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FOURIER-TRANSFORM RAMAN SPECTROSCOPY OF CARBOHYDRATES

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The aim of this paper is to show that Fourier-transform Raman is a valuable tool for the studies of carbohydrates. Most of the specimens examined, including raw materials, give excellent spectra rapidly and non-destructively. New spectra of various carbohydrates are presented along with some spectroscopic analysis to demonstrate the significance of this recently developed technique.

INTRODUCTION

Carbohydrates are a biologically important group of compounds. They serve as an energy store (glycogen, starch) or as a source of energy for immediate use in the living cell (glucose). Some of them are the building elements of the cell walls. It would be impossible to overestimate the significance of these compounds for humans as even the cells of our body could not function without them (ribose, glucose).

Over the past few decades extensive studies have been made to correlate IR spectra of carbohydrates with their structural features. Despite certain difficulties and experimental limitations (Kuhn, 1950) some success has been attained especially in such areas as the determination of the glycosidic linkage configuration.

In recent years laser-Raman spectroscopy has become a powerful tool in the structural study of biologically important compounds. However most such studies were on proteins and nucleic acids and only a few on carbohydrates.

Among the saccharides, D-glucose has been the most extensively studied compound (Mathlouthi & Seuvre, 1988; Mathlouthi & Luu, 1980; Mathlouthi, Luu & Luu, 1979; Mathlouthi, Luu, Meffroy-Bigget & Luu, 1980; Longhi, Zerbi, Paterlini, Ricard & Abbate, 1987). The reason for the apparent lack of interest in many other carbohydrates has been the difficulty in obtaining reasonably good spectra, especially for the naturally occurring sugars and sugars in food products. As these materials are not pure, they frequently fluoresce when excited with visible laser light. Thus Raman spectra, superimposed on a strong fluorescent background are practically useless for any analytical purpose. Moreover, such sensitive samples as plants may decompose (dehydrate or burn) under the strong laser radiation. Hence, though Raman spectroscopy has several advantages over infrared (IR), it cannot be used in some cases successfully.

There is a great aticipation connected with the application of new Raman technique - NIR FT - R which seems to be a remedy for these problems. It has already been shown that the use of near - infrared Nd/YAG laser at 1064 nm as an excitation source drastically reduces the fluorescence that so hampers conventional Raman spectroscopy (Ellis, Hendra, Hodges, Jawhari, Jones, Barazer, Passingham, Royaud, Sanchez-Blasquez & Warnes, 1989). Furthermore, the measurements are practically non-destructive and no sample preparation is required prior to FT - Raman examination. All this makes the method much more attractive as a routine analytical tool than conventional Raman.

Despite the "short curriculum vitae" of the FT - R it would be difficult to survey all the applications of this technique in analysis. However, a variety of them can be given to support

the thesis that Raman spectroscopy has become as versatile as IR spectroscopy. In this publication a few such examples will be presented to show that the method can be applied not only to the studies of chemically pure carbohydrates but also various naturally occurring and industrially-processed products containing saccharides may successfully be examined.

INSTRUMENTATION

In 1987 a new Raman system based on FT - IR Perkin Elmer interferometer was developed at the University of Southampton and a year later it was decided to develop the prototype as an analytical instrument. The apparatus shown schematically in a diagram above (Fig. 1) is a modified Perkin-Elmer 1720-X FT-IR spectrometer (Ellis *et al.*, 1989).

The emergent beam from the Spectron model 301 c.w. Nd^{+3} /YAG laser passes through a spike filter (F_1) and small prism (P), and then is focused onto a sample. As a 180° scattering geometry is used, the back-scattered radiation emanating from the sample is collected by a lens combination (L_3) and is focused on to the entrance aperture (Jacquinot stop) of the inteferometer.

Two transmission filters (F_2 and F_3) are incorporated to reject visible light (F_4).

The InGaAs detector used in this system operates at room temperature or, if necessary, may be cryogenically cooled to minimize detector noise.

Spectra are recorded at 12, 6, 3 or 1 cm^{-1} resolution as required. Sample cells are mounted on standard IR "cards" which are easily positioned in a pre - aligned slot in the spectrometer (Ellis *et al.*, 1989).

MATERIALS AND MEASUREMENTS

The samples of D - glucose (α, β), maltose, sucrose, dextran and Sephadex were from Sigma Chemical Company. Cane sugar (white and brown), cotton wool and tissue were commercially available products while plant materials (lime and cypressus wood) were collected from the Southampton area.

No sample preparation was required prior to FT - R measurements and the spectra were recorded directly on the samples as supplied,

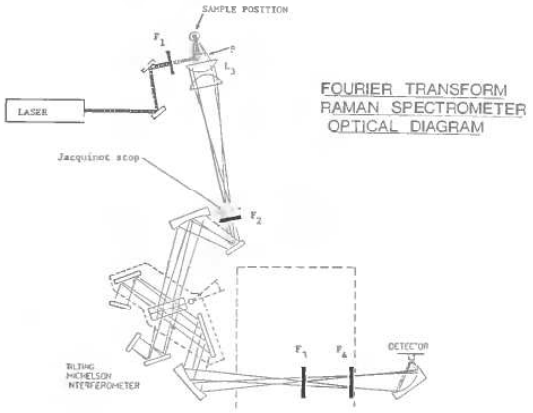


Fig. 1 FT Raman spectrometer optical diagram.

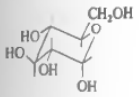
with minimal or practically no fluorescence background.

All of the spectra, measured in the range 3500 - 300 cm^{-1} at 6 cm^{-1} resolution, are displayed graphically as an intensity of scatter (arbitrary units) versus frequency shift (cm^{-1}). FT-Raman spectra shown here are corrected for filters and detector characteristics.

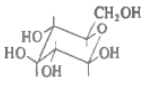
RESULTS AND DISCUSSION

Saccharides are designed to fulfill a particular biological role. The most common structural property of sugars closely related to their functions is the ability to adapt one of the two anomeric forms: alpha or beta. Each of the anomers is characterized by a different configuration of the hydrogen in the C(1) position

as it is shown for D-glucose:



alpha



beta

The determination of the anomeric configuration is an important aspect in the structural study of carbohydrates. Until recently this could be done using H^1-NMR (Allinger, Cava, Jogh, Johnson, Lebel & Stevens, 1971) and optical rotation measurements (Allinger *et al.*, 1971). Now, FT-Raman spectra are available and, as will be shown, the method gives excellent results on saccharides.

The FT-R spectra of crystalline alpha- and beta-D-glucose (dry and wet) are presented in Figs 2 and 3. It may be surprising that such a subtle difference in the geometry of the molecule (axial or equatorial position of hydrogen) results in such massive spectral changes as observed here.

