



Traditions, anomalies, mistakes and recommendations in infrared spectrum measurement for wood

Laszlo Tolvaj¹

Received: 9 April 2022 / Accepted: 21 October 2022
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Abstract

This paper deals with the difficulties of infrared spectroscopy measurement and suggests ways of dealing with them. Many problems appear when applying ATR (attenuated total reflection) measurement for determining the absorbance spectrum of wood, especially the highly porous nature of wood which does not fulfil the requirements of ATR measurement. Correct ATR spectrum determination requires wavelength dependence correction, but some authors miss out doing this. Normalisation of the infrared (IR) spectrum is a useful data manipulation method for correct evaluation of the spectra, but the incorrect normalisation can destroy the spectrum preventing the evaluation of the spectrum appropriately. Examples are given to teach the correct normalisation process. The difference spectrum method is an excellent tool to present the changes in IR spectra, but only a few scientists use it. Usage of wavenumbers during IR spectrum presentation is a traditional method nowadays. However, the usage of wavelength gives a more expressive spectrum presentation than wavenumber if the whole wavelength interval is presented in one diagram.

Introduction

Infrared (IR) spectroscopy is an excellent tool to follow the chemical changes in a material generated by different treatments. Wood is an opaque material in the IR, and consequently, the traditional absorption measurement is not usable in most cases. The first generation of IR spectrophotometers contained a prism to disperse the IR beam, but this technology was not sufficiently sensitive enough to detect diffusely reflected IR radiation. Scientists had to create new methods to measure the IR absorption spectrum of wood. In this regard, the pellet method was designed. It needed a bearer material which was transparent for IR radiation. The commonly used bearer material was potassium bromide (KBr). Powdered wood was mixed

✉ Laszlo Tolvaj
tolvaj.laszlo@uni-sopron.hu

¹ Institute of Wood Technology and Technical Sciences, University of Sopron, Bajcsy-Zs. U. 4, 9400 Sopron, Hungary

into KBr powder, and the mixture was pressed to form a disc. This semi-transparent disc was used to determine the absorption spectrum by the traditional transmittance measurement method. The absorption positions of the main chemical components for wood were determined by this pellet method (Hergert 1960; Sarkanen et al. 1967a, b, c; Obst 1982; Faix and Beinhoff 1988; Faix 1991).

As an independent variable, the wavenumber is often used instead of wavelength or frequency to visualise the absorption spectrum in IR spectroscopy these days. In contrast, the wavelength and the frequency are the usually used independent variables in wave physics.

The appearance of computers generated great progress in spectrophotometer manufacturing. With the advent of Fourier transform infrared (FTIR) instruments and different accessories, infrared spectra of solid samples can be obtained more rapidly with greater sensitivity. Dispersive spectrophotometers apply a narrow slit to achieve high spectral resolution, but this narrow slit significantly reduces the intensity of the passed light. For example, when applying 8 cm^{-1} resolution, only 8/3600th (0.2%) of the incident radiation reaches the detector (Michell 1988). The introduction of the Michelson interferometer and the computer data processing solved this problem. The prism was replaced by a Michelson interferometer to generate a powerful signal for reflectance measurement. The moving mirror of the interferometer generates an interferogram. This interferogram is analysed using Fourier transformation (FT) by the computer, and the result is the actual spectrum. This technique is suitable to determine the reflectance spectrum of wood. However, although the Michelson interferometer existed at the beginning of the twentieth century, the first results measured by FTIR technique were only published in the last part of the century in the field of wood science (Faix 1986; Faix and Beinhoff 1988; Michell 1988; 1991; Owen and Thomas 1989; Zavarin et al. 1990; Tolvaj 1991; Anderson et al 1991a, b, c). The reason of delay was the lack of computer-aided technology.

The Michelson interferometer and the use of computer analysis highly enhanced the usability of the attenuated total reflection (ATR) technique in IR spectroscopy as well. When a light beam travels from a medium of high refractive index (crystal) to a medium of low refractive index (sample), the beam is reflected totally back from the sample to the crystal if the incident angle of beam is high enough. The light extends a small distance beyond the surface of the sample, and the intensity of beam is partly reduced according to the absorption properties of the sample. This method requires excellent contact between the crystal and the measured material and therefore is a useful method for liquids or soft, easily deformable solids. Wood having high porosity and low deformability does not meet this requirement, although the ATR technique is suitable to measure the spectrum of small wooden chips. The ATR spectrum differs from a classic transmission spectrum because the light penetrates into the sample at a depth proportional to the wavelength. Consequently, the absorbance in an ATR-measured spectrum at longer wavelengths is proportionally greater than at shorter wavelengths when compared to a classic transmission spectrum. This problem can be eliminated by mathematical manipulation, which is available in the spectrophotometer data analysis program. However, many studies in the literature do not use this correction. As the ATR technique is a simple one, more and more researchers apply ATR to IR measurement of solid wood samples (Kuo et al. 1988;

Pandey and Theagarajan 1997; Müller et al. 2008; Fufa et al. 2013; Yldiz et al. 2013; Kubovsky and Kacik 2014; Santoni et al. 2015; Kacik et al. 2015; Zborowska et al. 2016; Timar et al. 2016; Kubovsky et al. 2020; Jnido et al. 2021). Unfortunately, most of the researchers ignored that this measurement technique is less suitable for measuring block type samples than for measuring chip type samples.

