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132 *Current Analytical Chemistry*, 2014, *10*, 132-139

Infrared Microspectroscopy Applications - Review

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Abstract: One of the most exciting recent developments in infrared spectroscopy has been the coupling of the spectrometer to an infrared microscope. The combination of the new infrared spectrometer and a microscope was a natural thought of scientists in these fields. This development has been so rewarding and so useful in solving today's chemical problems that infrared microspectroscopy has quickly become a significant subclassification of infrared spectroscopy. Infrared microspectroscopy has a much longer history than the recent enthusiasm would imply, however. The great interest in the use of infrared spectroscopy to solve chemical problems that occurred shortly after World War II spread into the medical and biological fields as well as pharmaceutical and forensic sciences. The objective of this article is to review new developments in applications of FTIR microspectroscopy in analysis, covering the period between 2005 and 2013.

Keywords: Microspectroscopy, basic principles, applications.

1. INTRODUCTION

In the recent years the analytical chemistry research was focused on the development of new methods and techniques for the identification and quantification of ever smaller amounts of various materials. General identification of compounds at the picogram level can now be fulfilled through the techniques of molecular microspectroscopy, i.e., molecular spectroscopy through a microscope. Even there are more different molecular spectroscopies; the most used are infrared (IR) and Raman ones.

IR spectroscopy has long been a mainstay of the analytical chemist's tool for identification of molecular systems because of its high selectivity and applicability to essentially all molecules and in all physical states [1-9]. Fourier Transform Infrared (FTIR) spectroscopy represents one of the most powerful tools in the study of different materials, since it is able to investigate simultaneously organic and inorganic compounds. Until the advent of molecular microscopy, IR was generally considered applicable to microgram quantities of substances with use of good sampling technique and special accessories. The development of microscopic IR makes it possible to identify picogram quantities and even to detect femtogram levels for many substances. Historically, FTIR microspectroscopy has been used to investigate organic components in the cross sections with a microscope working in total reflectance or attenuated total reflectance (ATR) modalities, but these methodologies are affected by some disadvantages. First, total reflectance spectra often suffer distortions due to anomalous dispersion and reststrahlen phenomena [10, 11]. Secondly, in order to obtain significant signal intensity, the dimension of the spot investigated must be around 100 μm and the crystal/sample contact surface can easily damage the analysed substrate [10].

The objective of this article is to review new developments in applications of FTIR microspectroscopy in different types of analysis, covering the period between 2005 and 2013. Prior to a review on this subject, it is useful to give a short introduction to the concept of the FTIR microspectroscopy technique, while in the major section quantitative and qualitative determinations will be presented.

2. THEORETICAL ASPECTS

An important aspect in infrared microspectroscopy is the identification of spectral patters, e.g. spectral bands that are characteristics for a constituent [12, 13]. Based on these specific bands the researchers can investigate the distribution of a substance in a mixture, but for extracting the important data a chemometrical analysis of the data must be performed [14].

The IR microscope (shown schematically in Fig. (**1**)) is very similar to the optical microscope [15].

As there is no means to visualize the infrared image abinitio, the sample has to be first visualized by using optical methods. To obtain IR spectra from microscopic regions, apertures are employed first to selectively limit the wide field of view of the optical signal. IR radiation can then only pass through the slits and spectra are acquired by measuring this throughput as a function of interferometer retardation. The dependence of the infrared sampling on the optical image requires that the optical and infrared paths be parfocal

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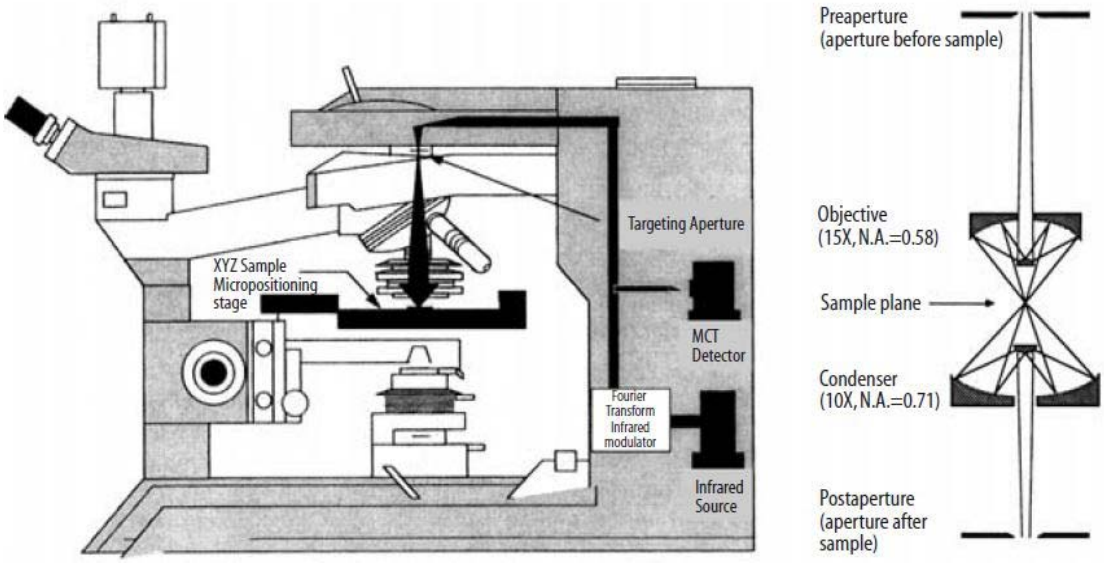