It is generally accepted that several bands in the Raman spectrum are characteristic for alpha and beta anomers, and that they appear in the range 1000 — 700 cm^{-1} (Mathlouthi & Seuvre, 1988; Mathlouthi & Luu, 1980; Mathlouthi *et al.*, 1979; Mathlouthi *et al.*, 1980; Longhi *et al.*, 1987). The anomeric C-H vibration (bending) is observed at 840 and 897 cm^{-1} for α- and β-glucose respectively (Fig. 2 and 3). Two other "diagnostic" bands assigned to ring vibrations are seen at 770, 912 and 918 cm^{-1} in alpha and beta-isomer respectively (Fig. 2a, 3). Some of the skeletal vibrations are also characteristic of this particular anomer. The corresponding bands are observed at 403 and 538 cm^{-1} in alpha-glucose spectrum (Fig. 2a) whilst in beta — at 425 and 518 cm^{-1} (Fig. 3).

Previous Raman and IR studies of sugars have not generally considered the C-H stretching region as conformationally interesting, except for one report published recently (Longhi *et al.*, 1987).

Only from the spectra shown here can we see how sensitive the ν(C-H) vibrations are to any change in the pyranose ring conformation. Thus it should be surprising that such dramatic changes are seen in this range of the spectrum of α-glucose when the sample is wet (Fig. 2b). Obviously, the addition of water results in spectral changes observed in the whole range e.g. 3500—300 cm^{-1} (Fig. 2). Beta-glucose seems to be "water-resistant" when compared to alpha as its Raman spectrum remains practically the same for both dry and wet samples (Fig. 3).

The anomeric forms of monosaccharide units in di- and poly-saccharides may be easily identified if only their FT-Raman spectra are available.

In Fig. 4 the spectra of dry and wet maltose are presented. Maltose, known as malt sugar, is a disaccharide composed of α-1,4-linked glucose units. From the spectra shown here it is clear that the sample examined was a mixture of the two anomers. The corresponding bands are observed at 847 and 898 cm^{-1}. The beta configuration of the second glucose may "protect" maltose against water and thus the spectrum of a wet sample should not essentially differ from this of dry sugar. Indeed, apart from the intensity change caused by water absorption, these spectra are very similar to each other.

That the alpha-glucose is a component of two polisaccharides - dextran and Sephadex - may be seen from their FT-R spectra shown in Figures 5 and 6. The bands characteristic of the alpha anomer are observed at 847, 537 and 400 cm^{-1}. These two polymers are close relatives as the main constituent of Sephadex is dextran and thus these spectra are similar to each other as expected.

Carbohydrates in food products

In food industry laboratories even today, classical analytical methods are still in use. They were certainly good enough some years ago but now they can not compete with currently used chromatography and spectroscopy methods.

Frequently, IR spectroscopy techniques are employed in food analysis for recognition, qualitative identification and quantitative analyses of foodstuffs. Food products are complex mixtures and their IR spectra are usually not of the best quality as each of the components contributes to the observed picture. Furthermore, most foodstuffs contain water which shows strong absorption bands in the whole range e.g. 4000 - 200 cm^{-1} . These bands frequently obscure the absorption peaks due to other constituents of the product examined.

The IR analysis of carbohydrates is rather difficult even if they are chemically pure and dry. The spectra are dominated by overlapping bands which are due to OH and CH vibrations (Kuhn, 1950; Baker, Bourne, Stephens & Whif-

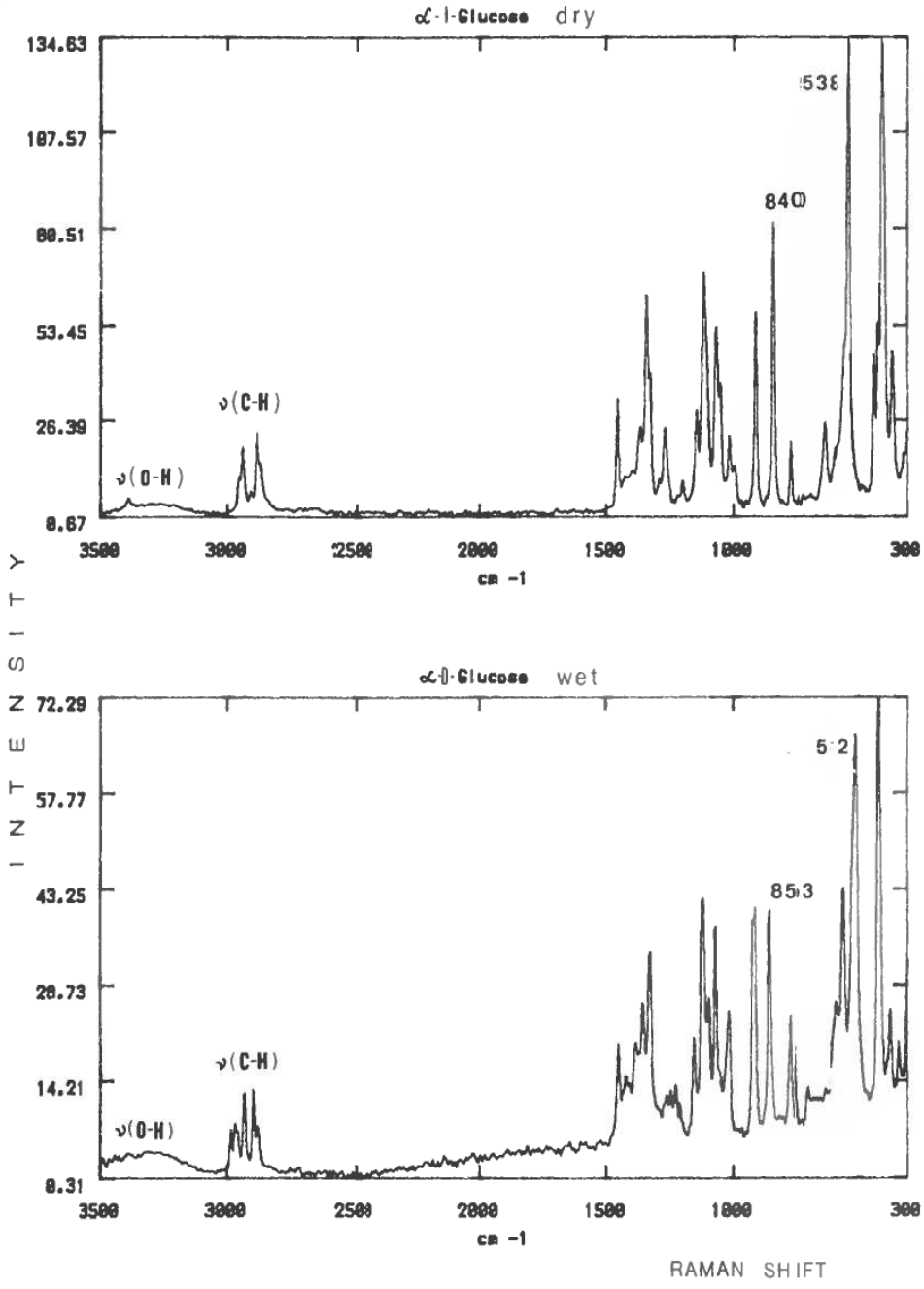


Fig. 2 FT-Raman spectra of dry and wet alpha-D-glucose in the range 3500-300 cm^{-1}.