Wavenumber or wavelength?

In physics, wavelength is the usually used independent variable to present a spectrum, although both wavelength and wavenumber were used in analytical IR spectroscopy in the 1960s. The spectra were presented with two axes representing both wavelength and wavenumber in many cases (Harrington et al. 1964; Marton and Sparks 1967; Sarkanen et al. 1967a, b, c; O'Brain and Hartman 1969; Owen and Thomas 1989; Anderson et al. 1991a, b; Anderson et al. 1991a, b, c). However, the use of wavelength completely disappeared in the twenty-first century. (The wavelength is used in near IR spectroscopy these days as well). The wavenumber itself is acceptable because it represents one kind of frequency, only the use of the unit 1/cm does not fit to the International System of Units (SI). In the case of wavelength, either nanometre (nm) or micrometre (micron, μm) is SI units. The correlation between wavenumber $f(\text{cm}^{-1})$ and wavelength $\lambda(\text{nm})$ is determined by the following equation.

$$f(\text{cm}^{-1}) * \lambda(\text{nm}) = 10000000$$

The real question for wood scientists is which independent variable represents more clearly the IR spectrum of wood. Figure 1 shows the baseline corrected Kubelka–Munk (K–M) spectrum of poplar (*Populus x euramericana cv. pannonia*) measured by the diffuse reflectance method as a function of wavenumber, and Fig. 2 presents the same spectrum as a function of wavelength. The two spectra contain the same K–M values, but the appearances are different. The fingerprint region (right side) is compressed, and the hydroxyl-methyl region (left side) is expanded in Fig. 1. The opposite situation happens in Fig. 2, the fingerprint region is expanded, and the hydroxyl-methyl region is compressed. These appearances are generated by the reciprocal relationship between the wavenumber and wavelength. The fingerprint region contains plenty of overlapped absorption bands. This region occupies 29% of the horizontal axis if the independent variable is wavenumber. In contrast, the fingerprint region takes up 73% of the horizontal axis if the independent variable is wavelength. Meanwhile, the horizontal interval of hydroxyl-methyl region changes from 36 to 13%. The expansion is excellent for the fingerprint region, and the 13% is sufficient for representing the hydroxyl-methyl region. The main advantage of presentation by wavelength is that the most important fingerprint region is enlarged (Figs. 1 and 2 do not display the whole analytical IR region. The introduced interval contains all important absorption bands of wood). It can be concluded that the presentation of IR absorption spectrum of wood is much more useful by wavelength than by wavenumber if the whole wavelength interval is presented in one diagram. Both

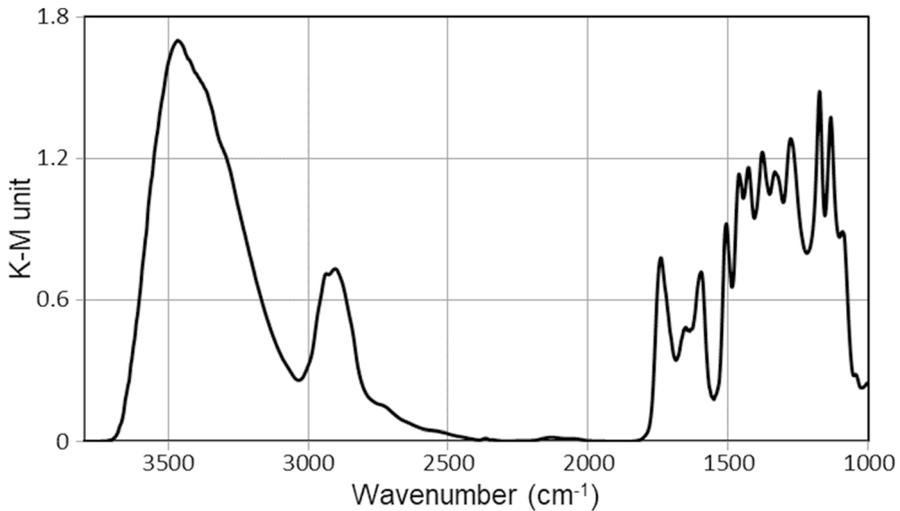


Fig. 1 Absorption spectrum of poplar presented in 1000–3800 cm^{-1} wavenumber interval

