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Identification of celluloses with Fourier-Transform (FT) mid-infrared, FT-Raman and near-infrared spectrometry*

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Abstract: Five different celluloses are studied with FT-IR, FT-Raman and near-infrared (NIR) spectrometry, and the fingerprint region of the spectra is assigned. Identification of each of the five celluloses is possible with each of the three vibrational methods. Four different batches of hydroxyethyl cellulose yield very similar spectra with each of the three methods. The methods are compared with respect to their capability to discriminate between celluloses and batches, and with respect to ease and feasibility of sample preparation.

Keywords: *Celluloses; identification; FT-IR; FT-Raman; near-infrared; spectrometry.*

Introduction

Identification of bioactive substances and formulation excipients in pharmaceuticals is done using various analytical and physical chemical methods, in accordance with pharmacopoeia monographs; for a recent discussion, see [1]. Among these methods is Fourier-transform mid-infrared spectrometry (FT-IR).

FT-IR is able to discriminate between even closely related compounds, and it is sensitive to impurities in low concentrations. However, FT-IR is strongly dependent on proper sample preparation, so sample preparation may be difficult or time-consuming. In some cases, the sample may not even be amenable to FT-IR analysis. Distortion of FT-IR spectra by specular reflectance, or change in crystal structure by grinding of substances for KBr tablets, are examples of such problems [2, 3]. These properties of FT-IR have led us to investigate FT-Raman and near-infrared (NIR) spectrometry as alternatives to FT-IR. Both methods have, in principle, the same discriminating capability as FT-IR.

In the present study, five different celluloses were investigated using both FT-IR, FT-Raman and NIR spectrometry; these were: ethyl- (EC), hydroxyethyl- (HEC), ethylhydroxyethyl- (EHEC), hydroxypropyl- (LF-HPC) and hydroxypropylmethyl-cellulose (HPMC). For HEC, spectra of four different batches were studied. The contrast between spectra of different celluloses, and the degree of sample preparation, was compared between the FT-IR, FT-Raman, and NIR methods.

Experimental

Materials

Ethyl cellulose was 10 cps CR from Dow Chemical Co.; ethylhydroxyethyl cellulose was Bermocoll E 320 G from Berol Nobel; hydroxypropyl cellulose was type LF from Aqualon/Hercules; and hydroxypropylmethyl cellulose was 6 cps from Dow Chemical Co. Hydroxyethyl cellulose was Aqualon Natrosol; the four batches were: 250 G-Pharm., 250 M-Pharm., 250 HX-Pharm. and 250 HHX-Pharm.

For FT-IR, 20 mg cellulose was dissolved in 5 ml of a suitable solvent, either H_2O, CH_2Cl_2, or CH_3OH–CH_2Cl_2 (3:7, v/v). Of this solution, 0.25 ml was ground with 300 mg KBr under a heat lamp, and pressed into a tablet.

Methods

FT-IR spectra were obtained on a Perkin–Elmer 1710 FT-IR spectrometer, at a reso-

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lution of 4 cm^{-1}, with 12 scans. The spectra were obtained in transmission mode and converted to absorbance. FT-Raman spectra were obtained from the pure cellulose in 5 mm diameter NMR-tubes, on a Perkin–Elmer 2000 FT-Raman spectrometer, at a resoluition of 4 cm^{-1}, with 32 scans. NIR spectra were obtained from the pure celluloses in cuvettes with a front surface window with 36 mm diameter and a depth of 10 mm, on a NIR Systems 6500 scanning spectrometer, with a spectral bandwidth of 10 nm, 2 nm data interval, and 50 scans per spectrum. The NIR spectra were obtained in diffuse reflectance mode (R), and afterwards converted to apparent absorbance (A), using the formula $A = \log(1/R)$.

The vibrational spectra were interpreted according to [4], with molecular structure of the celluloses from [5]. Comparison with a previous study of the FT-Raman spectrum of cotton (unsubstituted cellulose) was also used [6].

Results and Discussion

In Fig. 1, FT-IR spectra are displayed for LF-HPC, HPMC, EC, HEC and EHEC. All five spectra differ clearly from each other.