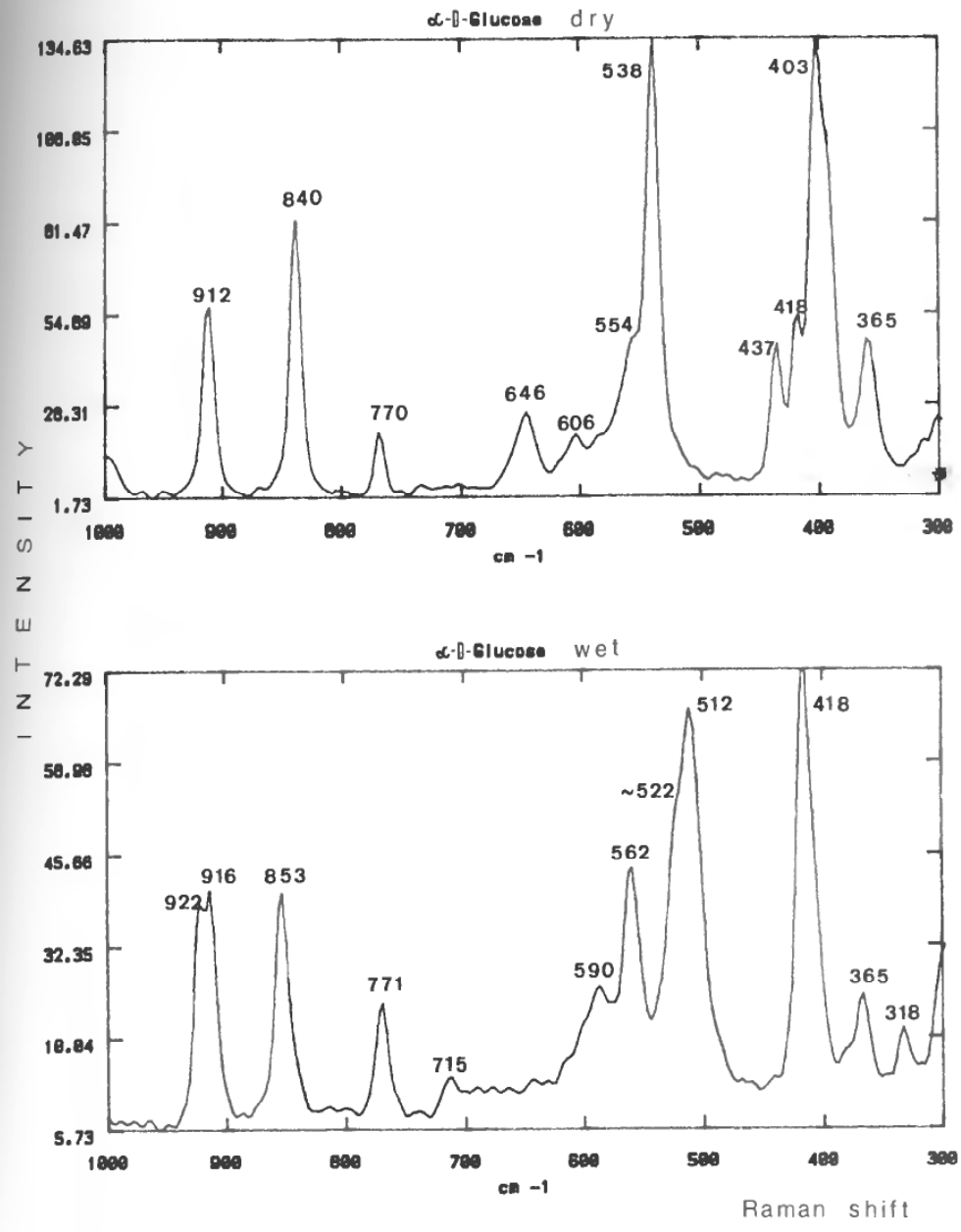


Fig. 2a FT-Raman spectra of dry and wet alpha-D-glucose in the range 1000-300 cm^{-1}.