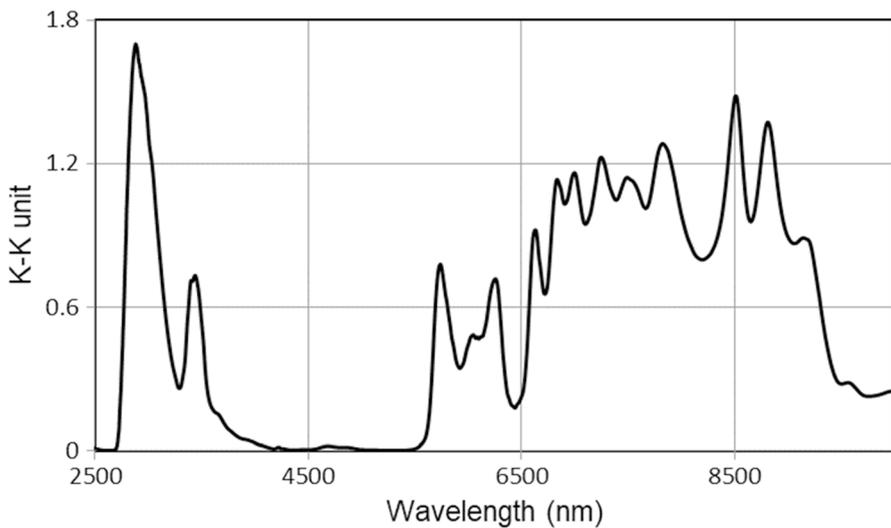


Fig. 2 Absorption spectrum of poplar presented in 2500–10,000 nm (or 2.5–10 μ) wavelength interval

representations (by wavelength or by wavenumber) give similarly detailed information if a zoomed (such as fingerprint) region is presented.

The K–M equation was used to convert the reflectance spectrum to absorbance spectrum in Figs. 1 and 2. The K–M transformation has validity limits. It is important to keep in mind these limits. This transformation works well if the absorbance values are low, but it can create anomalies if the reflectance values are low (high absorbance). Details can be found in a previous work (Tolvaj et al. 2011). Table 1

shows the list of absorption peaks for poplar wood in both $1/\text{cm}$ and nm for seeing the equivalence.

Problems of ATR measurement

The ATR measurement method requires perfect contact between the crystal and the measured material, but wooden blocks (as samples) are not soft enough to produce close contact between the crystal and the sample by pressure. The only crystal, which can be used, is diamond as it can tolerate high pressure, but the active surface of the diamond crystal is no larger than $1\text{--}3\text{ mm}^2$. Consequently, the researcher must keep in mind, if the surface of the sample is considerably larger than the surface of the crystal, then the increasing pressure does not generate closer contact between the crystal and the sample. This is because the sample holder around the crystal “absorbs” the pressure and the contact consequently remains poor. Using a small wooden chip, the ATR technique gives better spectrum than for solid wood sample. Figure 3 shows the absorbance spectra of poplar wood in both block and chip format (the diffuse reflectance FTIR spectrum of the same block sample is presented in Fig. 2). The chip was 3 mm in diameter and 0.5 mm in thickness. The surface of the block was $30 \times 10\text{ (mm}^2\text{)}$, and it was 5 mm thick. The spectrum of block type sample gives all information which is visible in chip type spectrum, but the intensities are extremely low in case of the spectrum of the block. Low intensities are because of the poor contact between the sample and the crystal. The results in Fig. 3 show that the ATR technique is much less suitable to determine the absorption properties of block type wood sample compared to chip type sample.

Table 1 Characteristic IR bands of poplar wood presented in Fig. 1 and Fig. 2 (place of maximum) and band assignments (Faix 1991; Csanady et al. 2015)

Wavenumber (cm^{-1})	Wavelength (nm)	Assignment
3461	2890	O–H stretching
2904	3443	CH stretching
1739	5749	C=O stretching vibration of non-conjugated carbonyls
1597	6263	Aromatic skeletal breathing with CO stretching (syringyl lignin)
1507	6635	Aromatic skeletal vibrations (guaiacyl lignin)
1462	6841	C–H deformation
1428	7003	Aromatic skeletal vibration combined with C–H in-plane deformation
1379	7252	Aliphatic C–H stretching in CH_3
1334	7499	Syringyl ring breathing
1277	7833	Guaiacyl ring breathing
1174	8515	C–O–C stretching (asymm.) in cellulose and hemicelluloses
1134	8819	C–O–C stretching (symm.), arom. C–H in-plane deformation, glucose ring vibration

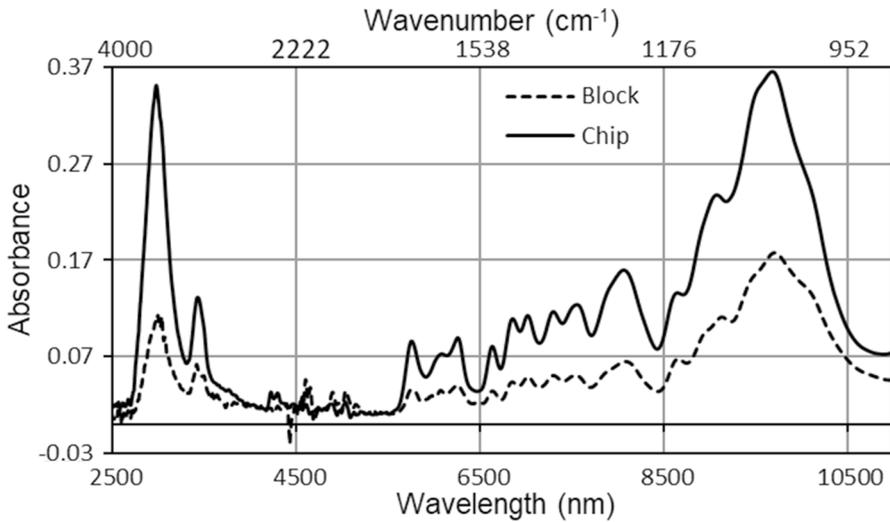


Fig. 3 ATR spectra of poplar chip and block sample (the wavelength dependence of the absorption was corrected for both spectra)

There is a small negative peak visible close to 4500 nm. This phenomenon is abnormal in case of an absorption spectrum. The reason is due to the background spectrum measurement. If the surface of crystal is not clean during the background spectrum measurement, the recorded background spectrum can contain absorbance information. These absorbance values are missing during sample spectrum measurement, hence generating negative absorbance values.

