrations of the methylene groups to merge into one single signal. In contrast to the investigations of Atalla & co-workers, we recorded the Raman vibrational spectra by means of NIR laser excitation. Thus, fluorescence-free Raman spectra of cellulose, pulps and plant material were measured without special sample preparations. Furthermore, using methods of derivative spectrometry already small differences in the vibrational behaviour of cellulose modifications or cellulose forms were identified at their FT Raman spectra. For instance, the alkaline treatments of cellulose indicated a frequency shift $\Delta \nu$ of 13 cm$^{-1}$ of the most intensive Raman line (2893 cm$^{-1}$), as can be seen from the second derivatives, see Figure 1 (below). After the treatments ($c_{NaOH} = 16\%$) the intensity maximum appeared at 2880 cm$^{-1}$, which is a characteristic of cellulose modification II.$^{[14]}$

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{FT Raman spectra following the polymorphic transformation cellulose I into cellulose II due to cellulose treatments with different alkaline concentrations; (on top): low frequency range; (below): CH stretching region of the spectra and their second derivatives.}
\end{figure}

(*) cellulose modification I; (+) cellulose modification II

The polymorphic transformation of cellulose I into cellulose II is caused also by dissolving cellulose in different molten inorganic salt hydrates. Previously, it was reported that several molten inorganic salt
hydrates may serve as solutes for cellulose.\textsuperscript{[15-17]} Interestingly, from cellulose, regenerated from the melts of ZnCl$_2$•4H$_2$O, LiSCN•2.5H$_2$O and LiCl•2ZnCl$_2$•6H$_2$O, can be proven that dissolution leads to the transformation of cellulose I to cellulose II. This was confirmed by FT Raman spectra and WAXS investigations.\textsuperscript{[13]}

\textit{Amorphous cellulose}

The three molten salts ZnCl$_2$+4H$_2$O, LiSCN•2.5H$_2$O and LiCl+2ZnCl$_2$+6H$_2$O served as solvents for the cellulose for the dissolution experiments. Solutions of cellulose (5 \% w/w) were prepared using the melts. Whereas solutions of cellulose in ZnCl$_2$+4H$_2$O appeared liquid after rapid cooling to room temperature, solutions in LiSCN•2.5H$_2$O and LiCl+2ZnCl$_2$+6H$_2$O formed a glassy state. In all cases no precipitation of cellulose fibres was observed. It can be concluded, that the specific interactions between cellulose and molten salts within the solution are maintained also in the solid state after cooling.

The dissolving process could be controlled easily by online FT Raman spectroscopic investigations, because the pure molten salts do not show any vibrational mode at the observed frequency range of the FT Raman spectra. It turned out that the Raman lines of the dissolved cellulose are broader in the melt systems than for the pure polymers. Nevertheless, a Raman shift of the $\nu$(C-O-C) modes of cellulose ($\Delta\nu$ of $\sim$10 cm$^{-1}$) to lower wave numbers was observed indicating different vibrational coupling between the anhydroglucopyranose units when compared with the crystalline cellulose.

A comparison of the FT Raman spectra of amorphous cellulose with the spectra of cellulose dissolved in the hydrated melts is shown in Figure 2. It can easily be seen that the vibrational frequency of the $\nu$(C-O-C) mode of cellulose in the melts and that of amorphous cellulose are nearly the same. Additionally, typical vibrations of amorphous cellulose were observed at 1260 cm$^{-1}$ and 1460 cm$^{-1}$. This was also the case for the cellulose in the hydrated melts. Therefore it was deduced, that cellulose dissolved in molten salts undergoes a transition into an amorphous state. This was also confirmed by $^{13}$C NMR measurements.\textsuperscript{[16]} No indication for the formation of additional compounds have been observed.

\textit{Crystalline and amorphous cellulose - Determination of $X_c$Raman/\%}

In view of those results, it became interesting to examine the vibrational behaviour of crystalline cellulose I, and compare it with the amorphous forms, which were produced by grinding of the crystalline ones.

Two model systems of cellulose I, a bacterial cellulose as well as an Eucalyptus sulphite pulp system were investigated. These also served as calibration models which represented entire ranges of cellulose I crystallinity of 0-69\% and 0-40\%.