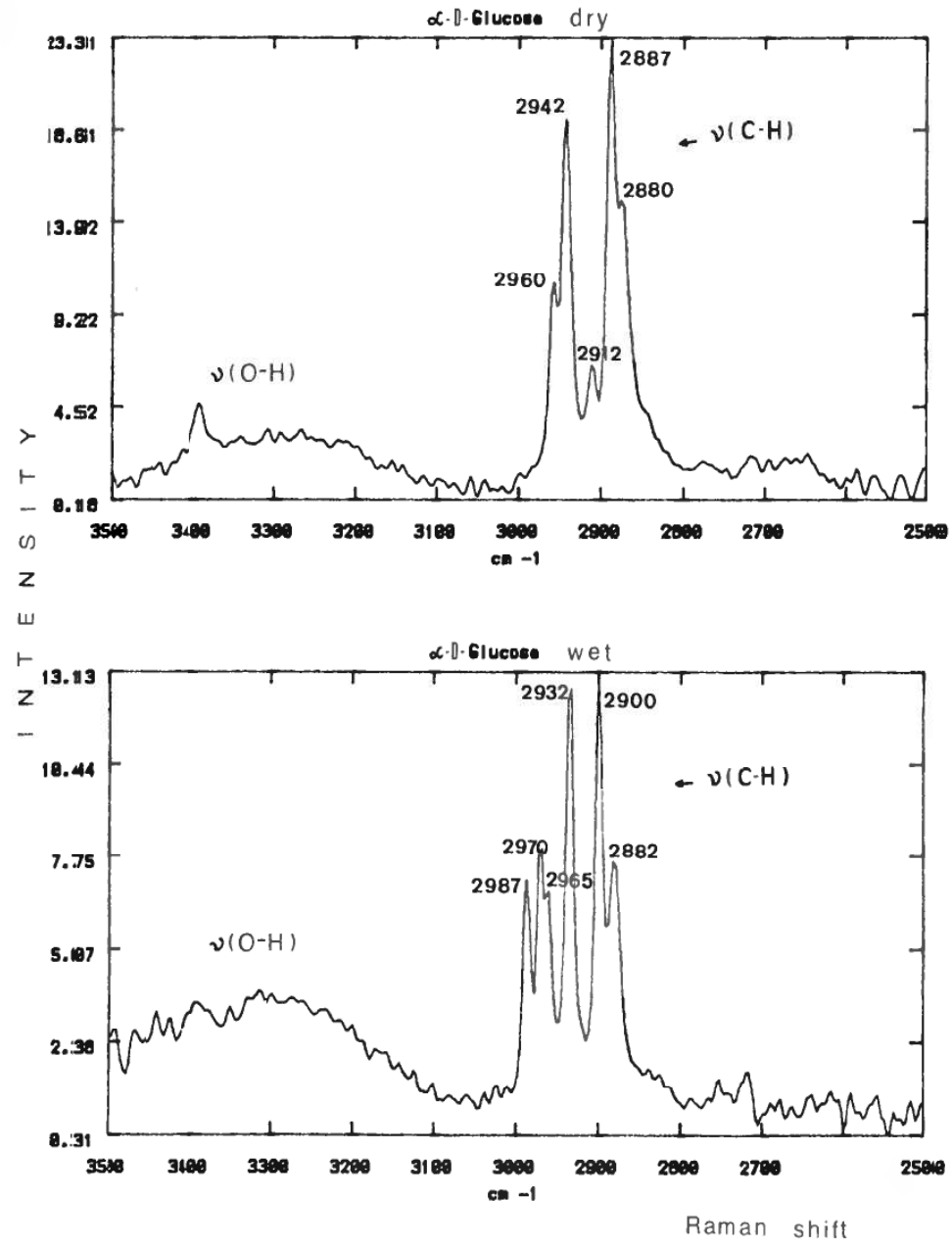


Fig. 2b FT-Raman spectra of dry and wet alpha-D-glucose in the range 3500-2500 cm^{-1}.

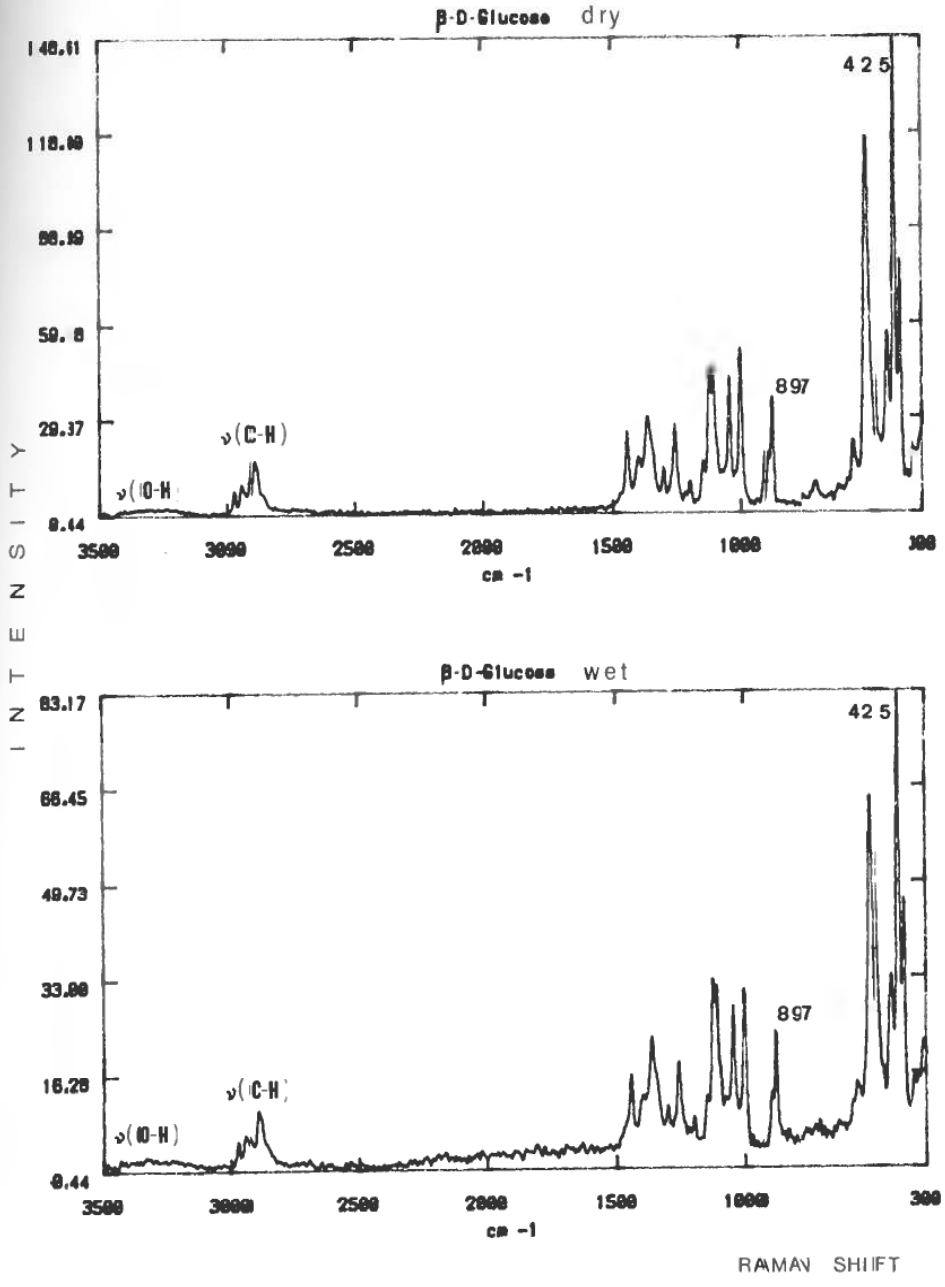


Fig. 3 FT-Raman spectra of beta-D-glucose (dry and wet) in the range 3500-300 cm^{-1}.