The bands due to ether COC stretch (IR, 1150–1060 cm^{-1}, very strong) are strong in all five spectra. For EC the bands are quite pure, since EC has few OH groups. This lack of OH groups is also reflected in the EC spectrum above 3000 cm^{-1} (OH stretch).

Alcohols absorb strongly in the IR at 1075–1000 cm^{-1} (primary) and 1150–1075 cm^{-1} (secondary alcohols). LF-HPC and HPMC only contain secondary alcohol groups, and in Fig. 1 they both have an intense band around 1075 cm^{-1}. This band is also found for the secondary alcohol groups of HEC and EHEC, but HEC and EHEC have an additional shoulder around 1030 cm^{-1} due to their primary alcohol groups. Methoxy groups (IR, 1200–1185 cm^{-1}) are only found in HPMC, and for this compound a band is observed around 1188 cm^{-1} in Fig. 1.

The bands above 1200 cm^{-1} and below 1000 cm^{-1} are stronger in Raman and shall be discussed below.

Figure 2 compares FT-IR spectra of four different batches of HEC: G-Pharm., M-Pharm., HX-Pharm. and HHX-Pharm. The

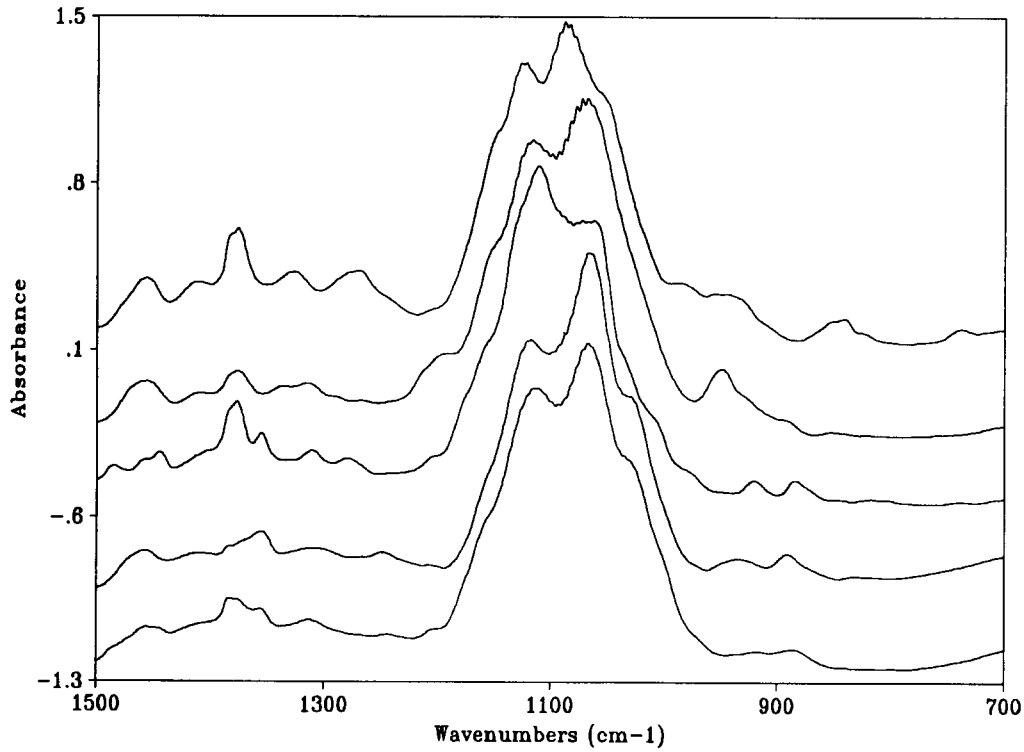

Figure 1
FT-IR spectra of (top to bottom): LF-HPC, HPMC, EC, HEC and EHEC, in the region 700–1500 cm^{-1}. Absorbance scale: 2.8, 3.2, 1.75, 2.2 and 2.4, respectively; spectra are offset for clarity. Concentrations: 1–1.5 mg cellulose in 300 mg KBr. For abbreviations of celluloses, see text.