Once more, using methods of fine resolution of the convoluted FT Raman spectra of cellulose, significant differences
between crystalline cellulose I and its amorphous form were observed in the range of $\delta CH_2$ of the CH$_2$OH side chains of the cellulose skeletons. Vibrational modes of methylene bendings $\delta CH_2$, characteristic of each form, crystalline (1481 cm$^{-1}$) and amorphous (1462 cm$^{-1}$), were determined, see Figure 3 (on top). A strong intensity dependence of these two peaks was observed due to the transition from crystalline to amorphous state, as it is presented in Figure 3 (below) by physical mixtures of cellulose I with different degrees of adjusted crystallinity, $Xc/\%$: 0%; 10%; ...60%.

As a result, the intensity ratio of the two characteristic lines is strongly influenced by the degree of crystallinity of cellulose I, and therefore also suitable for its quantification. Taking this into account, a Raman crystallinity index, $Xc_{\text{Raman}} / \% = (I_c / I_c + I_a) \times 10^2$, was defined to determine the percentage crystallinity -$Xc_{\text{Raman}}$- of cellulose I.

Intensities of the Raman modes of crystalline ($I_c$) and amorphous ($I_a$) content were obtained by peak fitting using Gaussian bands and linear baselines. Linear calibration curves illustrating the relationship between experimentally determined percentage crystallinities, $Xc_{\text{Raman}}$ and adjusted crystallinities, $Xc$, were obtained by least square analyses of the data for both model systems.

In Figure 4, the Eucalyptus sulphite pulp system was included into the bacterial cellulose calibration set. KBr was used for homogenisation of the powder blends to keep effects of light diffraction due to different refractive indices at a minimum. Thus, it became possible to describe the two cellulosic systems by one calibration model.

Microcrystalline cellulose with known $Xc_{\text{NMR}}$ values was examined for validation of this univariate calibration model.

Figure 3. FT Raman spectra and second derivatives of cellulose I model compounds, (top). FT Raman spectra of generated mixtures of cellulose I with different adjusted degrees of crystallinity, $Xc/\%$, (bottom).

Figure 4. Eucalyptus sulphite pulp model (V) included into the bacterial cellulose calibration set (■), described by $Xc_{\text{Raman}} = 0.962 \times Xc + 1.34$. 
Generally, $X_c^{\text{Raman}}$ values and $X_c^{\text{NMR}}$ values agree to within $\pm$ 5%. These investigations using methods of univariate spectra analysis led to the development of a new method for quantification of cellulose I crystallinity. This method is described in full somewhere else.\textsuperscript{[16]}

**Quantification and prediction of cellulose properties**

In accordance with the new spectroscopic approaches, it is necessary to establish reliable and rapid methods to perform FT Raman analyses for quantification or for prediction of physical and chemical properties also of such complex molecules like cellulose and high variable systems like biological materials. For these purposes, methods of multivariate calibration, classification and clustering of the analytical data may be used.

**Determination of $X_c^{\text{Raman}}$ - a multivariate calibration model**

The cellulose I model systems with known percentual crystallinity values $X_c^{\text{Raman}}/\%$ that were obtained by means of the univariate calibration model as described above were examined using the Bruker OPUS/Quant 2 software. A partial least square algorithm (PLS) was utilized in order to find the best correlation function between spectral and concentration matrix. A very fast multivariate chemometric calibration model for predicting the percentage of cellulose crystallinity, $X_c^{\text{Raman}}/\%$ was developed by applying cross-validation.\textsuperscript{[19]} In Table 1 the $X_c^{\text{Raman}}/\%$ values of different cellulose determined by means of the univariate as well as the multivariate calibration model are presented and compared with the corresponding $X_c^{\text{NMR}}$ values.

It is necessary that the number of calibration spectra exceeds the number of components in the mixture in order to obtain statistically significant results for PLS software. 22 FT Raman spectra, one for each generated cellulose I mixture with known degree of crystallinity $X_c^{\text{Raman}}/\%$ as predicted by the univariate chemometric model, were used for generating the multivariate calibration model. FT Raman spectra were vector normalized and the first derivatives of the spectra were calculated for all pre-calibrations. Not the total spectral range from 3500- 150 cm$^{-1}$ was used to perform the PLS studies. We preferred to select the small spectral region 1510-1210 cm$^{-1}$ because of its strong crystallinity dependence, which turned out to improve the prediction of results. The accuracy of the established calibration model it was validated by using a cross-validation method. The validation statistics described the resultant calibration model with good fits ($R^2 = 0.9805$) and with low error between modelled and reference values (RMSECV= 2.04).