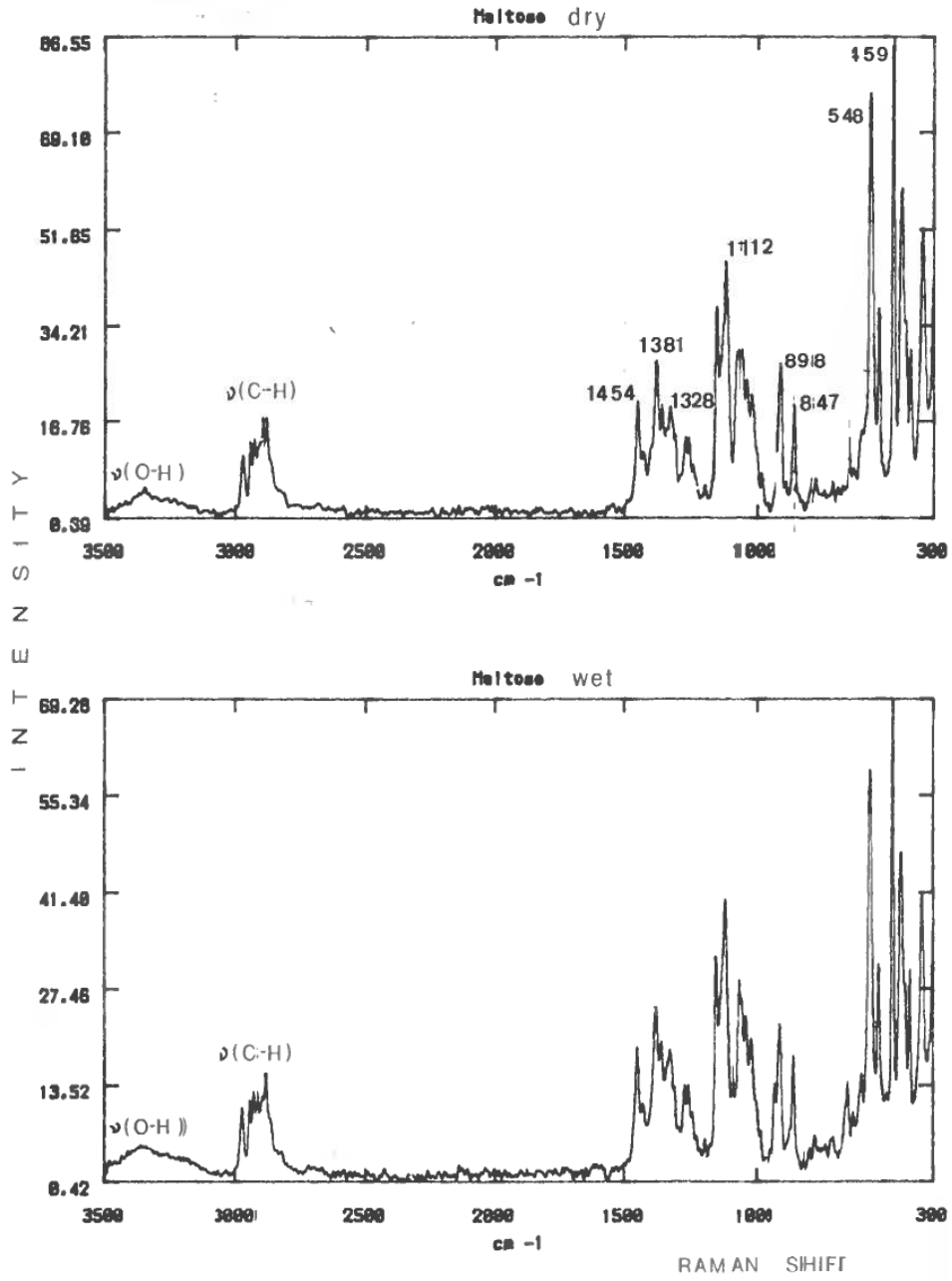


Fig. 4 FT-Raman spectra of dry and wet maltose in the range 3500-300 cm^{-1}.

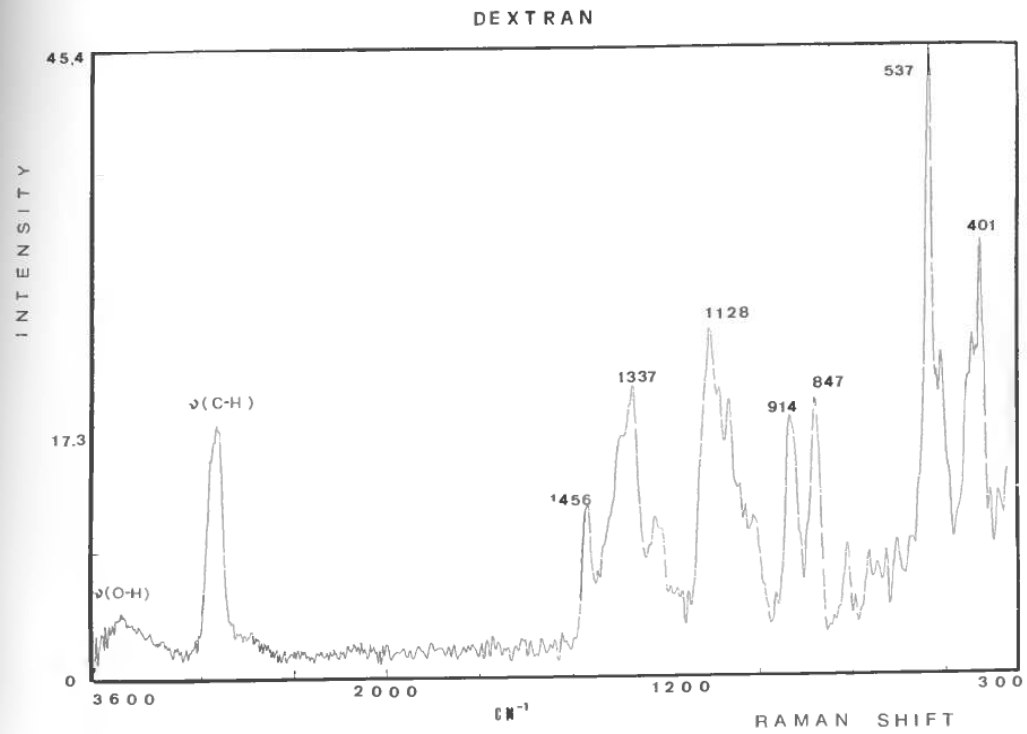


Fig. 5 FT-Raman spectrum of dextran in the range 3600-300 cm^{-1}.