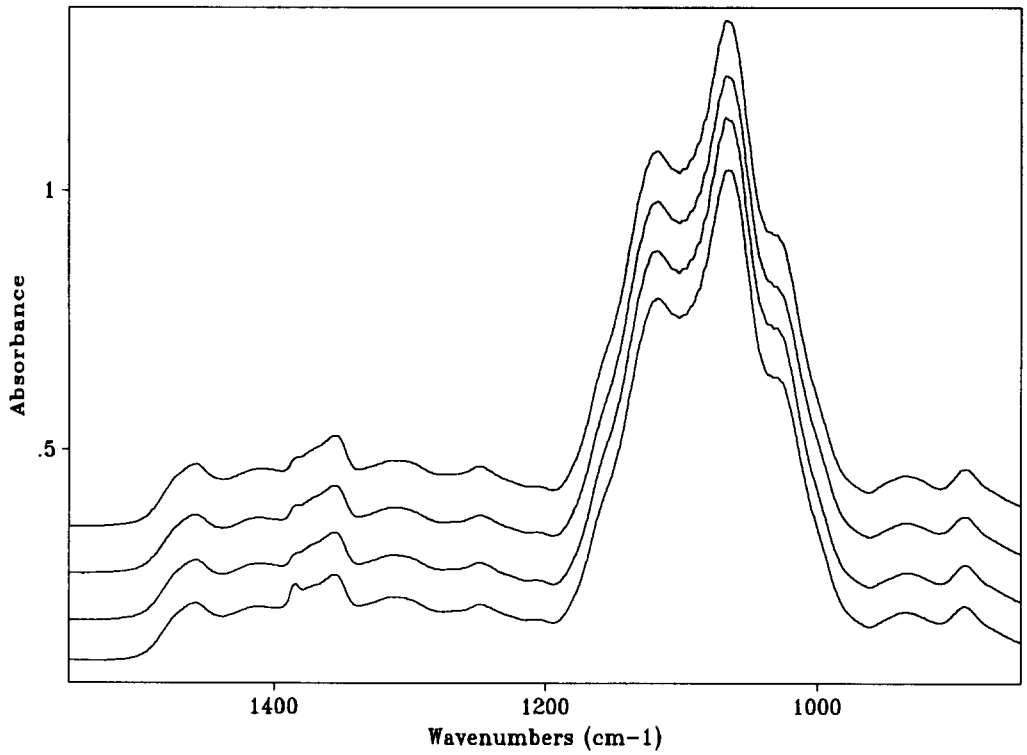

Figure 2
FT-IR spectra of HEC batches (top to bottom): G-Pharm., M-Pharm., HX-Pharm. and HHX-Pharm., in the region 850–1550 cm^{-1}. Absorbance scale: 1.5, 1.5, 1.8 and 1.3, respectively; spectra are offset for clarity. Concentrations: 1 mg cellulose in 300 mg KBr.