Fig. (1). Schematic diagram of an infrared microscope (left), the details of the focusing optics (right).

and collinear. The major difference between optical microscopes and the one required for IR use is the incorporation of all-reflecting optics to allow maximal IR transmission and the incorporation of aspherical reflecting surfaces in a Cassegrain-type configuration to minimize optical aberrations. Refractive elements, if required for some special needs, are made from IR transmitting materials that are resistant to moisture (e.g. CaF_2). Apertures used are usually coated with highly absorbing carbon black to eliminate stray radiation. Polarizers or any other filters can be inserted into the beam path and polarized infrared measurements can be conducted similar to polarized measurements using visible radiation [16].

Radiation incident on a sample results in radiation interacting with the sample to be transmitted, reflected, refracted, absorbed or emitted. Almost any of these modes [17] (as can be seen in Fig. (**2**)) may be used for examining the absorbance characteristics in a microscopic configuration.

Reflectance spectroscopy is a common place for samples that cannot be prepared for transmittance measurements. However, reflectance measurements must be carefully conducted as the reflected beam is not only indicative of the composition of the sample but is also affected by surface conditions at the sample plane. This makes the reflectance spectra, though indicative of material chemistry, difficult to interpret and generally less useful for quantitative analysis. Since the polarization of the beam is maintained for reflectance, especially specular reflectance methods, examination of orientation at polymer surfaces using reflection techniques is attractive [18]. Reflection-absorption modes involve the transmission of the infrared beam through the sample and subsequent reflection to pass through the sample again. Usu-

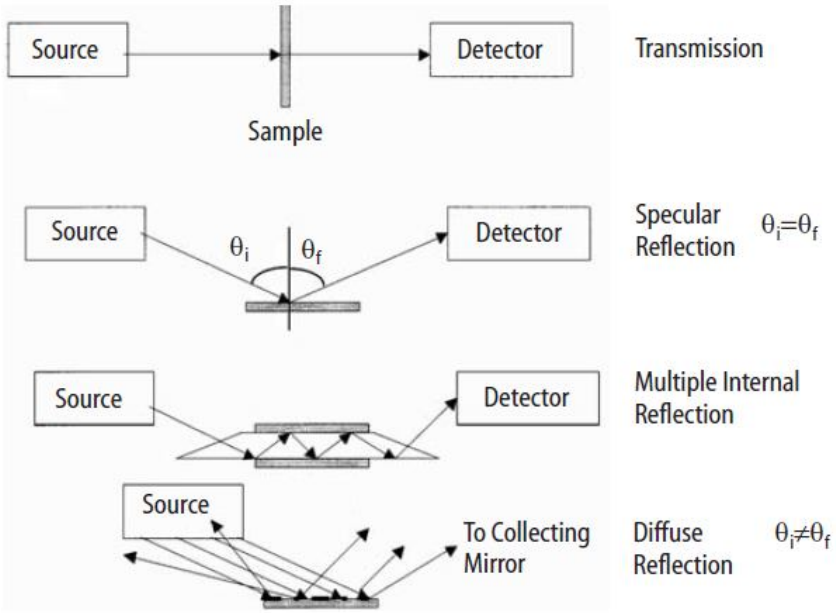

Fig. (2). Major Sampling Techniques for Infrared Spectra Collection in a Microscopic configuration.

ally, sample preparation is difficult for such experiments and they are useful when samples are thin in their native state and/or supported on a reflecting surface.

In the transmission mode the infrared beam passes the sample and is detected behind the sample [19]. Infrared microspectroscopy experiments in the transmission mode provide high data quality without any distortions based on e.g. reflection artifacts. An important requirement for transmission experiments lies in the sample preparation. Scatterings effects should be avoided by polishing the sample (for hard sample like bones) or microtomed (for soft samples like tissues) and/or sandwiched between infrared transparent supports. The sample should be prepared in order to meet the absorption criteria of the Lambert-Beer law. As a rule of thumb, the sample thickness should be sufficiently thin in order to keep absorbance values in the range between 0.4 to 0.8 absorbance units.