The penetration of IR radiation into the sample during total reflection is wavelength dependent. Longer wavelengths penetrate deeper than the shorter wavelengths. The penetration decrease reduces the absorption value. This phenomenon can be seen in Fig. 4 (not corrected spectrum). The absorbance value of hydroxyl groups at 2890 nm is low although the concentration of hydroxyl groups is relatively the highest in wood. As a consequence, the absorbance at 2890 nm should be the highest. Both diffuse reflectance and KBr pellet method exhibit high absorbance in the OH region (Michell 1988; Zavarin et al. 1990; Anderson et al. 1991a, b, c; Tolvaj and Faix 1995; Zanuttini et al. 1998; Huang et al. 2008; Tolvaj et al. 2014) (Fig. 2 shows it as well). This distortion can be minimalised by wavelength dependence correction. All FTIR spectrophotometers can do this correction. Figure 4 shows how the correction eliminates the distortion. This wavelength correction of ATR spectra is important to get correct intensity ratios among the bands. Unfortunately, many scientists ignore this important correction.

Comparing the IR spectra of a poplar block sample measured by both the ATR and diffuse reflectance (DR) technique, some anomalies can be seen (Fig. 5 shows these spectra). The presented ATR spectrum was subjected to wavelength dependence correction, and the DR spectrum was baseline corrected. The measured values of the ATR spectrum were much smaller (approximately 9 times) than the values of DR spectrum. The ATR spectrum was multiplied by 9.12 for correct comparison of

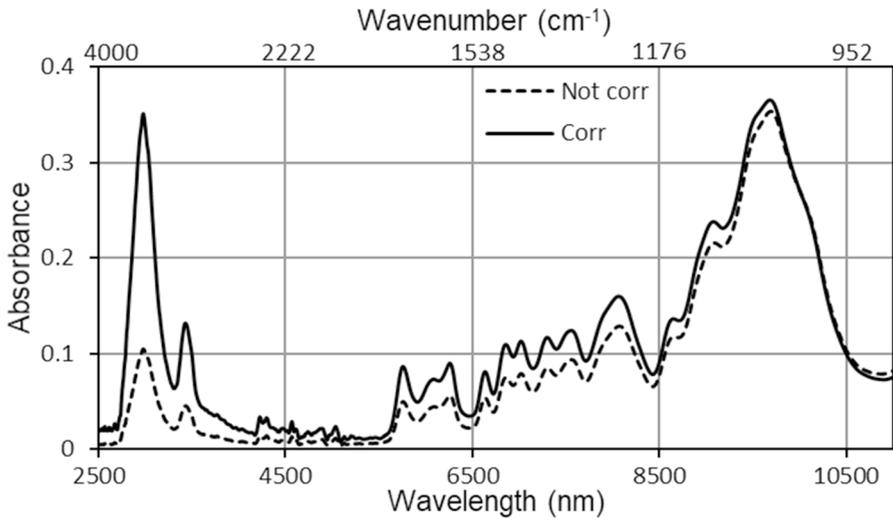


Fig. 4 ATR spectra of poplar chip before and after wavelength dependence correction

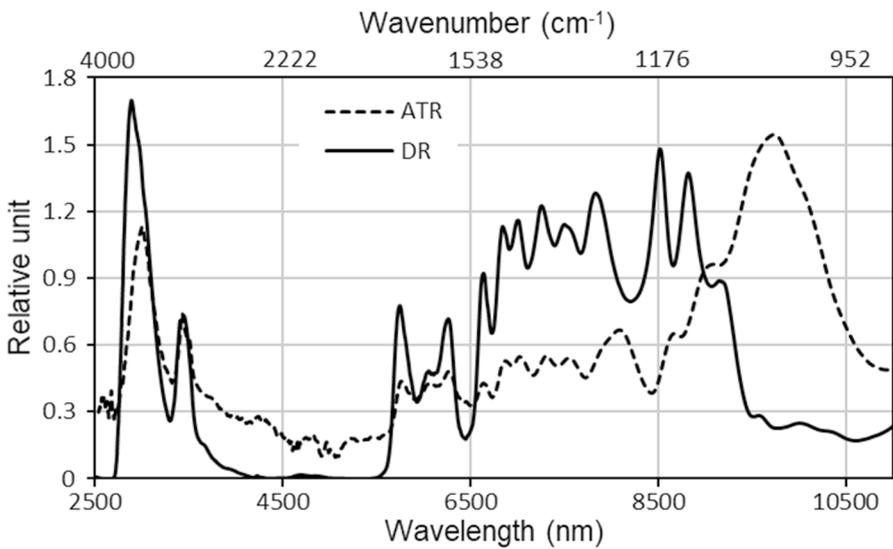


Fig. 5 Absorbance spectrum of block type poplar sample measured by ATR and DR technique