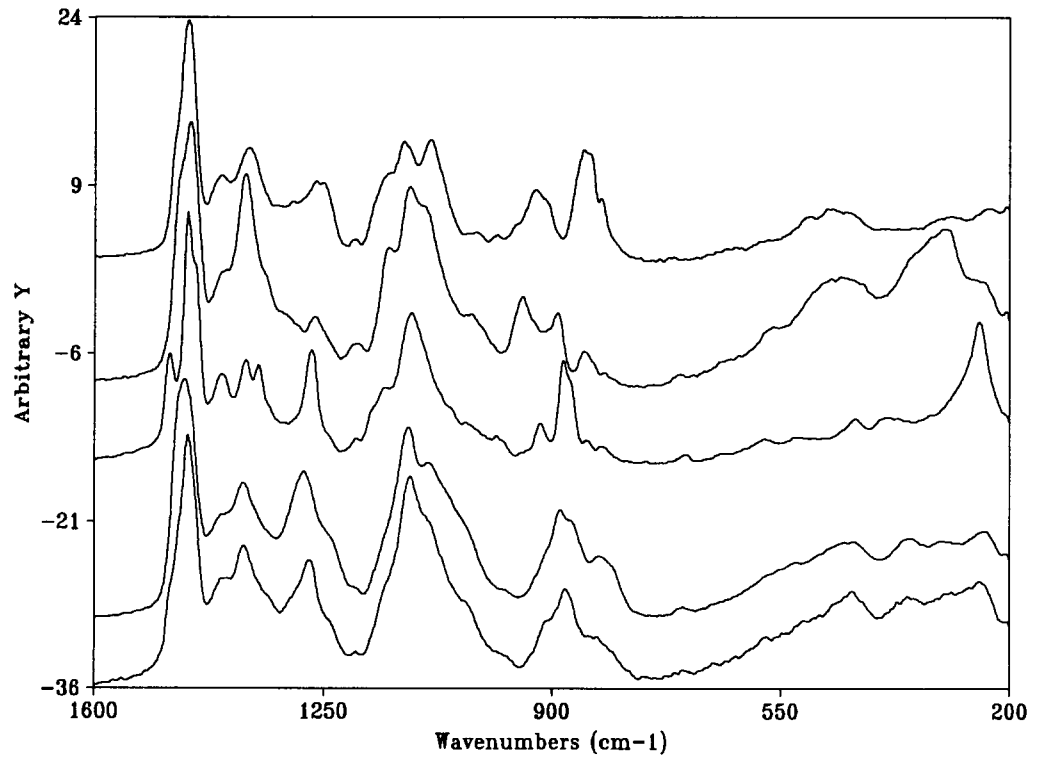

Figure 3
FT-Raman spectra of (top to bottom): LF-HPC, HPMC, EC, HEC and EHEC, in the region 200–1600 cm^{-1}. Intensity scale: 60, 110, 60, 65 and 40, respectively; spectra are offset for clarity. Pure cellulose in 5 mm NMR tubes.

four spectra are nearly identical. One difference is the intensity of the band at 1385 cm^{-1}. This band is due to nitrate, and was found to be quantitatively proportional to the amount of nitrate in the sample.

FT-Raman spectra are displayed in Fig. 3 for LF-HPC, HPMC, EC, HEC and EHEC. All five spectra are seen to differ from each other. Notably, the difference between HEC and EHEC seems larger from FT-Raman in Fig. 3 than from FT-IR in Fig. 1.

Ether COC stretch gives rise to strong Raman bands in the 890–820 cm^{-1} region [4], and unsubstituted cellulose has a Raman band at 900 cm^{-1} [6]. All five substituted cellulose in the present study have bands close to this frequency, although the frequencies are shifted due to ether groups in the substituents. For EC, the band at 883 cm^{-1} must be a rather pure ether band, and it is nearly unshifted for HEC and EHEC.

Cotton [6] and EC only have weak bands between 850 and 600 cm^{-1}, so for HEC, EHEC, HPMC and LF-HPC, the bands in the 850–750 cm^{-1} region are likely due to substituents. Both primary and secondary alcohols have strong Raman intensity in the 900–800 cm^{-1} region, and give rise to the bands around 840 cm^{-1}. These bands are similar for HEC and EHEC which contain both primary and secondary alcohols. Branched alkanes also have C–C stretch Raman bands in the 850–750 cm^{-1} region, so C–C stretches in the substituents may contribute intensity in this region. C–C stretch is the dominant contribution in the 950–900 cm^{-1} region with the strongest bands for the most branched celluloses, HPMC and LF-HPC.

The bands in the 1200–1000 cm^{-1} region also have contributions from C–C and C–O stretches. Unsubstituted cellulose has strong Raman bands from ether at 1097 and 1122 cm^{-1}, and from C–C at 1153 cm^{-1} (medium). The bands around 1120 and 1150 cm^{-1} are similar for the five substituted celluloses and comparable to unsubstituted cellulose. Since EC is the only compound with low intensity around 1090 cm^{-1}, this band probably gains intensity from secondary alcohol in the substituted celluloses.