<table>
<thead>
<tr>
<th>Cellulose</th>
<th>$X_c^{\text{NMR}}/%$</th>
<th>$X_c^{\text{Raman}}/%$</th>
<th>$X_c^{\text{Raman}}/%$ multivariat</th>
</tr>
</thead>
<tbody>
<tr>
<td>ElcemaF100</td>
<td>26 %</td>
<td>23 %</td>
<td>28 %</td>
</tr>
<tr>
<td>ElcemaF150</td>
<td>32 %</td>
<td>18 %</td>
<td>30 %</td>
</tr>
<tr>
<td>VitaceLL A300</td>
<td>29 %</td>
<td>21 %</td>
<td>30 %</td>
</tr>
<tr>
<td>Vivapur 102</td>
<td>60 %</td>
<td>59 %</td>
<td>55 %</td>
</tr>
<tr>
<td>Avicel PH200</td>
<td>59 %</td>
<td>59 %</td>
<td>58 %</td>
</tr>
<tr>
<td>Avicel PH301</td>
<td>62 %</td>
<td>52 %</td>
<td>57 %</td>
</tr>
<tr>
<td>Bacterial cell.</td>
<td>72 %</td>
<td>69 %</td>
<td>69 %</td>
</tr>
<tr>
<td>BuckeyLinters</td>
<td>60 %</td>
<td>56 %</td>
<td>58 %</td>
</tr>
<tr>
<td>Cotton Linters</td>
<td>67 %</td>
<td>50 %</td>
<td>61 %</td>
</tr>
<tr>
<td>Borregard</td>
<td>55 %</td>
<td>51 %</td>
<td>54 %</td>
</tr>
</tbody>
</table>

Table 1: Degree of crystallinity $X_c/\%$ of different cellulose materials determined by $^{13}$C NMR and FT Raman spectroscopy utilizing univariate and multivariate methods for the analysis of FT Raman spectral data.
Determination of the degree of substitution (DS) of cellulose derivates - multivariate calibration models

FT Raman spectra of cellulose derivates like carboxymethylcellulose (CMC) and cellulose acetate (CA) were also recorded. The degrees of substitution of CMC\textsuperscript{20} and CA\textsuperscript{21} were determined through NMR measurements. Multivariate chemometric models for the determination of DS\textsubscript{CMC} and DS\textsubscript{CA} were obtained by applying the PLS algorithm of the BRUKER software OPUS/Quant 2 to the FT Raman spectral data of the calibration samples. In both cases cross-validation was used for the verification of the predictions of the calibration model.\textsuperscript{19}

In Figure 5 (top) an example of a FT Raman spectrum of a carboxymethylcellulose is presented. Typical Raman signals which characterise the pattern of substitution were observed at the frequency ranges of υ(COO\textsuperscript{-}), υ(C-O) and γ(C-C) modes. Consequently, the wave number range 1730-800 cm\textsuperscript{-1} of the spectra was utilized for the development of the multivariate calibration model. A PLS regression algorithm was applied for finding the best correlation function between the vector normalized FT Raman data and the corresponding DS values of the carboxymethylcellulose.

The calibration model was evaluated by cross-validation.

As a result, a multivariate calibration model for predicting DS\textsubscript{CMC} values in the range of 0.39–2.00 with high accuracy ($R^2=0.9779$) and low error between modelled and reference values (RMSECV= 0.0815) could be developed. A similar calibration model for determining the degree of substitution of cellulose acetate DS\textsubscript{CA} in the range of 1.25–2.90 was generated. For this purpose, the FT Raman spectral data were pre-calibrated by calculating their second derivatives. Here, the frequency range of 1860-1275 cm\textsuperscript{-1} of υ (C=O) is most suitable for the calibration procedure. The final cross-validation statistics certified a calibration model of high accuracy ($R^2=0.959$) and with low error between the modelled and reference values (RMSECV=0.119) for the fast prediction of DS\textsubscript{CA} values.

Conclusion

The results reported in this work clearly demonstrate that FT Raman spectroscopy is an effective method for the characterization of native cellulose, pulps, as well as cellulose derivates. The important advantages of FT Raman spectroscopy are the ease of sample preparation and the short time required for the measurements. The effective combination of macroscopic and microscopic tools as well as the possibility for on line process control will establish this analytical method for production and quality control of all processes in which changes of conformational arrangements and molecular compositions of cellulose occur.

For the first time univariate and multivariate calibration models were developed utilizing FT Raman spectral data for determining the degree of cellulose I crystallinity, X\textsubscript{C\textsubscript{Raman}} and the degrees of substitution DS\textsubscript{CMC} as well as DS\textsubscript{CA} for native cellulose and cellulose derivates.
References