Vibrational spectroscopy: a 'vanishing' discipline?

Robert J. Meier†[ab]

Received 26th May 2005
First published as an Advance Article on the web 19th July 2005
DOI: 10.1039/b503880d

The aim of this *tutorial review* is to convince a broad readership that vibrational spectroscopy, although according to some vibrational spectroscopists seemingly less in focus nowadays than in days past, is far from 'dead'. It may seem to some that infrared and Raman spectroscopy are less in focus than in times past, despite the unique analytical capabilities. Vibrational spectroscopy is particularly powerful for non-destructive characterisation of substances, including living material. But compared to the past, a shift in applications has taken place, bringing new opportunities. This is partly due to the introduction of *new features*, including imaging and 2D correlation spectroscopy. Another factor is the *recognition* that vibrational spectroscopy can play a role in new rather than only in the traditional fields of application, *e.g.* new applications in the life-science field (living cells, cancer research), the characterisation of soil. But also the traditional application in catalysis sees new development within the context of *Operando* spectroscopy.

1 Introduction

Over the years, a large variety of analytical tools have become available for the analysis of chemicals and (bio)polymers. With the advent of new techniques, sometimes more accurate, sometimes more versatile, older techniques may lose their significance or charm. Whether this is appropriate is another matter. Titrimetry is a technique hardly exercised in academia anymore, while still very relevant in industry, and further development is still desirable. A few years ago, Prof. Guiseppe Zerbi, in the preface of a book entitled "Modern Polymer Spectroscopy",[1] wrote "For unfortunate reasons the success of vibrational infrared and Raman spectroscopy in industrial and university laboratories seems to fade quickly in favour of other physical techniques which aim at chemical or structural diagnosis of unknown samples." It is certainly true that less emphasis is put on vibrational spectroscopy in many respects. This is partly caused by the strongly growing interest in biochemical research, which tends to use tools including mass spectrometry in high-throughput mode.

Although perhaps not always realised, a series of other applications does make use of the strengths of vibrational spectroscopy. IR and Raman spectroscopy can be used to study the constitution of materials, rather than overall constitution in terms of molecular composition that can also be studied by chromatography, l-NMR or mass spectroscopy. The optical spectroscopies can be used to study materials directly and provide molecular information. This may lead to the determination of, *e.g.*, relations between a molecular structure and the mechanical properties of the material. When Zerbi mentioned that other techniques are used for chemical or structural diagnosis of unknown samples, we now see that with the advent of spectrum libraries IR and Raman spectroscopy are also becoming more powerful in the identification of unknown samples. This may be particularly relevant in forensic investigations and the study of old paintings, for instance. Forensic applications generally come down to identification of chemicals (drugs), or traces of materials such as fibers, rubber, paint, and therefore closely relate to traditional analysis of chemicals and polymers. Application emerges in the paper and pulp industry. Over the last decade, the combination of mid-IR or Raman with chemometric techniques has also been developed. An emerging field profiting herefrom is the characterisation of soil. Also the current availability of IR and Raman imaging instruments has widened fields of application.

Finally, very interestingly, there are emerging fields in which vibrational spectroscopy may become an important tool. This includes bio-medical research, *e.g.* cancer research, the study of blood samples, *etc.*. There are definitely opportunities here, although one should be careful making the right choices: in

Rob Meier

Rob Meier is a Corporate Scientist in the DSM R&D organisation, a Visiting Scientist at the Research Center Jülich, Germany, and an Honorary Visiting Professor in the Chemistry Department of the University of York, U.K. He studied Theoretical Chemistry at the University of Amsterdam, and graduated from the same university on a subject in experimental condensed matter physics. He joined DSM in 1985. His interests include various experimental spectroscopies, computational chemistry, polymer physics and catalysis.

---

[a]*Institute of Chemistry and Dynamics of the Geosphere IV: Agrosphere, Forschungszentrum Jülich GmbH, D-52425 Jülich, Germany*
[b]*DSM Research, P.O. Box 18, 6160 MD Geleen, Netherlands. E-mail: r.meier@wxs.nl*

† This paper is dedicated to Prof. Guiseppe Zerbi to honour his significant contributions to vibrational spectroscopy.

new fields of application it takes time before it is settled which techniques are in fact best choice. First publications are often over-optimistic, selling a specific technique. This, however, applies to any technique, and vibrational spectroscopy will certainly also find its way here.

In the present review we limit ourselves to mid-infrared and Raman spectroscopy, although there are ongoing interesting developments in far-infared spectroscopy (also known as TeraHertz spectroscopy, in particular for food and life science research) and near-infrared spectroscopy as well. Most examples we will use for illustration have been studied using standard type equipment, and no specialized techniques were involved. With special accessories many more applications become within reach, invoking techniques such as Resonance Raman spectroscopy or near-field approaches. Furthermore, it will be evident that in a review of the current size only a selection of fields of application can be highlighted. We encourage the reader to refer to the literature for other examples and other applications. In this respect we can definitely recommend the recently issued impressive five-volume set Handbook of Vibrational Spectroscopy[2] in which practically all issues and applications are covered to a greater or lesser extent.

Whereas IR spectroscopy is better known and more often applied than Raman spectroscopy, the latter is becoming increasingly popular thanks to the introduction of Raman imaging and, last but not least, the recognition that it has a unique contribution in a series of applications. The higher spatial resolution is one important difference, and the sensitivity for specific molecular vibrations another one. On the other hand, Raman spectroscopy may suffer from fluorescence interference. IR is much more sensitive, *i.e.* lower levels of detection are feasible, and libraries with IR spectra for interpretation of unknown species are widely available. In the next section we will briefly introduce modern IR and Raman instrumentation. Then a selection of examples will be presented illustrating specific applications.

2 IR and Raman instrumentation

Only some main features of instrumentation will be described, because this review focuses on applications. Modern IR instruments are, almost without exception, of the Fourier-Transform type. These are robust, easy to handle, and availability is generally very good. These may be equipped with a microscope to allow for micro-spectroscopy with a spatial, lateral, resolution in the 10 μm range. More recent are infrared imaging instruments, enabling the recording of an image of a sample.

Portable Raman instruments have become available allowing for in-process analysis in plants and other studies outside the laboratory. Raman spectrometers of the Fourier-Transform type have been available for some time now, but dispersive instruments have made a comeback with the introduction of imaging instruments. The much shorter wavelength used in Raman spectroscopy makes the spatial resolution one order of magnitude better compared to IR (~ 1 μm). Because Raman spectroscopy is a scattering technique, it allows for what is known as confocal Raman micro-spectroscopy, limiting *depth*-resolution to the μm range.