The region 1500–1200 cm^{-1} is typical for CH bends, many of which have strong Raman intensity. In unsubstituted cellulose [6], bands in this region are found at 1478 (weak), 1462 (w), 1410 (w), 1380 (strong), 1338 (medium) and 1294 cm^{-1} (w). For the five substituted celluloses, the bands around 1370 (m) and 1405 cm^{-1} (w) are likely due to the glucose part, but for the bands around 1460 and 1270 cm^{-1} the intensity is much larger than for cotton. LF-HPC, HEC and EHEC have large substituents and similar intensity patterns. For

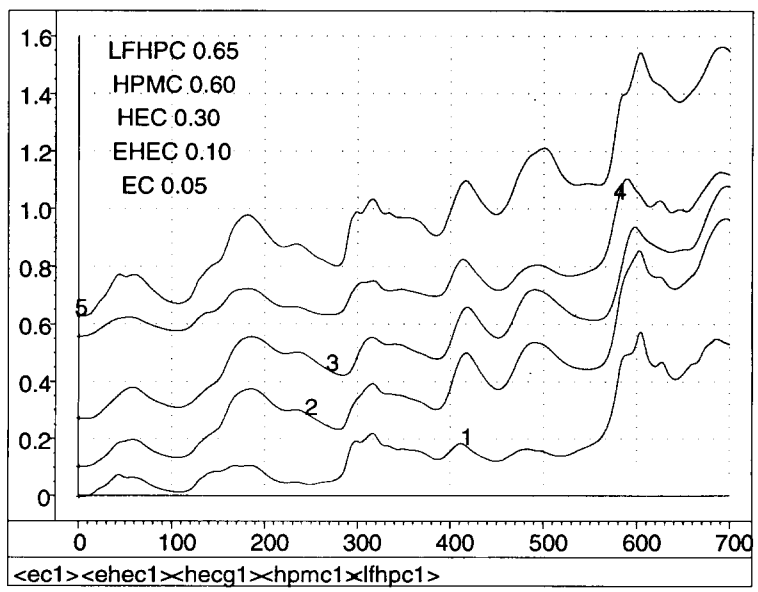

Figure 4
NIR spectra of (top to bottom): LF-HPC, HPMC, HEC, EHEC and EC, in the region 1100–2500 nm. Abscissa scale shown is channel number (CN), where wavelength = (1100 + 2 × CN) nm. Absorbance scale 0–1.6; spectra are offset for clarity by 0.65, 0.60, 0.30, 0.10 and 0.05 absorbance units, respectively. Pure celluloses in cuvettes.

HPMC, the intensity of the band at 1370 cm^{-1} is large compared to the band at 1264 cm^{-1}. Since 1370 cm^{-1} derives intensity from the glucose part, and 1264 cm^{-1} does not, this may be due to the rather low degree of substitution in HPMC.

Methyl and methylene groups have strong Raman bands in the region 1475–1440 cm^{-1}, also with neighbouring oxygen [4], and the large intensity of the bands around 1460 cm^{-1} is characteristic for the alkane parts in the substituted celluloses. Also the very high frequency band of EC at 1485 cm^{-1} must be due to CH bend.

The FT-Raman spectra of the four different batches of HEC showed only small differences. Nitrate, which was found in the FT-IR spectrum at 1385 cm^{-1}, has a strong Raman band at 1050 cm^{-1}, but this nitrate band is masked by HEC bands.

NIR spectra are displayed in Fig. 4 for LF-HPC, HPMC, HEC, EHEC and EC. Again, all five spectra differ from each other. EC lacks alcohol groups, and has consequently low intensity around 1450, 1900 and 2100 nm (channels 175, 400 and 500 in Fig. 4), characteristic for ROH vibrations. In NIR spectra, CH_3, CH_2 and CH vibrations are found in order of increasing wavelength. This phenomenon is seen at 1200 and 1700 nm (channels 50 and 300), and in the region of C–H combinations, 2200–2500 nm (channels 550–700). HPC with much branched substituents has intensity at longer wavelengths compared to the other celluloses.