the spectra. The methyl band at 3435 nm was the only position where the intensity of ATR spectrum was high enough and the wavelength location of maximum was equal to the DR spectrum. This point gave the multiplication position for the ATR spectrum. Both spectra give the bands and their position correctly in the 2500–7800 nm wavelength interval. However, the intensity differences within the ATR spectrum are extremely small. The DR spectrum shows much more differentiated intensities. The

left part of the hydroxyl band below 3000 nm is missing in the ATR spectrum. A huge peak is visible in the ATR spectrum at 9710 nm. This band modifies the bands located in the 8400–9500 nm interval. Two strong characteristic bands of wood are completely missing at 8540 and 8850 nm, representing the absorption of ether linkages. This is an anomaly generated by the ATR measurement. Unfortunately, the DR measurement method also generates an anomaly in this region. The absorption band of C-O stretching at 9710 nm is completely missing in the spectrum measured by DR measurement method (in contrast, it is the highest band of ATR spectrum). Fortunately, this region is not crucial to the analysis of the main components of wood. This anomaly was recognised by many scientists (Zavarin et al. 1990; Anderson et al. 1991a, b; Anderson et al 1991a, b, c; Faix and Böttcher 1992; Pandey and Theagrajan 1997). The magnification of the ATR spectrum raised the values in the 4000–5500 nm interval where wood has no absorption. It happened below 2700 nm as well. Figure 5 shows that the DR technique gives a much more detailed spectrum in the main region of the analytical IR interval than the ATR technique.

The small measured area (1–3 mm²) is a disadvantage of ATR technique if an inhomogeneous surface (like wood) is measured. It is hardly possible to measure exactly the same surface area before treatment and after treatment.

The ATR technique using a diamond crystal has an additional disadvantage. Diamond has a wide and strong absorption band in the middle of the analytical IR region. This absorption can be visualised by measuring the background spectra of the FTIR spectrophotometer in an empty sample compartment and using a diamond ATR crystal. These two spectra are visible in Fig. 6. The background intensities measured by diamond are approximately 20 times smaller than the background

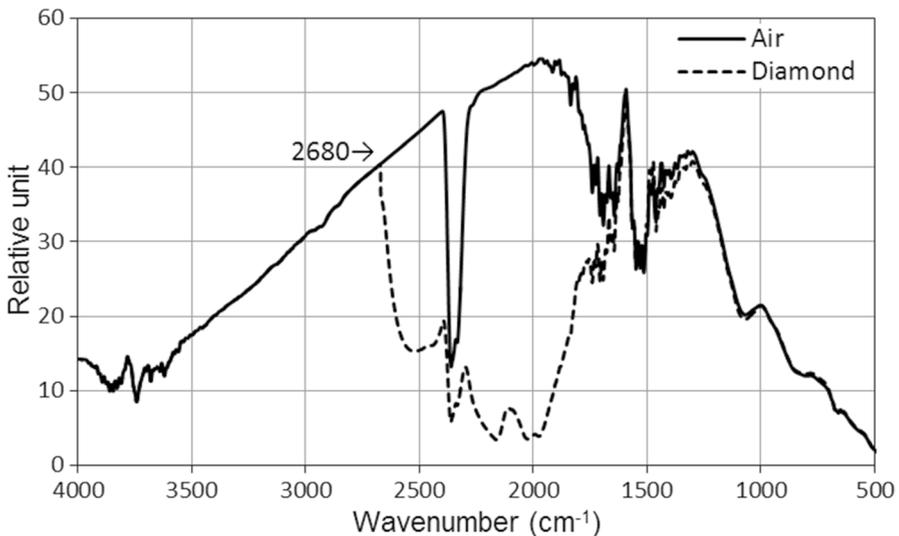


Fig. 6 Background spectra of the FTIR spectrophotometer measured by empty sample compartment (Air) and by diamond ATR crystal (the ATR background was multiplied to fit to the air background at 2680 cm⁻¹)

intensities of empty sample compartment. The background values of diamond were multiplied to generate equal values at the 2680 cm^{-1} wavenumber for the correct comparison. Diamond shows strong absorption between 1600 and 2600 cm^{-1} . This absorption reduces the sensitivity of ATR measurement in this region considerably. Fortunately, wood hardly has any absorption in this region, only the unconjugated and conjugated carbonyl region is affected between 1600 and 1800 cm^{-1} . The sensitivity decrease in the absorption region of diamond is well demonstrated by the absorption band of CO_2 at 2360 cm^{-1} . The background measured in empty sample compartment shows high absorption by CO_2 . The background measured in the presence of the diamond crystal exhibits only a small negative peak representing the absorption of CO_2 . (The two background measurements were taken after each other.)