Imaging, *i.e.* the recording of a series of spectra originating from a set of points at the sample, may be accomplished in various ways. The simplest way is to shift the sample stepwise, using an automated sample stage, recording a spectrum after every step. Another approach is to use moving optics to scan the laser beam over the sample surface. Other, more efficient ways involve two-dimensional detectors capable of directly recording the signal from many points at the sample. Alternatively, a line of points at the sample can be imaged using cylindrical optics while the other direction on the CCD detector can be used to record a full spectrum. Various set-ups have their individual limitations, related to sensitivity, overall time to record the image, and retaining true confocality.

Over the last decade the combination of synchrotron radiation and IR micro-spectroscopy has been introduced. The brightness of the synchrotron source leads to a high S/N ratio which is retained when performing microscopy experiments at the diffraction limit. This has turned out particularly useful in the study of biological cells, *e.g.* the distribution of lipids and proteins in the cell.

Unfortunately there has not yet been a breakthrough in UV Raman spectroscopy. That technique has the potential to circumvent the fluorescence problem that may show up in Raman spectra, while the scattering intensity is high because the intensity is inversely proportional to the fourth power of the wavelength.

3 Applications

Well-known and well-appreciated applications in polymer science include the characterization of branching and unsaturation in polyolefins, the effect of oxidative degradation as studied by the sensitivity of carbonyls in the IR spectra, Raman spectroscopy on the degradation of PVC (the formation of polyene sequences), and the extensive literature on chain defects and its detection by IR spectroscopy, although most of this literature is limited to polyolefins.[3] The possibility of studying chain orientation using polarized IR and Raman spectroscopy is also well-covered in the older literature, *e.g.* liquid crystal work or orientation in film and fiber. Also known for a longer period of time is the use of time-resolved vibrational spectroscopy to monitor *in-situ* real-time polymerisation reaction kinetics.[4] Some potentially powerful applications seem almost forgotten, such as accurate bond length determination from vibrational spectra.[5]

The characterisation of inorganic materials by vibrational spectroscopy has been well appreciated. This includes applications in inorganic materials such as zeolites and metal oxides for catalysis, ceramics, the important field of semi-conductor structures, and application in geology.

The current strong interest in life sciences related research had led to a large number of papers in the literature involving application of vibrational spectroscopy in that field. The topics vary from the characterisation of relevant organic species, including polymorphs, the characterisation of formulations including tablet, the study of tissue and blood, but *ex-situ* as well as *in-situ*, micro-spectroscopic studies of biological cells, *e.g.*, for cancer research.

More recently a sub-field known as *Operando* spectroscopy has emerged. It is the field in which a catalyst can be studied at its working place under real catalytic conditions.

In what follows, we will illustrate some known but in particular some new developments.

3.1 Infrared and Raman microscopy on polymers

Infrared microscopy is very suitable for studying gels and other type impurities in polymers, in particular polymer films. This so-called trouble shooting work is of high importance in the industrial environment. Skilled operators may directly recognize the impurity type from the spectrum recorded, or with the help of an IR spectral library.

Whereas both IR and Raman microscopy are useful options, the higher spatial resolution of Raman spectroscopy makes it the preferred choice in quite a few applications, assuming both techniques are present in the laboratory. The C–C stretch vibrations of the carbon–carbon backbones of any polymer chain are particularly Raman active. Therefore these vibrations have been often used to study the relation between molecular structure and macroscopic mechanical properties (stress or strain, plastically deformed material), including fibre-based composites. Examples can be found in numerous papers, *e.g.* a review.[6] In addition, Raman spectroscopy can be applied to study the composition of multi-layer systems, *e.g.* polymer film, coatings, human skin, etcetera, without the need to microtome the sample. When using an automated sample stage, which can also move in the z-direction (along the optical axis), a complete characterisation of a multi-layer system is possible. Using confocal Raman spectroscopy, depth resolution can be significantly enhanced. Refraction at the surface will lower the attainable depth resolution, but can be avoided by using an oil-immersion objective. The latter is nicely illustrated in Fig. 1 showing the characterisation of a three-layer polymer sample and the substantial differences in layer thickness while using dry or oil immersion optics. The same approach allows for the study of the curing of coatings, including the drying process as a function of depth into the sample (see, *e.g.*, Fig. 14 in Ref. 6). It should be mentioned that optical effects, in particular refraction, are very important especially when attempting the limits of spatial resolution. Several papers, however, have addressed this problem in recent years.

Melamine reveals some very strong Raman active bands, and this chemical species as well as melamine based resins, including the final application in fibreboard, offer particularly interesting opportunities for both IR and Raman micro-spectroscopy (see Ref. 7 and references therein).

3.2 Infrared and Raman imaging

This is most definitely one of the most important developments in vibrational spectroscopy of the last decade. Imaging is a particularly powerful technique to study homogeneity of materials that might be related to bulk properties such as mechanical properties or to surface properties such as gloss. This may apply to impurities as well as to differences in orientation, crystallinity, or distribution of additives. With vibrational imaging both the distribution as well as chemical information on the inhomogeneity is obtained.

Imaging may be applied directly to the sample. Alternatively, a microtomed coupe may be used for inspection. It depends on the question and the sample characteristics whether slicing is acceptable or necessary to obtain IR or Raman images. More extensive experience reveals that one more advantage of imaging shows up when looking for differences in composition or morphology (inhomogeneities) that lead to minor differences in the spectra. An example could be skin–core differences in a polymeric material. When individual spectra are compared, differences can be so small that it is not justified to draw definite conclusions. However, when many data points are recorded, the small difference can be found persistent throughout the data set therewith allowing for a statement on, *e.g.*, difference in skin and core.

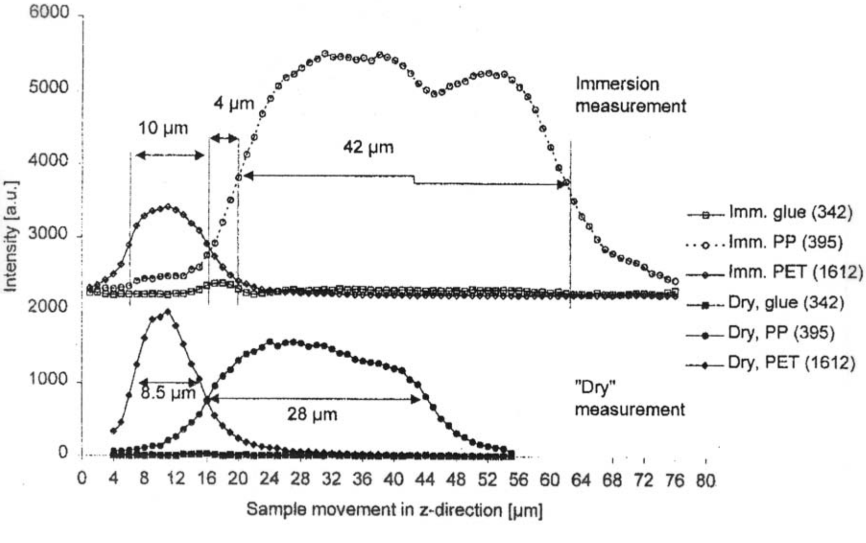


Fig. 1 Three-layer polymer sample measured with immersion optics and traditional 'dry' optics. No traces of the glue can be seen in the 'dry' measurement. (Reproduced with permission from Ref. 31. Copyright 2002, Society for Applied Spectroscopy.)