In Fig. 5, the NIR spectra of the four different batches of HEC are compared. The four spectra are very similar, and for that reason first derivative spectra are shown. It is seen that in some regions the four spectra are identical, notably above 2100 nm. However, there are differences around 1150, 1350, 1700 and especially 1900 nm (channels 25, 125, 300 and 400). The first three wavelength regions correspond to CH vibrations, whereas 1900 nm is the wavelength of the dominant NIR band from water. It thus seems that there are some differences in the substitution pattern and in water content between the four batches.

Conclusions

It was found possible to distinguish reliably between the five different celluloses with each of the three methods FT-IR (Fig. 1), FT-Raman (Fig. 3) and NIR (Fig. 4). Interpretation of the FT-IR and FT-Raman spectra in combination gave comprehensive structural information on the celluloses. An important aspect is that the ability to distinguish was found to be at least as high for FT-Raman and NIR as it was for FT-IR.

All three methods, FT-IR (Fig. 2), FT-Raman and NIR (Fig. 5), showed only minor differences between the spectra of the four different batches of HEC. On the basis of the vibrational spectra, all four batches would be identified as HEC. The most notable differences were the amount of nitrate which could be determined by FT-IR, and the water con-

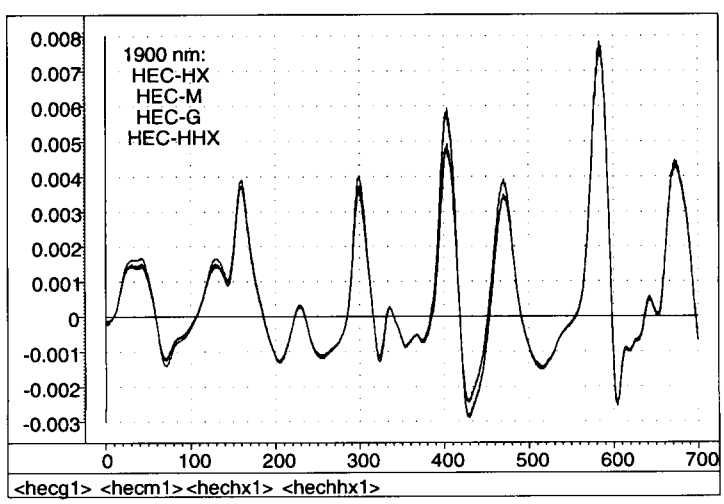

Figure 5
First derivative NIR spectra of HEC batches in the region 1100–2500 nm. At 1900 nm, the order is from above: HX-Pharm., M-Pharm., G-Pharm. and HHX-Pharm. Abscissa scale as in Fig. 4. Absorbance scale −0.003 to 0.008.

tent in the batches which could be distinguished by NIR, but apart from this the three methods must be judged to show the same amount of difference between batches.

It may be desirable to study samples in their naturally occurring state, i.e., to be able to study not only their chemical but also their physical properties. In this respect, NIR, which requires minimal sample preparation, is generally quite sensitive to the physical properties of the sample, like particle size and packing density. For FT-Raman, the chemical information in the spectra is generally less perturbed by the physical properties, although differences in crystallinity often show up clearly. For pharmacopoeial purposes, IR-identity is defined chemically, not physically, sometimes requiring extensive sample preparation, e.g. film formation or recrystallization.

Another aspect is that of sample preparation. In the present study, FT-Raman and NIR spectra were obtained directly from the celluloses, whereas FT-IR spectra demanded sample preparation. This is not only a matter of time consumption. Problems with sample preparation, e.g. with respect to obtaining a small enough particle size for the compound under study, more often render samples unsuited for analysis with FT-IR than with FT-Raman or NIR.

In the present work, the spectra have been evaluated directly by inspection. However, for practical use of the vibrations methods, vibrational spectrometry must be combined with statistical or chemometrics methods for acceptance or rejection of samples as having a certain identity. Here, principal components analysis (PCA) in combination with Mahalanobis distance is a possibility, but this aspect is beyond the scope of the present paper.

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