Problems of normalisation

The measured IR spectrum is generated by the superposition of absorption and scattering. Whereas absorption represents the loss of a photon, scattering is the traveling direction change of a photon without crossing the medium border. Both phenomena reduce the number of collected photons during the reflectance spectrum measurement. Consequently, the measured spectrum contains information according to the absorption and to the scattering as well. The separation of these two effects is difficult. The absorbance values are usually calculated by the Kubelka–Munk equation. There is a hyperbolic (reciprocal) relationship between the reflectance values and absorbance (K–M units) values. The diffuse reflectance units built in the IR spectrophotometers are unable to collect all of the reflected photons. The collection of reflected photons happens within a well-defined square angle determined by the size of the spherical mirror. Any changes in the surface properties modify the number of the mirror-collected photons modulating the measured reflectance values. These changes appear in the absorbance spectrum; nonetheless, the absorption itself does not change. This distortion is a multiplicative type because of the reciprocal relationship between the reflectance and absorbance values. The roughness change is one of the usual surface property alterations during different treatments of wood. The increase in roughness causes more diffuse reflection, thereby reducing the number of the mirror-collected photons. The measured intensity of reflectance is therefore decreasing because of the roughness increase. The K–M equation interprets this as an absorption increase. This phenomenon increases the absorbance values generating distortion. The multiplicative type anomaly can be recognised by the diverse distortions within the spectrum. The small absorbance values suffer small distortions while high absorbance values undergo great distortions. This phenomenon is visible in Fig. 7. This figure presents the absorbance spectra of poplar wood measured before and after 5-day UV irradiation. The deviations between the plotted spectra are generated by the photodegradation and by the distortion caused by the roughness increase. The photodegradation of the aromatic ring in lignin reduced the absorption at 1505 and 1596 cm^{-1} wavenumbers. As a consequence, the intensity of unconjugated carbonyl groups increased at 1740 cm^{-1} (Sudiyani et al. 2003, Pandey

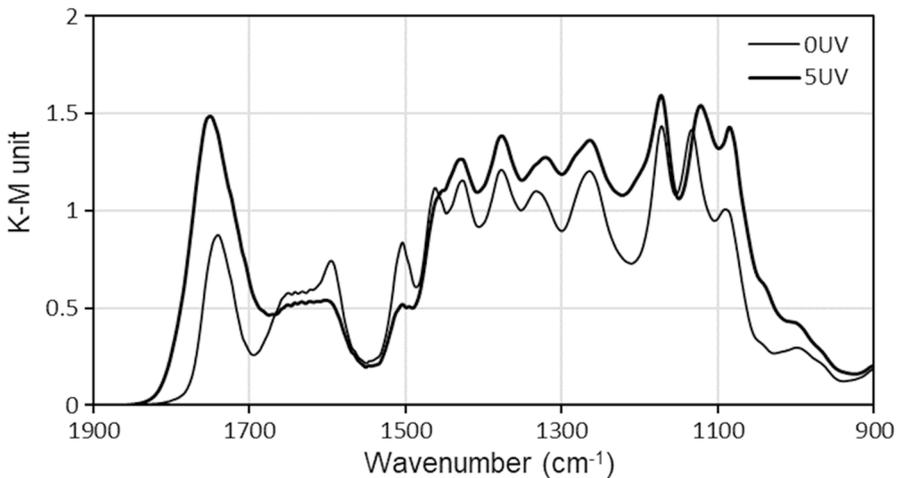


Fig. 7 Absorbance spectra of poplar measured before and after 5-day UV irradiation

2005, Pandey and Vuorinen 2008, Popescu et al. 2013, Agresti et al 2013, Yildiz et al. 2013, Broda and Popescu 2019, Liu et al. 2019a, Liu et al. 2016, 2019b). However, the spectrum of treated sample is lifted up in the 1450–900 cm^{-1} region. (Lifting happened in the carbonyl and aromatic regions as well, but it is not evident because the photodegradation caused changes at the same places.)

This anomaly confuses the interpretation of the photodegradation generated changes in the 1450–900 cm^{-1} region.

However, normalisation can help to reduce this anomaly. For correct normalisation it is necessary to find an absorption peak which is not affected by the applied treatment. If there is more than one choice it is better to choose a peak in a central position. During the normalisation, the spectrum of the treated sample is multiplied by a constantly obtained equal value with the initial spectrum at the chosen peak. The other possibility is to multiply all of the spectra getting one unit value at the chosen peak. The second option is the better choice if it is intended to compare the spectra of different samples or species. The normalisation eliminates the anomaly at the place of the chosen peak and reduces it considerably in other places.

The spectra of Fig. 7 were normalised to the band maximum at 1376 cm^{-1} . The intensities of spectra were adjusted to 1.0 by this normalisation at maximum at 1376 cm^{-1} . This C–H band of cellulose is often used as internal standard because of its high intensity, central position and strong stability. The normalised spectra are visible in Fig. 8. This data manipulation eliminated the lifting effect of the roughness increase. The effectiveness is only questionable around 1100 cm^{-1} . The remained lifting around 1100 cm^{-1} demonstrates that the normalisation is not a perfect solution. It helps only close to the chosen peak. That is why the central position of the chosen peak is important.