Many IR imaging studies have been reported in the polymer field including papers on blend composition, photo-crosslinking[8] (discussed in the HTE paragraph below), fibre-reinforced composites,[9] and the processing of images forming morphology visualization.[10]

In the life-sciences field, FT-IR imaging can be directly applied, without any sample preparation, to obtain the distribution of the active component in a pharmaceutical tablet. Fig. 2 shows an example, recorded in 15 minutes, where red indicates the highest concentration of the active ingredient.

The next application is one of two, one being the example of very low concentration of a small molecule penetrating a membrane to be discussed later, in which we will see that the effect of the addition of a (very) low concentration of an additive, unlikely to be detectable directly by Raman spectroscopy, can be studied by the effect it has on other constituents of the sample. Hybrane is a hyperbranched polymer with application as a paper additive. Its function, as claimed, is to force calcium carbonate to concentrate at the surface of the paper rather than evenly distributed throughout the paper sample. This is precisely what can be concluded from a Raman image from a cross-section of the corresponding paper sample, *viz*. Fig. 3.

3.3 Polymorphism

An excellent introduction to this phenomenon and vibrational spectroscopy can be found in Ref. 2. Polymorphism occurs in a wide variety of materials, including pure sulfur, oxides, and organic compounds that may find application in pharmaceutical tablets. Polymorphism can be studied by various techniques, with X-ray diffraction traditionally one of the key techniques. Other techniques include solid state NMR, thermal analysis (DSC) and IR and Raman spectroscopy. In specific cases vibrational spectroscopy may have a distinct advantage. Different hydrates, *e.g.* anhydrous *versus* monohydrate forms, of organic crystalline materials for instance can often be hard or impossible to distinguished by powder X-ray diffraction. Here both NMR and vibrational spectroscopy can reveal the presence of solvent molecules.

Another very characteristic example is titanium dioxide, TiO_2, a material that is used in a wide variety of applications including paints, polymers, sun-creams, as agent in the remediation of waste water, the mineralisation of trichloromethane.

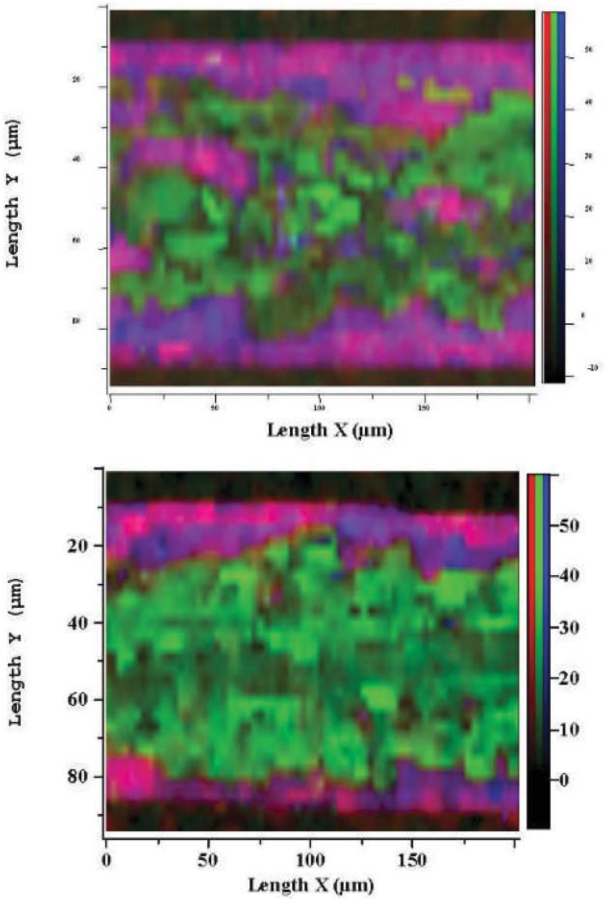
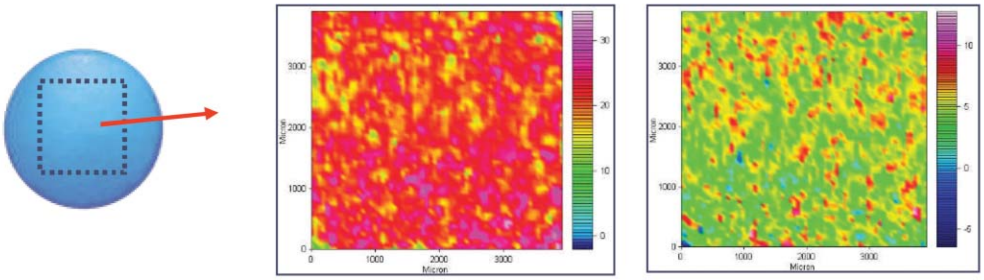


Fig. 3 Cross sections of two different paper samples: a Hybrane free paper (upper) and a paper containing Hybrane. The Hybrane paper contains a pre-coat with 0.6 parts Hybrane per 100 parts of dry pigment (color with latex/starch as binder, applied by a size press), a mid-coat (latex/starch) and a latex only top-coat. On these Raman images one can see that the pigment $CaCO_3$ (red) and the latex (blue) penetrate less into the base paper (green, cellulose) when Hybrane is used. When the pre-coat is applied on the base paper, water starts to penetrate into the base paper, resulting in the formation of an immobilized layer at the interface between the base paper and the coating. The Hybrane induced denser packing at this interface results in less migration of the remaining liquid of the pre-coat into the paper. Due to a denser packing of the pre-coat the next two coating layers also migrate less into the inner part of the paper.



$\int$ 1250-1050 cm^{-1} (starch) $\int$ 1790-1650 cm^{-1} (drug)

Fig. 2 Active component in a tablet, with red indicating the highest concentration and blue the lowest concentration. Spectra were kindly provided by Bruker Optics.