3. APPLICATIONS OF INFRARED MICROSPECTROSCOPY

The literature studied shows a great number of papers dedicated to infrared microscopy analysis. Most of the papers are related to quantitative and qualitative assay of a compound, but there are also many papers dedicated to quantitative methods.

The literature studied shows a great number of papers dedicated to pharmaceutical counterfeits drug analysis using different analytical techniques. There are some reviews dedicated to this subject [12, 13, 20-23], that illustrate the impact and promise of this spectroscopy methodology. In these reviews the fundamental principles of the technique in the context of FTIR spectroscopy was presented as well as new applications ranging from the physical chemistry of macromolecular systems to the detection of different compounds (cells and tissue, polymers, historical manuscripts, pharmaceutical drugs, herbal, latent print and trace evidence, pigment analysis, etc.).

Now we will present some of the most important papers related to the infrared microscopy application: pharmaceuticals (drugs and cosmetics), forensic, biomedical and some other different field of interest.

Pharmaceutical applications. When a sample is analyzed by infrared (IR) (MIR or NIR spectroscopy), its homogeneity is an important issue. A spectrometer integrates spatial information. However, the use of a mean spectrum on a surface can be a drawback when analyzing a solid form. For example, in the pharmaceutical industry it is important to map the distribution of active ingredients and excipients in a tablet [24]. The authors conducted a quantitative study of the feasibility of NIRS in predicting coating time and classifying production tablet cores in terms of their dissolution properties. IR multispectral imaging is a recent development that combines the chemical information from spectroscopy with spatial information [25, 26].

Synchrotron IR micro-spectroscopy is an appropriately method that can be used for the analysis of small particles of archaeological cosmetics [27]. The sample originates from old Egyptian cosmetic remains. Five different particles were analyzed and delivered similar findings: the core is fat rich, and surrounded with a mineral phase. In two particles, lead palmitate was clearly identified as a major component. Phosgenite ($Pb_2CO_3Cl_2$), a lead salt synthesized in aqueous solution, was also located in region near the surface of the particles. The discovery of this latter salt demonstrated that the Egyptians mastered some practices of synthesis in aqueous solution, already 33 centuries ago [28].

Near-infrared imaging systems (NIRS) simultaneously record spectral and spatial information. Each measurement generates a data cube containing several thousand spectra, so chemometric methods are required to extract qualitative and quantitative information. Gendrin, Roggo and Collet in their study [29], tried to determine the feasibility of quantifying active pharmaceutical ingredient (API) and excipients content in pharmaceutical formulations using hyperspectral imaging. Two kinds of tablets with a range of API content were analysed: a binary mixture of API and cellulose, and a pharmaceutical formulation with seven different compounds. Best content predictions were achieved using 40 μm/pixel resolution and the PLS2 method with SNV normalized spectra.

By using FT-IR and microRaman spectrometry a large number of black to gray powders have been analysed [30]. The difficulty in analysing archaeological samples is due to their composition which generally involves a mixture of different inorganic and organic materials, some times degraded by the environmental conditions. So it has been possible to understand the different nature and composition of the materials employed in preparation of ancient cosmetic powders, found inside Pompeian unguentaries situated in depository of "Casa Bacco". Dark black powders were essentially composed of amorphous carbon probably due to the carbonization of vegetal or animal tissues. On the basis of the FT-IR and microRaman spectra, it has been possible to identify some specific old cosmetic materials and some binders (ex: wax) used in cosmetic preparations. In the study of ancient black powders, the main result was the identification of the principal component: the amorphous carbon, which, in the case of black eyeliners of Pompeii, has been characterized as fuligo.

Water soluble polymers are often used in tablet compaction for their desirable compaction and dissolution properties [31]. ATR-FTIR spectroscopic imaging has been used to analyze in situ the spatial distribution of different components in tablets with different compositions. Caffeine tablets made of three different polymer matrices, microcrystalline cellulose, hydroxypropyl methylcellulose (HPMC) and lactose, were investigated. It was found that the distribution of caffeine is strongly affected by the composition of polymer matrix used in the tablet.