The usefulness of normalisation is well demonstrated by the difference spectra (Fig. 9). The difference spectrum was calculated by the subtraction of the

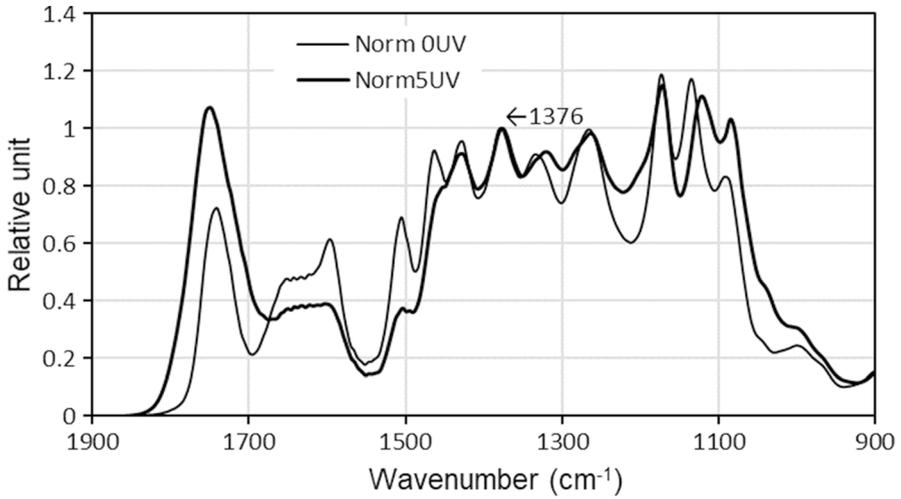


Fig. 8 Normalised absorbance spectra of poplar measured before and after 5-day UV irradiation

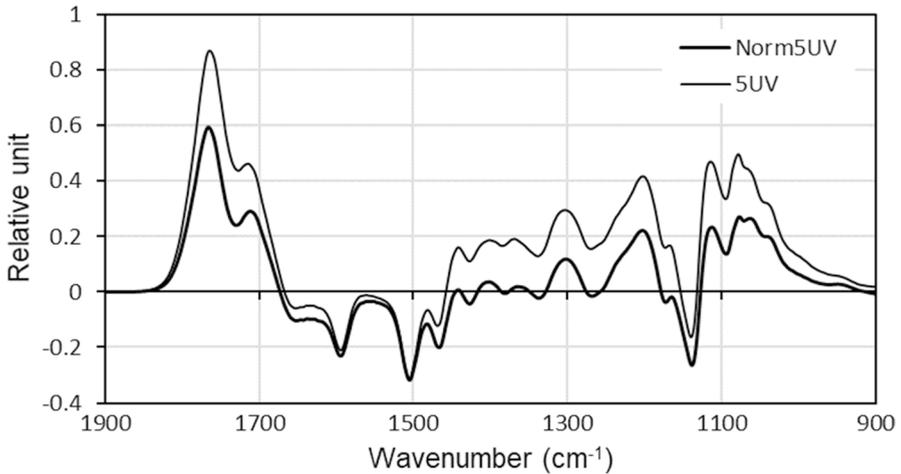


Fig. 9 Difference absorbance spectra of poplar determined by using the normalised spectra (Norm5UV) and calculated from the initial (not normalised) spectra (5UV). The changes were generated by 5-day UV irradiation

spectrum of the treated sample from the initial one. In this case, an absorption increase is represented by a positive band, while a negative band represents an absorption decrease. Figure 9 shows clearly the multiplication anomaly and how it was reduced by normalisation. Photodegradation generates absorption decrease in the aromatic C-H deformation at 1429 cm^{-1} . This negative difference peak is

a valley in the positive surrounding, if the spectra are not normalised (thin line). This phenomenon confirms the multiplicative effect.

The calculation of difference spectrum is a useful method if the spectrum contains highly overlapped bands, for example, wood in the fingerprint region. The prime advantage of the difference spectrum method is that only the changed bands are visible, reducing the number of overlapped bands. It is important for getting a correct difference spectrum that the measurement must be taken on the same surface area before and after the treatments. If this important requirement is ignored, the inhomogeneity of wood surface can destroy the difference spectrum. The usefulness of the difference spectrum method is demonstrated by the papers dealing with the temperature dependence of the photodegradation for wood (Tolvaj et al. 2013; Varga et al. 2017; Preklet et al. 2018) and the leaching effect of water during photodegradation of wood (Bejo et al. 2019; Pasztory et al. 2020; Varga et al. 2020).

Figure 9 contains two types of difference spectra generated by 5-day UV irradiation of wood samples. One was calculated before normalisation, and the other was determined after normalisation. The interpretation of the difference spectrum is difficult without normalisation in the $1450\text{--}900\text{ cm}^{-1}$ wavenumber interval. The negative peaks are visible as a valley between two positive peaks. Normalisation reduces this anomaly but does not eliminate it completely. The reliability of the data remained questionable around 1100 cm^{-1} and further on.

Normalisation is a useful method to evaluate a spectrum, but it can cause abnormal changes if it is applied incorrectly. An important requirement is that the chosen peak must remain intact by the applied treatment. Unfortunately, some researchers apply normalisation knowing that the chosen peak is altering during the applied treatment.