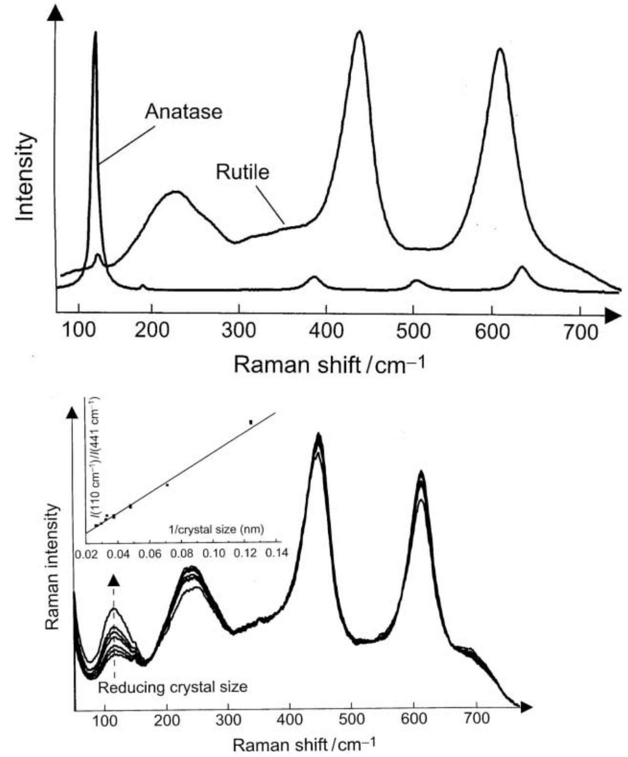


Fig. 4 Upper picture: Raman spectra of rutile and anatase forms of TiO_2. Lower picture: a band near 110 cm^{-1} correlates with the reciprocal crystal size for rutile TiO_2. (Reproduced with permission from Ref. 2 (N. J. Everall, pp. 143 & 145). Copyright 2002, John Wiley & Sons.)

Its photo-activity gives it very special properties. These properties are modified by suitable coatings, but also by the polymorph and particle size. Particle size is often in the range of tens of nanometers. Whereas polymorphy is otherwise preferentially studied by other analytical techniques, polymorphy and particle size of TiO_2 can be very well studied by Raman spectroscopy. Fig. 4 shows the difference between the spectra for the anatase and rutile polymorphs of TiO_2. The relative intensity of the low frequency anatase band can be correlated with the anatase content. More recently the use of Raman spectroscopy to measure the composition on-line was reported.[11] For the rutile form, the particle size can be determined from the relative ratio of intensities of the low frequency band (near 110 cm^{-1}) and the band near 450 cm^{-1}. For anatase, information on particle size in the nanometer range can be obtained from the line-width of the Raman bands.

3.4 Double bond reactivity: studying vitamin A degradation by Raman spectroscopy

With Raman spectroscopy the *in-situ* evolution of an all-*trans* retinol solution can be followed when submitted to UV light in the presence of oxygen. In this way it is possible to study vitamin A degradation by monitoring the decrease of the double bond from the 1593 cm^{-1} Raman band, *viz*. Fig. 5. The specific sensitivity for double bonds makes the technique very suitable for studying this degradation process, the curing of resins where double bonds react away, or the formation of double bonds (polyene sequences) as they take place upon the degradation of PVC.

3.5 Living cells and tissue studies

Over the last decade vibrational spectroscopy has started to become of interest for the study of living cells and tissue. This is obviously related to some inherent advantages of these techniques: spectroscopy can be used without the need for staining the sample, both *in vitro* and *in vivo* studies are possible (use of an inverted microscope for *in vivo* studies), information can be obtained on the molecular composition of skin layers (lipids, proteins, ...), and effects of disease as seen by Raman spectroscopy have been reported. Information on tissue structure, cellular components, and the state of health of cells and tissue is coming within the capabilities of vibrational spectroscopy as recently reviewed.[12] As an explicit example,

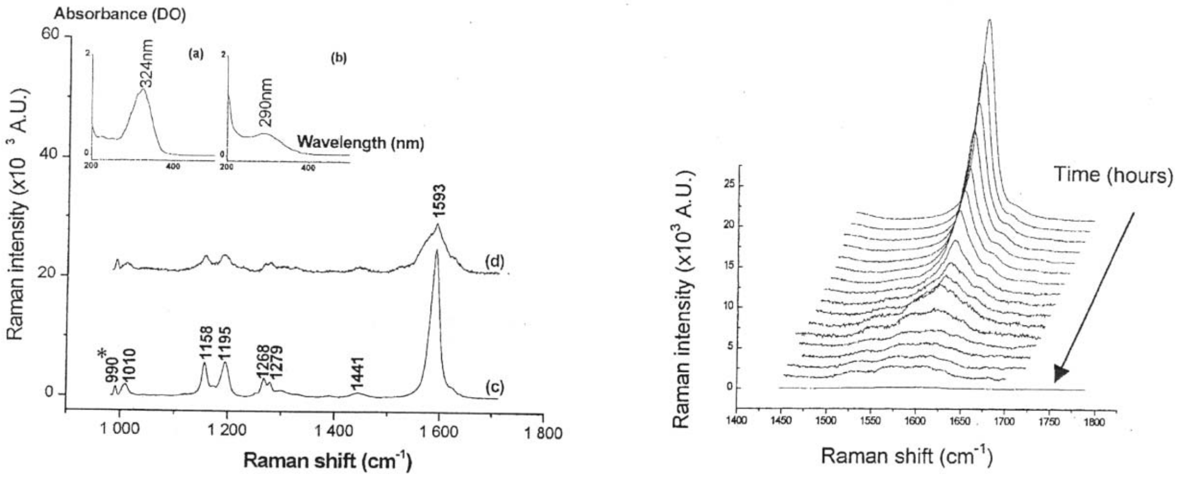


Fig. 5 Left: UV spectra of all-*trans* retinol in caprilic/capric triglyceride (a) before and (b) after irradiation at 365 nm for 15 h, and likewise the Raman spectra. Right: The time dependence of the 1593 cm^{-1} C=C band as a function of irradiation time. (Reproduced with permission from Ref. 32. Copyright 2003, Society for Applied Spectroscopy.)