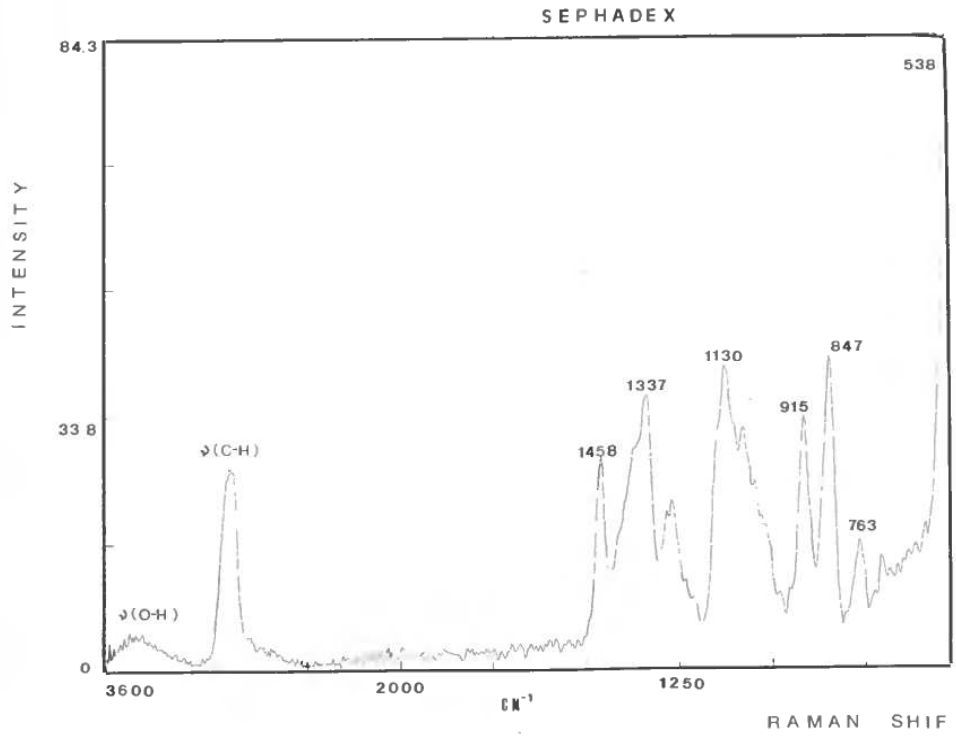


Fig. 6 FT-Raman spectrum of Sephadex in the range 3600-300 cm^{-1}.

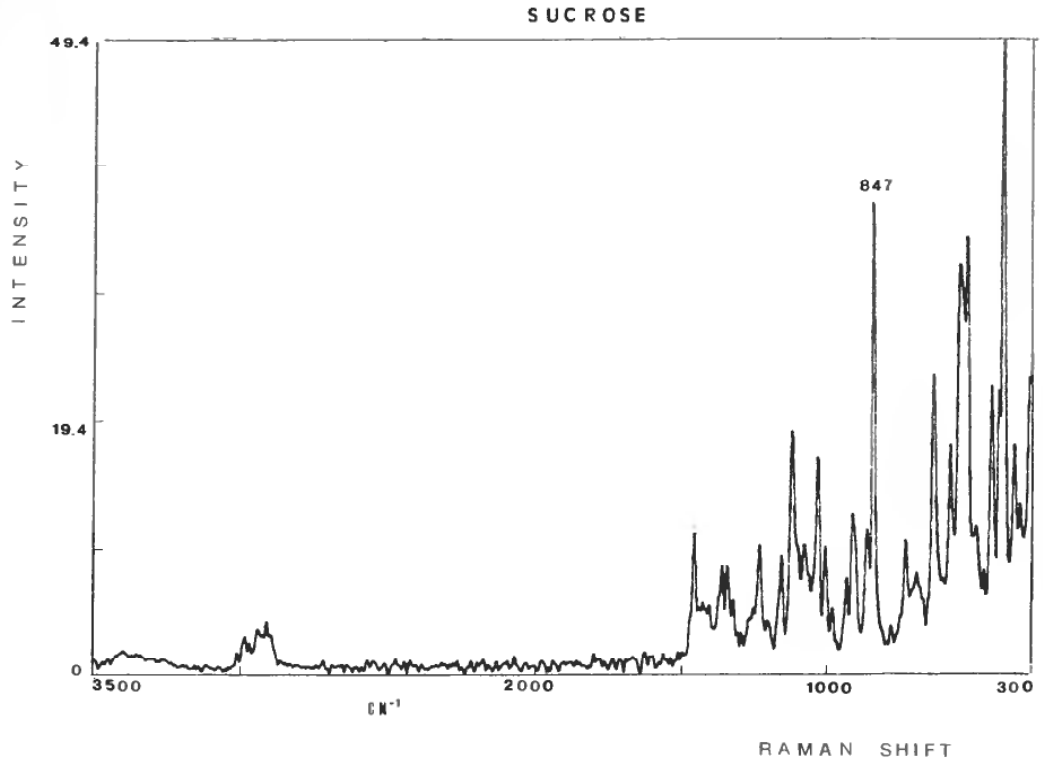


Fig. 7 FT-Raman spectrum of crystalline sucrose in the range 3500-300 cm^{-1}.

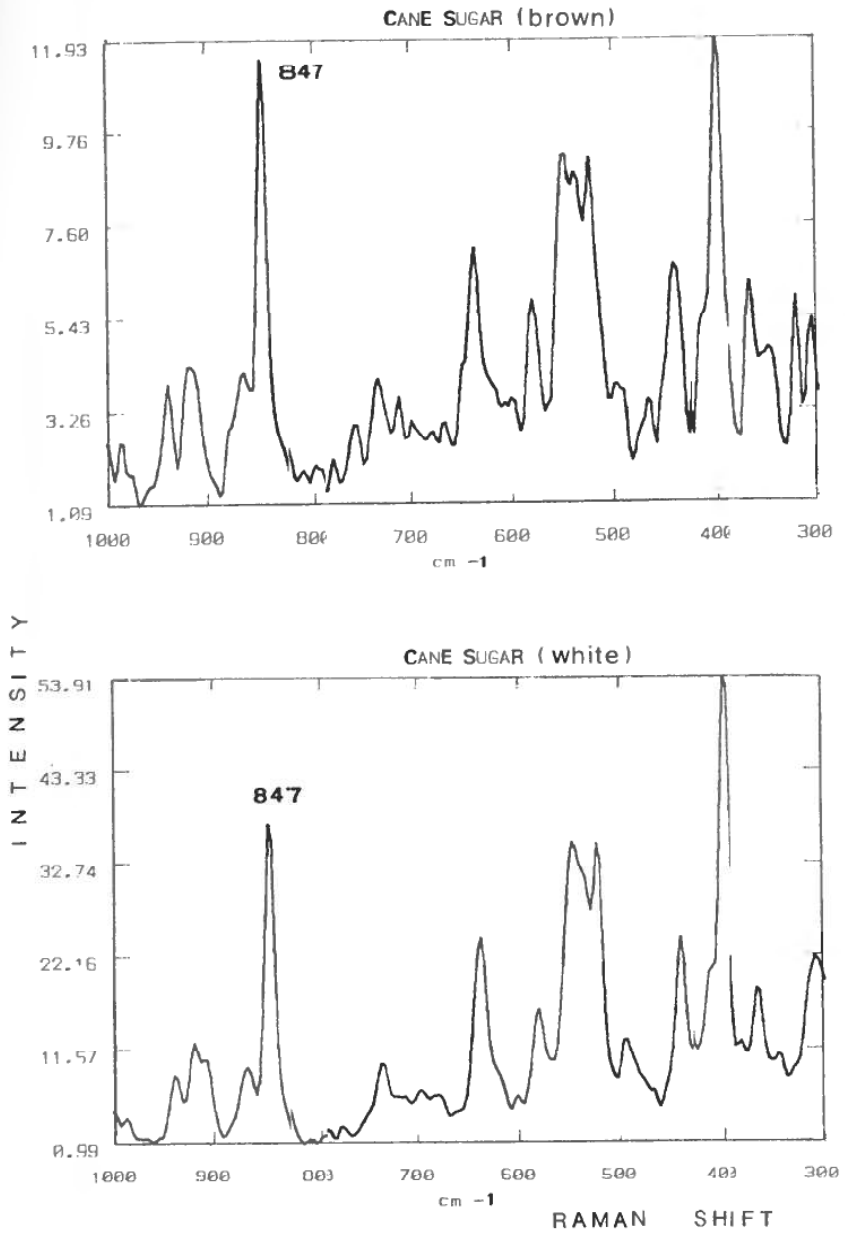


Fig. 8 FT-Raman spectra of brown and white cane sugar in the range 1000-300 cm^{-1}.