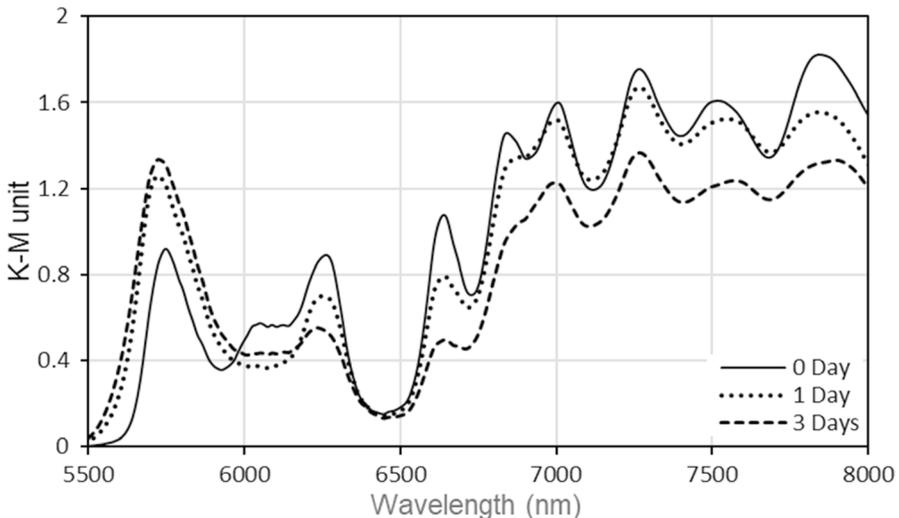


Fig. 10 K–M spectrum of poplar wood before and after UV irradiation

In this regard, an example can be seen to present the consequence of using the wrong normalisation. Figure 10 presents the K–M spectrum of poplar before and after UV irradiation. The comparison of spectra shows the degradation of lignin absorbing at 1505 and 1596 cm^{-1} wavenumbers. As a consequence, the intensity of unconjugated carbonyl groups increased at 1740 cm^{-1} . The right part of the spectra presents a parallel shift as a superposition of scattering change and surface inhomogeneity of the sample. The absorption changes are located on the top of the parallel shifts. The alteration of scattering was generated by the changing roughness. It is difficult to measure exactly the same area of the specimen before and after treatment, and the inhomogeneity of the surface also causes a scattering change. Proper normalisation is able to minimise the effect of scattering (see Fig. 8).

Figure 11 presents the effect of incorrect normalisation. It was mentioned above that the spectrum intensity at a proper normalisation location does not change during the treatment. Here, the result of normalisation at 5740 nm is presented where the absorption intensity increased during photodegradation. The normalisation of the spectra made the values equal at 5740 nm, but the other regions became confused. This process produced great distortion of the spectra. The normalisation spread out the trendlines proportionally to the intensity differences at 5740 nm. This example clearly shows the possible results of a wrong spectrum normalisation.

Conclusion

It is important to know that most of the spectroscopic measurement methods and data manipulation techniques have validity limits, and in some cases, they can generate anomalies as well. Scientists must keep these in mind and take care of avoiding wrong interpretations.

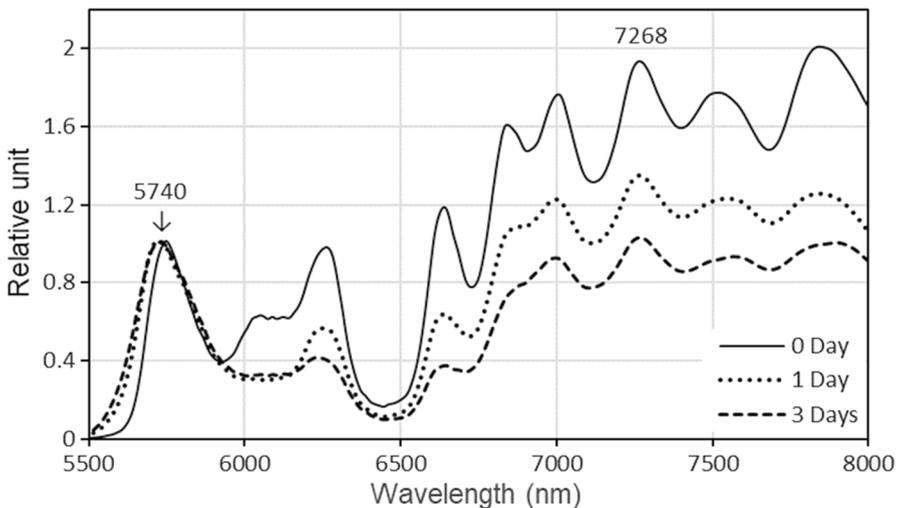


Fig. 11 Normalised K–M spectra of poplar wood before and after UV irradiation

The use of an incorrect method is not validated because it is present in the peer review literature. Never copy a method used by other researchers without scepticism, whether it is correct or not in your case.

Funding Open access funding provided by University of Sopron. No funds, grants, or other support was received.

Declarations

Conflict of interest The author has no financial or proprietary interests in any material discussed in this article.

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