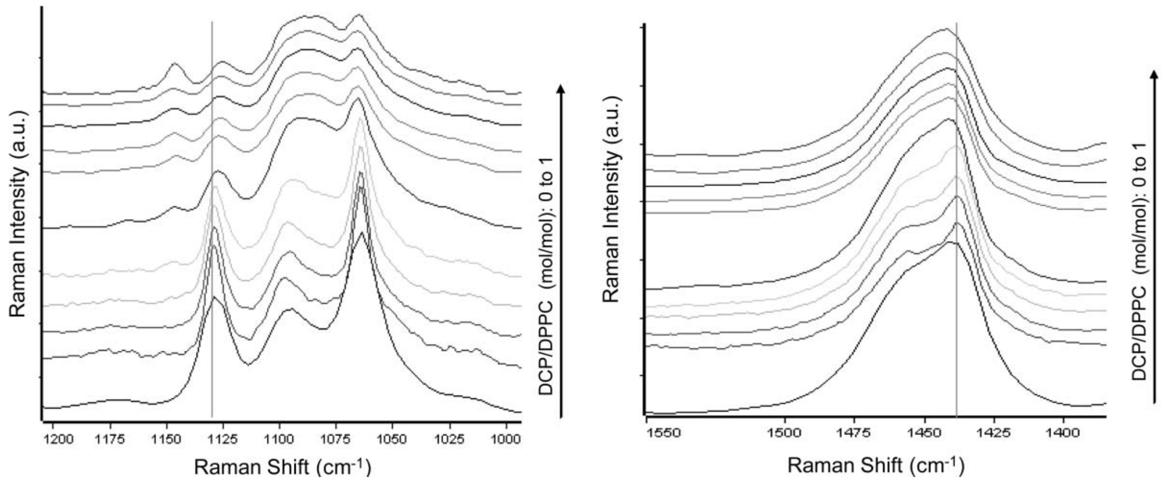


Fig. 6 Left: Spectral range of C–C stretching bands. Right: CH_2 scissoring band of DCP/DPPC liposomes at the molar ratio of 0/100, 0.04/100, 4/100, 10/100, 20/100, 30/100, 40/100, 50/100, 60/100, 80/100, and 100/100 at 26 °C.

spectral changes in the levels of nucleic acids, proteins, lipids and carbohydrates in colonic cancer crypts were reported.[13]

The basic structure of cell membranes is formed by a phospholipid bilayer. Apart from useful nutritional ingredients, harmful components might be taken up by the membrane and affect its structure. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC)/water liposomes can be used to study the structure of the alkyl chains in lipid bilayer membranes. A recent Raman study[14] revealed that it is possible to detect the presence of a 0.04% DCP (dichlorophenol) in a DPPC bilayer. The observed changes are due to the effect of the very low amount of DCP on the structure of the alkyl chain in the bilayer. From the Raman spectra in Fig. 6 it is revealed that there is a change in the spectrum. In the C–C stretch spectral range this is expressed by a narrowing of the 1060 and 1130 cm^{-1} band when going from 0 to 0.04% DCP. In the methylene scissoring region we see more of a bimodal character for 0.04% DCP. This example shows that normal Raman spectroscopy can be used as a very sensitive probe at concentrations realistic in real living cells.

3.6 Food

According to a recent overview, application in food characterisation is not yet that successful.[15] However, developments go fast, in particular when appropriate data processing tools become available and are applied. With the use of statistical methods (so-called self-modelling curve resolution) Pudney *et al.* quantified microstructures of complex soft materials including dairy spread.[16] Secondly, whereas Near-Infrared Spectroscopy is often attempted in these applications, mid-IR and Raman sometimes directly provide characteristic bands allowing for the discrimination of components in complex mixtures, *e.g.* vegetable oils.[17]

3.7 Soil characterisation

The characterisation of soil is an elaborate task which traditionally involves extraction procedures followed by elemental analysis, liquid phase NMR, *etc.*. The problem is, obviously, that soil is both a very complex and a very inhomogeneous material. It would therefore be very relevant to have access to a technique that can readily and without sample preparation provide a good indication on some key properties of soil. More recently, mid-infrared spectroscopy has been applied in this field.[18] It was shown feasible to obtain information on different treatments originating from different land management practices. Diffuse reflection IR spectroscopy (DRIFT) may become a tool for rapidly and accurately following fresh organic matter degradation in a soil matrix.[19] In combination with chemometric techniques, the results look promising, although further development and validation is definitely required. This looks an altogether very promising field of application under current development.

On the other hand, attempts to characterise the structure of humic acid, a key organic C component in soil, thus far do not reveal a high potential for vibrational spectroscopy (see Ref. 2, Volume 4, page 3201).

3.8 Correlation spectroscopy

Over the last decade 2D-correlation spectroscopy has been one of the new trends in vibrational spectroscopy. Even dedicated international meetings are now organized around this technique, *i.e.* 2DCOSII in Nottingham (August 2003). A recent review is available.[20]

In general in a 2D correlation experiment as defined in the context of vibrational spectroscopy[20] an external perturbation is applied to the system under study. This perturbation can be a mechanical or electric perturbation, it can also be a temperature or pressure variation, or a chemical perturbation such as variation in composition of an A-B copolymer (set of samples with different composition A/B), or the change in a system when it is polymerising (time-dependent spectra). The set of spectra obtained have been named the dynamic spectra. What is important is to realize that a series of spectra is obtained from samples that have some mutual relation. The correlation analysis, which involves some mathematical manipulations including Fourier transformation and the evaluation of correlation functions which have been described in detail in the literature (see Ref. 20 and references therein), leads to a plot in which the spectral intensity is plotted as a function of two independent spectral parameters, *i.e.* the wavenumber for IR and Raman spectra. An overview of the steps involved is shown in Scheme 1.

Normally two different plots are generated from this analysis: the synchronous and the asynchronous 2D correlation map. The synchronous map reveal spectral changes that occur similarly, *i.e.* a peak is positive if two intensity variations occur in the same direction, negative when they change in opposite direction. The asynchronous map emphasizes dissimilarities: an asynchronous peak when a change in spectral intensity at ν_1 happens earlier than at ν_2, whilst the peak is negative when the time-order is reversed. What this all means is that we obtain relations, correlations, between variations in a series of spectra. Let us illustrate this using a real example.

The photopolymerisation of acrylic and epoxy monomers with 1,6-hexanediol diacrylate (HDDA) as the acrylate and 3,4-epoxycyclohexylmethyl-3′,4′-epoxycyclohexane carboxylate (epoxy) as epoxy has been studied using real-time FT-IR. Spectra were recorded with a time-resolution of 78 ms and are displayed in Fig. 7a. From such spectra it is difficult to observe the changes upon polymerisation. However, by correlating the data and producing 2D-IR plots this information becomes much more apparent. Figs. 7b and 7c show the synchronous and asynchronous 2D-IR plots. The synchronous cross-peaks indicate vibrational bands that change simultaneously with time. Also note that a considerable improvement in spectral resolution is obtained. For this specific chemical system more interesting information can be revealed from the asynchronous plot. If both monomers disappear at the same rate, the absorption bands corresponding with the two monomers would decay at equal rates. As a consequence, with respect to time they would both show ‘in-phase’ behaviour with respect to each other, and consequently a strong signal in the synchronous 2D-IR correlation map. However, the actual asynchronous correlation map displayed in Fig. 7c evidently shows that the rates of polymerisation of the two monomers are different (cross-peaks (off-diagonal) in an asynchronous correlation map indicate *different* rates). It was then concluded that the two polymerisation reactions occur independently.