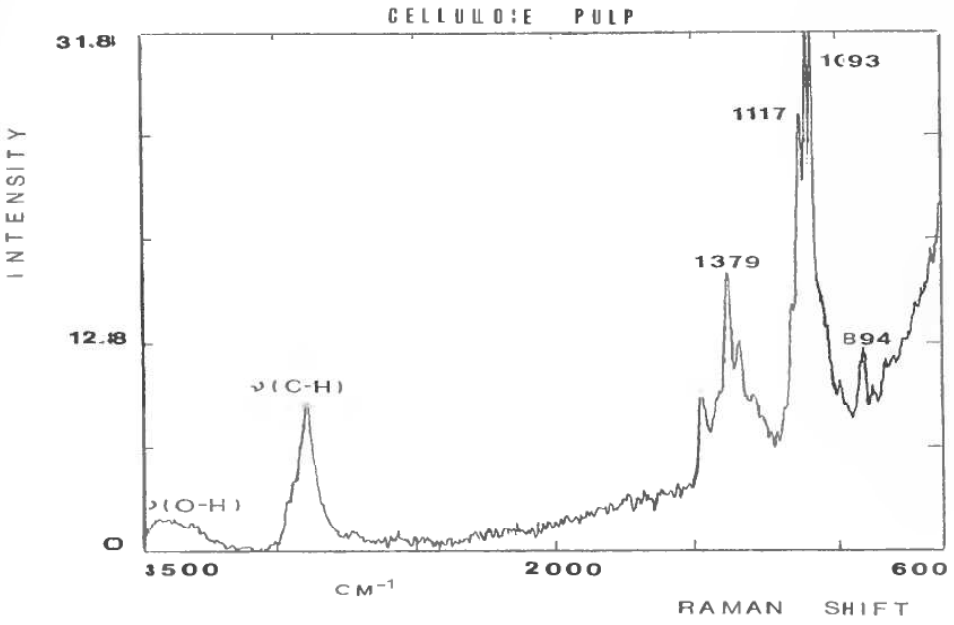


Fig. 9 FT-Raman spectrum of cellulose in the range 3500-600 cm^{-1}

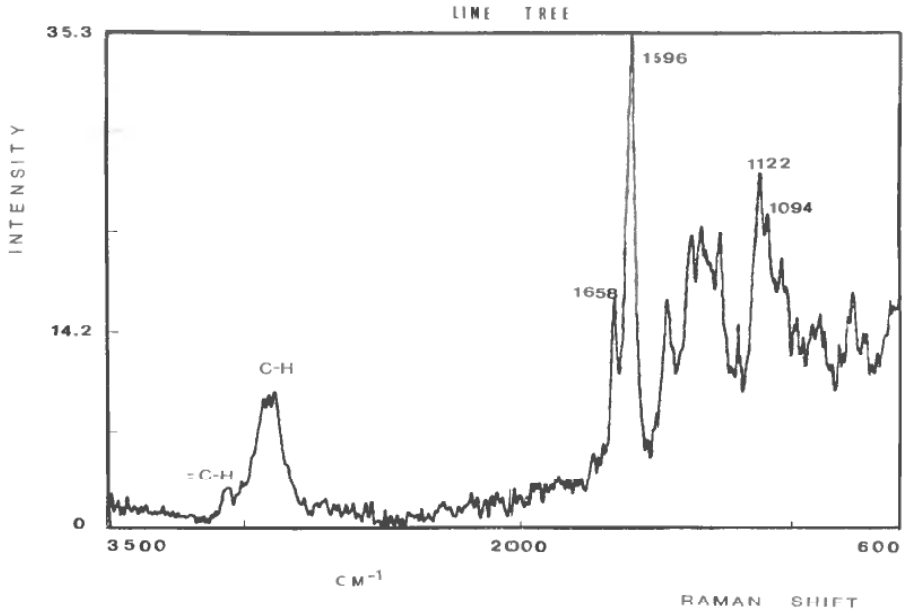


Fig. 10 FT-Raman spectrum of lime tree wood in the range 3500-600 cm^{-1}.

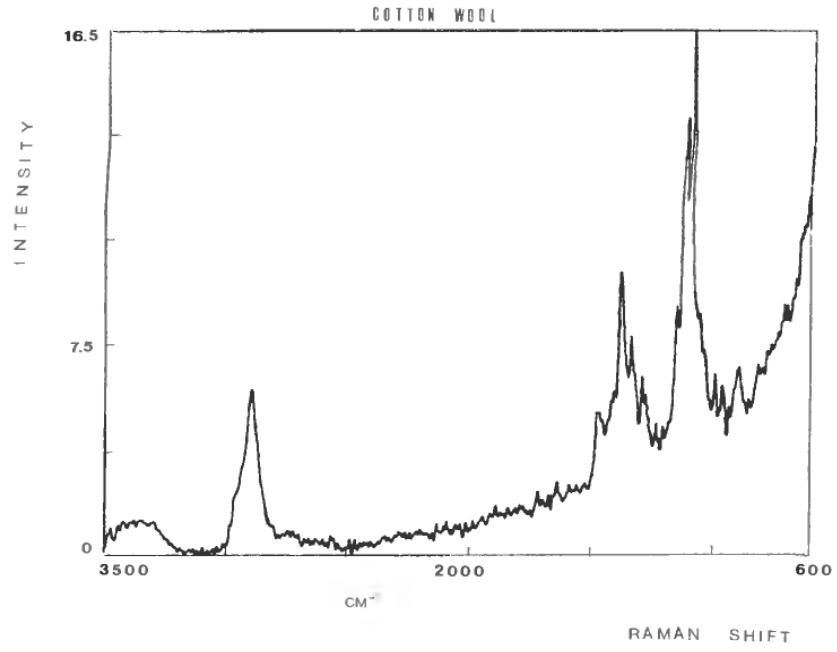


Fig. 11 FT-Raman spectrum of cyppressus wood in the range 3500-600 cm^{-1}.