We have thus seen that a main advantage of 2D correlation spectroscopy is a better analysis of highly overlapping spectral features by using a second dimension. A second use is in determining the order, in time, in which certain phenomena occur.

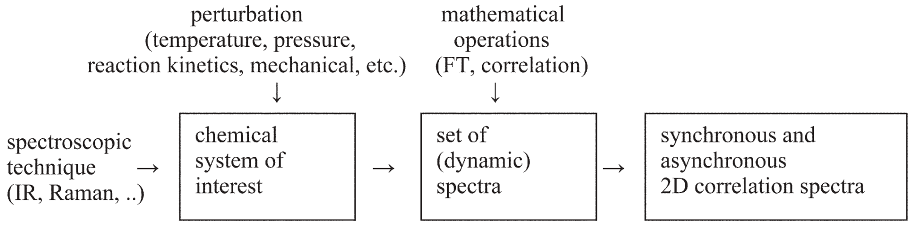
For polymer blends correlation spectroscopy can reveal miscibility at the molecular level (or disprove such miscibility).[21] Phase transition in lipid bilayers were studied using 2D IR correlation analysis.[22] In the field of medicine, 2D IR correlation spectroscopy has been applied to study traditional Chinese medicine ‘Qing Kai Ling’ injections from different manufacturers.[23] 2D correlation analysis of the spectra, revealing mostly broad features, led to ready discrimination between batches from different manufacturers, and even from different batches originating from the same producer.

One of the early applications by Noda *et al.* involved mechanical perturbation, *i.e.* the coupling of polarised IR spectroscopy and Dynamic Mechanical Analysis. An efficient set-up was developed later by Steeman c.s.[24] This, not often employed approach, is potentially very powerful in establishing relations between molecular constitution and macroscopic mechanical properties.

Certain application of the 2D correlation techniques approach have been criticised (see section 2.2 in Ref. 20) for being inappropriate. In other reports the evidence that a 2D correlation analysis has real added value compared to traditional 1D analysis and the use of, *e.g.*, deconvolution, is missing.

3.9 Catalyst characterisation

Infrared and Raman spectroscopy have been intensively used in catalyst characterisation, but also in the analysis of products of a catalytic reaction. This is well-known and well-documented in research papers and books, and includes the characterisation of zeolite structures and probing the acid sites by species such as ammonia *via* infrared spectroscopy, and the characterisation of metal oxide and supported metal catalysts. Also homogeneous catalysts may be studied using vibrational spectroscopy, but obviously NMR is a tremendous competitor. As soon as the homogeneous catalyst needs to be immobilised for application, vibrational spectroscopy comes into the picture again. Obviously advantages of vibrational spectroscopy include (i) specimens can be readily studied, whether in gas, solid or liquid form, (ii) *in-situ* experiments are accessible, in particularly when Raman is used one can use any optically transparent window, (iii) realistic catalytic conditions can be applied, *i.e.* (high) pressures and high temperatures, (iv) spectra can be recorded at the sub-second time frame. It is with respect to studying a catalyst under real working conditions that a new approach, *Operando* spectroscopy, has been introduced (for a review see Ref. 25). It refers to an *in-situ* like experiment (for the distinction between Operando and



Scheme 1

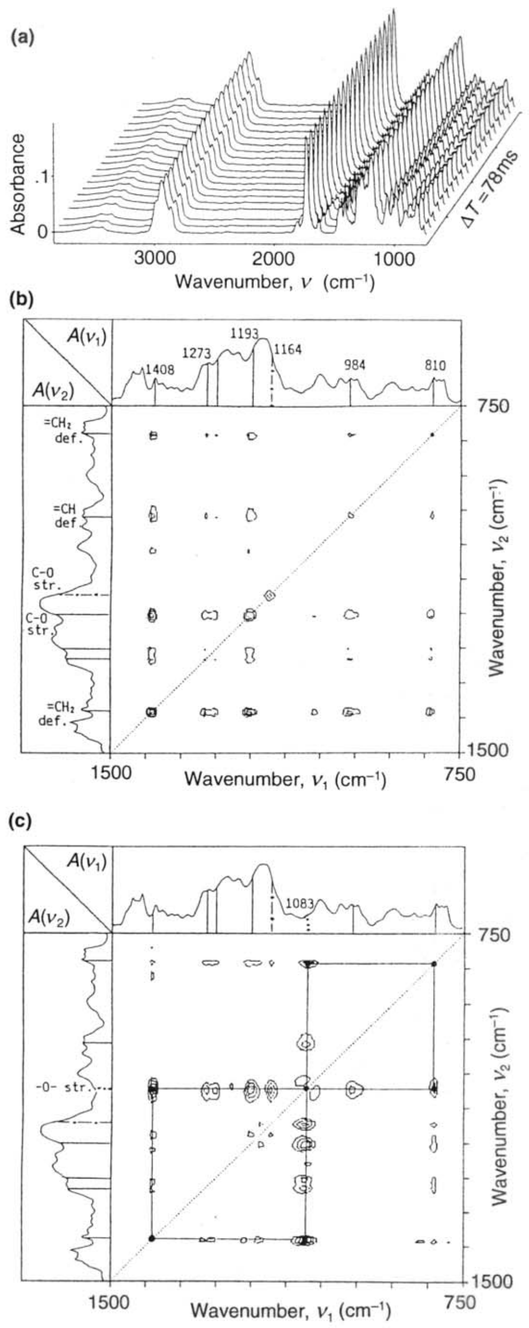


Fig. 7 (a) Real-time FT-IR spectra collected during the initial stage of UV curing of the 1:1 monomer blend of HDDA and epoxy. (b) Synchronous 2D-IR spectrum of HDDA–epoxy mixture. (c) Asynchronous 2D-IR spectrum of the same HDDA–epoxy mixture. In both (b) and (c) the 1D-IR spectrum is shown along both axes for comparison. In the 1D-IR spectra the bands arising from the acrylic monomer are indicated as (——), those originating from the acrylic polymer are characterised by (- · · - · · -), whereas finally the epoxy polymer bands are indicated by (· · · ·). (Reproduced with permission from Ref. 33. Copyright 1993, Society for Applied Spectroscopy.)

in-situ spectroscopy see Ref. 25), not in an industrial reactor but still in a manner that is relevant to reaction conditions and gives us insight into the details of the catalytic reaction mechanism. Such studies may, in the end, allow establishing quantitative structure–activity relationships for on-line process control. Whereas Operando spectroscopy does not exclusively refer to vibrational spectroscopy, this type of spectroscopy has a huge potential because of the arguments (i–iv) mentioned in the above. What is probably much more interesting is that more recently, within the context of Operando spectroscopy, multiple characterisation techniques are applied simultaneously to the catalytic reaction. For instance, three spectroscopic techniques simultaneously collecting data at a rate of 20 spectra per second produce 1200 spectra in one minute only. A set of appropriately chosen techniques thus provides a wealth of detailed information. An explicit example reported includes UV-VIS, Raman and EXAFS.[25] Also a powerful but perhaps underestimated technique as ESR may be suitably combined with, *e.g.*, Raman spectroscopy.

3.10 High-throughput experimentation

Parallel screening large series of samples is part of high-throughput experimentation (HTE). This popular experimental approach also requires efficient analytical tools. Though mostly other analytical techniques have been employed thus far, vibrational spectroscopy finds its specific application.

One example involves the characterisation of branching in polyethylenes using IR. As many laboratories, including industrial labs and specialised HTE companies study new polymerisation catalysts, this is a very relevant contemporary application.

Rafferty *et al.*[8] have used FT-IR imaging to characterize a series of photo-crosslinked poly(vinyl) cinnamate systems. This application is related to research into chemical sensors. By using a mask system, commonly used to make a series of different related materials on a single substrate, a variety of samples is produced exhibiting various crosslinking densities. The mask approach is illustrated in Fig. 8. By rotating the mask and applying a different UV exposure time in the second step, each small square on the substrate becomes a different sample. The IR image of such an array, part of which is displayed on the right in Fig. 8, illustrates that whereas the intensity of the alkene band in the poly(vinyl) cinnamate structure is an obvious choice to monitor cross-linking, the carbonyl band can be used equally well. Spatial resolution was about 6 μm and total acquisition time for a single image was 3.5 min.

Another example involves the relation between the Raman spectrum and mechanical properties of polyolefin samples.[26] Using multivariate data analysis, the Raman spectra of a series of metallocene-catalyzed polyethylenes were used to establish a correlation between features in the Raman spectrum and the density, the number of methyl branches per 1000 C atoms, the elastic modulus and the yield strength. The correlations for the mechanical properties are shown in Fig. 9. It may be argued that the correlation is far from perfect, but it should be realized that this application focuses on high-throughput analysis, *e.g.* 100 samples or more per day, for the purpose of screening potentially useful catalysts.

A rapid characterisation of the viscosity of waterborne automotive paint was reported by Ito *et al.*[27] FT-Raman spectroscopy in conjunction with partial least squares regression (PLS) was applied and led to reasonable correlation.

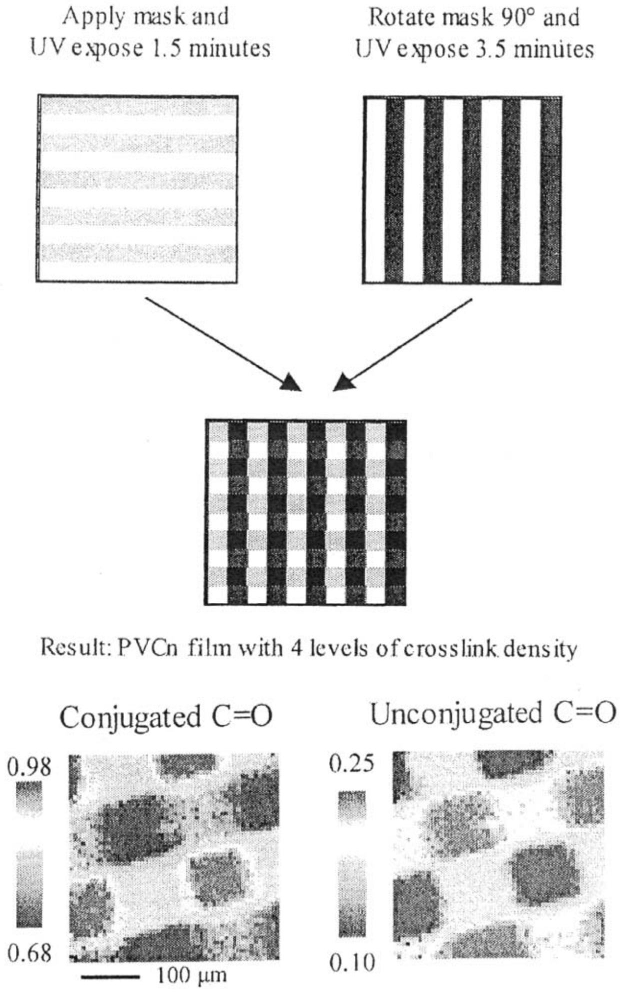


Fig. 8 Above: illustration of the application of a mask to produce different 'samples'. Below: FT-IR images of the carbonyl band of poly(vinyl cinnamate), showing different levels of crosslink density as revealed from a decrease in conjugated carbonyls. (Reproduced with permission from Ref. 8. Copyright 2002, Society for Applied Spectroscopy.)

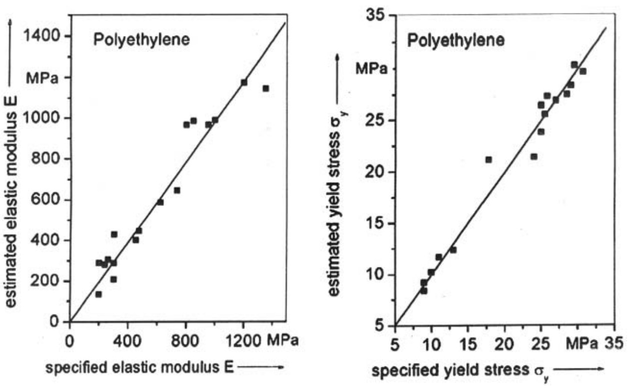


Fig. 9 Chemometric calibration of the elastic modulus (left) and the yield stress (right) of a series of polyethylenes. (Reproduced with permission from Ref. 26. Copyright 2003, Wiley-VCH Verlag GmbH.)