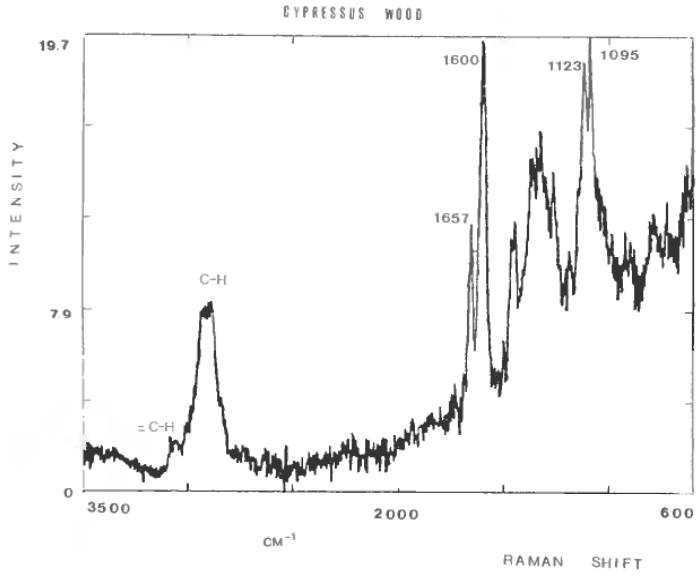


Fig. 12 FT-Raman spectrum of cotton wool in the range 3500-600 cm^{-1}.

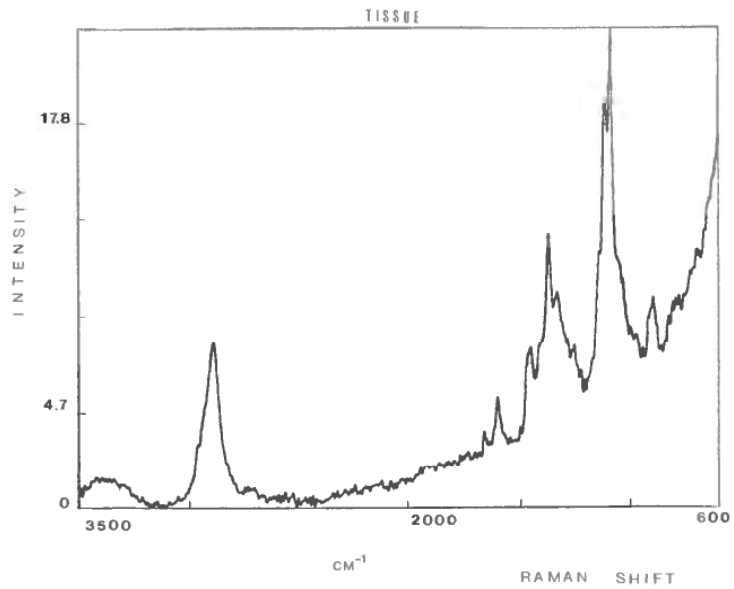


Fig. 13 FT Raman spectrum of tissue in the range 3500-600 cm^{-1}.

fen, 1954; DeMenna, 1989). Raman spectroscopy gives much better results on saccharides than IR does; observed bands are well defined and thus provide more precise information. However food additives and contaminants frequently fluoresce when excited at the visible wavelengths (Ellis *et al.*, 1989). This makes the application of the method limited to certain, relatively pure, products.

It has already been shown that Fourier-transform Raman with the use of near-infrared excitation may successfully be applied to the study of such "troublesome" samples as foodstuffs, including carbohydrate-based products (Ellis *et al.*, 1989; Longhi *et al.*, 1987).

For example FT-R can easily differentiate white cane sugar from brown. In white sugar the 847 cm^{-1} band characteristic of alpha-glucose vibration is of the same relative intensity as in chemically pure sucrose (Figures 7 and 8).

Furthermore, from the spectra shown here it may be seen that the crystallinity degree of white sugar is much higher than that of brown sugar and that the second (raw) product contains much more water than the first.

Cellulose in plant materials

Cellulose is a polysaccharide composed of many glucose units which are beta-1, 4-linked. It has an extended rigid chain structure with an inter- and intra-molecular hydrogen bond net. This polymer is a basic structural component of many plants and for example wood is made up by about 50% of it while cotton fibers are essentially pure cellulose.

Polysaccharides are among the most diffcult to study by means of infrared spectroscopy. One of the factors limiting resolutions of their spectra is the overlap of the CH and OH numerous modes. This problem is especially annoying in low-crystallinity sample such as cellulose where additionally, multiple hydrogen bonds lead to a large number of O-H modes. Thus it is understood that the IR spectra of many polysaccharides may be of little use for analytical purposes.

Despite these difficulties several attempts have been made to obtain the IR spectra of cellulose and wood polysaccharides (Zhbankov, 1966; Marchessault & Liang, 1966). it should be noted here that the samples had to be given special treatments (chemical and physical) prior to recording the IR spectra. The procedures applied were certainly destructive for the morphology of woody tissue.

The Raman spectroscopy offersan opportunity to investigate even individual morphological features in unperturbed plant tissue (Atalla & Agarwal, 1986). Unfortunately, Raman spectra of dry wood obtained by using conventional techniques with visible laser excitation are dominated by an overwhelming fluorescent background (Atalla & Agarwal, 1986). More-

over some special precaution have to be taken to avoid the decomposition of the sample (burning).

FT-Raman with near-infrared laser excitation allows the recording of reasonably good spectra of woody tissues with little fluorescence measured directly on the samples as supplied e.g. in their natural physical state.

Wood consists almost entirely of cellulose, lignin and hemicelluloses and the spectral features characteristic of these components may be observed in the spectra shown here (Figs 9, 10, 11).

Lignin can be easily detected in the FT-R spectrum as it gives a very distinct sharp band due to ν(C=C) vibration of its component — coniferyl alcohol. It is not difficult to distinguish the sample of cypressus wood from that of lime: the intensity of the ν(C=C) band is different in their spectra and thus the amount of lignin is different as well (Figs 3, 4). This observation could have forensic implications.

Finally, samples of cotton woll and paper tissue (as examples of purified and processed cellulosic materials) may also be examined by FR-Raman method. The corresponding spectra are presented in Figures 12 and 13.

Conclusions

It is clear that the principac limitations that have prevented the application of Raman spectroscopy as routine analytical tool have been overcome. The FT-Raman method allows the recording of spectra without fluorescent background (in most cases).

High quality spectra are easily obtained both from pure carbohydrates and from their mixtures found in various naturally occuring or industrially processed materials. The method is non-destructive and sampling is quite simple.

Some of the spectra presented here are completely novel (those of wood, tissue, brown sugar) whilst others have been reported before (see the literature data).

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