3.11 Interpretation of spectra

The availability of spectral libraries, more recently also of Raman spectra, is of great significance in the interpretation of spectra of unknown constituents. It is essentially a must in industrial laboratories, where the payback time for the investment will be comparatively short. Also quantum chemical *ab-initio* calculations have become a serious tool in interpretation, but only recently is accuracy becoming more or less acceptable. For small molecules differences of several percent between calculated and experimental frequencies are not a problem for interpretation. However, when molecules become a little larger, the number of vibrational bands becomes so large, and particularly dense in specific spectral regions, that it may not be unambiguous to assign even the correct order of vibrational bands on basis of the calculations. The main problem for isolated molecule spectra is the anharmonic effect. Two different approaches have recently been introduced that seem to cope well with this problem. The first approach is based on a second order perturbative treatment.[28] The second one involves analyses of trajectories from molecular dynamics simulations within the so-called *ab-initio* molecular dynamics method; for a review of that method with applications in chemistry see Ref. 29.

Despite the long history of vibrational spectroscopy, there are still serious gaps in available knowledge. Regarding microstructure of polymers, an overwhelming number of studies on polyethylene, polypropylene, PVC, nylon and a few other polymers, contrasts with the lack of information on many other polymers. For example, but the same applies to many copolymers (engineering plastics), for the technically important urea–formaldehyde resins various assignments are long-standing as tentative in literature.[30] When considering organometallics, information is mostly available on small model compounds, *e.g.* MX_4 *etc.*, whereas limited information is available on systems of real interest to the synthetic chemist. Therefore there is great opportunity for further studies involving model compounds, extended to real-life systems.

4 Conclusions and outlook

Whereas at least in some parts of the world vibrational spectroscopy seems to have suffered from a loss in interest, the field is still very much alive and we have seen highly relevant developments over the last decade. Imaging and two-dimensional correlation spectroscopy are probably the most important technical developments, next to the availability of spectral libraries enabling the characterisation of unknown constituents. As a result, the potential in materials research is significantly enhanced, whereas this is still developing in life-science applications (living cell, skin, membranes). At least as relevant, the potential in other fields has been recognised and new applications develop. And last but not least: today there is an ever-increasing need to solve complex problems. These require a multi-disciplinary approach in which vibrational spectroscopy can play a prominent role, *e.g.* the case of Operando spectroscopy to study working catalysts.

References

1 *Modern Polymer Spectroscopy*, Ed. G. Zerbi, Wiley-VCH, Weinheim, 1999.
2 *Handbook of Vibrational Spectroscopy*, J. M. Chalmers and P. R. Griffiths, Eds., John Wiley & Sons, 2002.
3 P. C. Painter, M. M. Coleman and J. L. Koenig, *The Theory of Vibrational Spectroscopy and its Application to Polymeric Materials*; John Wiley & Sons, New York, 1982.
4 C. Decker, *J. Polym. Sci. Part A Polym. Chem.*, 1992, **30**, 913.
5 J. A. Larsson and D. Cremer, *J. Mol. Struct.*, 1999, **485–486**, 385.
6 R. J. Meier and B. J. Kip, *Microbeam Anal.*, 1994, **3**, 61.
7 P. J. Larkin, M. P. Makowski, N. B. Colthup and L. A. Flood, *Vib. Spectrosc.*, 1998, **17**, 53.
8 D. W. Rafferty, J. L. Koenig, G. Magyar and J. L. West, *Appl. Spectrosc.*, 2002, **56**, 1549.
9 A. M. Mavrich, *Appl. Spectrosc.*, 1995, **49**, 149.
10 R. Bhargava, S.-Q. Wang and J. L. Koenig, *Appl. Spectrosc.*, 2000, **54**, 1690.
11 I. M. Clegg, N. J. Everall, B. King, H. Melvin and C. Norton, *Appl. Spectrosc.*, 2001, **55**, 1138.
12 M. Diem, M. Romeo, S. Boydston-White, M. Miljković and C. Matthäus, *Analyst*, 2004, **129**, 880.
13 A. Salman, R. K. Sahu, E. Bernshtain, U. Zelig, J. Goldstein, S. Walfish, S. Argov and S. Mordechai, *Vib. Spectrosc.*, 2004, **34**, 301.
14 A. Csiszár, E. Koglin, R. J. Meier and E. Klumpp, submitted for publication.
15 L. G. Thygesen, M. M. Løkke, E. Micklander and S. B. Engelsen, *Trends in Food Science & Technology*, 2003, **14**, 50.
16 P. D. A. Pudney, T. M. Hancewicz, D. G. Cunnigham and M. C. Brown, *Vib. Spectrosc.*, 2004, **34**, 123.
17 H. Schultz, M. Baranska, H.-H. Belz, P. Rösch, M. A. Strehle and J. Popp, *Vib. Spectrosc.*, 2004, **35**, 81.
18 R. S. Antil, M. H. Gerzabek, G. Haberhauer and G. Eder, *J. Plant Nutr. Soil Sci.*, 2005, **168**, 108.
19 R. Spaccini, A. Piccolo, G. Haberhauer, M. Stemmer and M. H. Gerbazek, *Geoderma*, 2001, **99**, 245.
20 I. Noda, *Vib. Spectrosc.*, 2004, **36**, 143.
21 C. Marcott, I. Noda and A. E. Dowrey, *Anal. Chim. Acta*, 1991, **250**, 131.
22 D. L. Elmore and R. A. Dluhy, *Colloids Surf. A*, 2000, **171**, 225.
23 Q. Zhou, S.-Q. Sun and L. Zuo, *Vib. Spectrosc.*, 2004, **36**, 207.
24 P. A. M. Steeman, R. J. Meier, A. Simon and J. Gast, *Polymer*, 1997, **38**, 5455.
25 B. M. Weckhuysen, *Phys. Chem. Chem. Phys.*, 2003, **5**, 4351.
26 C. Gabriel, D. Lilge and M. O. Kristen, *Macromol. Rapid Commun.*, 2003, **24**, 109.
27 K. Ito, T. Kato and T. Ona, *Vib. Spectrosc.*, 2004, **35**, 159.
28 V. Barone, G. Festa, A. Grandi, N. Rega and N. Sanna, *Chem. Phys. Lett.*, 2004, **388**, 279.
29 V. van Speybroeck and R. J. Meier, *Chem. Soc. Rev.*, 2003, **32**, 151.
30 C. G. Hill, A. M. Hedren, G. E. Myers and J. A. Koutsky, *J. Appl. Polym. Sci.*, 1984, **29**, 2749.
31 J. Vyörykkä, M. Halttunen, H. Iitti, J. Tenhunen, T. Vuorinen and P. Stenius, *Appl. Spectrosc.*, 2002, **56**, 776.
32 N. Failloux, I. Bonnet, M.-H. Baron and E. Perrier, *Appl. Spectrosc.*, 2003, **57**, 1117.
33 T. Nakano, S. Shimdada, R. Saitoh and I. Noda, *Appl. Spectrosc.*, 1993, **47**, 1337.