In the last years the traditional single point spectroscopy (mainly focused on near infrared, Fourier Transform Infrared and Raman Spectroscopy) has been successfully combined with mapping devices that allow obtaining a spectral pattern in each area unit of one surface (commonly denoted as pixel) [32]. The opportunity to obtain quality spectral information of tablets surface in a short period of time allows the direct analysis of the sample without needing further chemical analysis. Analysis of chemical images with mathematical tools makes possible to extract the desirable information

from the raw data [32, 33]. For example, a number of research works have been recently published focusing the interest in the analysis of pharmaceutical mixtures being blended to assure the perfect distribution of all the analytes on the surface of the product tablet [34]. The general scope was to introduce a new approach for better visualization of anomalies in surfaces as well as to study the surface itself, locating different zones, etc. The methodology is based on the mutual correlation between one specific pixel and the rest of the image. Therefore, the methodology can be used as a control technique to detect defects in final tablets and punctual contaminants, being a potential supplementary tool for quality controls. Near-infrared chemical imaging (NIR-CI) is the fusion of near-infrared spectroscopy and image analysis. It can be used to visualize the spatial distribution of the chemical compounds in a sample (providing a chemical image). This method [34] also provides guidance for hyperspectral image (or NIR-CI) analysis describing each of the typical steps involved.

Solid state characterization and physicochemical property of the active pharmaceutical ingredients (APIs) are fundamental elements in the pharmaceutical development from the beginning of drug discovery to the final market products [35, 36]. Metoclopramide HCL, chemically known as 4-amino-5-chloro-2-methoxy-N-[(-2-diethyl-amino)ethyl] benzamide HCL, is commonly used by an oral tablet to treat a short term basis for patients with heartburn, esophagitis, or different symptoms of nausea and vomiting [37]. In conclusion, a continuous process of dehydration, amorphization and recrystallization for the API of MCP H_2O in the solid state was easily determined using a simultaneous DSC-FTIR microspectroscopy. This one-step novel simultaneous DSC-FTIR microspectroscopy might be available for studying the changes in the progressive processes of the thermal-dependent solid-state characterizations of drugs.

Mid-infrared ATR FT-IR microspectroscopy is applied in conjunction with multivariate statistical analysis on petal samples of Petunia hybrida from wild-type and from two transgenic lines in which the PhEXPA1expansin gene expression was down-regulated and up-regulated, respectively [38]. In this work, the authors explored the potentialities of mid-infrared FT-IR microspectroscopy to highlight molecular processes in plant cell walls through an original combination of multivariate data analysis techniques. FT-IR spectroscopy has also been quite extensively used in studies on plant cell walls, which contain a great variety of macromolecular compounds, including cellulose, hemicellulose and pectins [39 - 41].

Illicium verum (Chinese star anise) dried fruit is popularly used as a remedy to treat infant colic. However, instances of life-threatening adverse events in infants have been recorded after use, in some cases due to substitution and/or adulteration of I. verum with I. anisatum (Japanese star anise), which is toxic. It is evident that rapid and efficient quality control methods are very important to prevent re-occurrence of such dire consequences. The potential of short wave infrared (SWIR) hyperspectral imaging and image analysis as a rapid quality control method to distinguish between I. anisatum and I. verum whole dried fruit was investigated [42]. This method has the potential to detect I. anisatum whole dried fruits within large batches of I. Verum through upscaling to a conveyor belt system.

ATR-FTIR spectroscopic imaging method was used also to study the dissolution of delayed release and pH resistant compressed coating pharmaceutical tablets [43]. Tablets with an inner core and outer shell were constructed using a custom designed compaction cell. FTIR imaging produced important information about the physical and chemical processes occurring at the interface between tablet sections during dissolution.

Forensic analysis. Forensic analysis is another field of large applications for infrared microspectroscopy. For this field of applications we can mentione some of the following analysis: paint chips [44, 45], latent fingerprints [46 - 48], autentification of papers [22, 49, 50] and other different materials, such as ink characterization of stamps [51] or artistic prints [52 - 55].

The potential of infrared chemical (hyperspectral) imaging as a technique for the forensic analysis in the automotive paint chips, in particular, and multicomponent samples was proofed [44]. Sample preparation procedures for the infrared analysis of paint chips are detailed, with the recommendation that where mounting resins are chemically incompatible with the sample, it is better to mount and section the sample in a soft wax from which the sections can be removed and pressed into a KBr disk for transmission analysis. Infrared chemical images of multilayered paint chips have been successfully obtained, with the chief advantage over conventional infrared analysis being that thousands of infrared spectra are collected in a few minutes across the whole sample, at a spatial resolution of around 5 μm. In other work [45] it were evaluated the capabilities and limitations of chemometric methods and other mathematical treatments applied on spectroscopic data and more specifically on paint samples. The uniqueness of the spectroscopic data comes from the fact that they are multivariate – a few thousands variables – and highly correlated. Statistical methods are used to study and discriminate samples. In conclusion, the use of chemometrics for the forensic analysis of paints provides a valuable tool for objective decision-making, a reduction of the possible classification errors, and a better efficiency, having robust results with time saving data treatments.

Current latent print and trace evidence collecting technologies are usually invasive and can be destructive to the original deposits. A non-invasive vibrational spectroscopic approach that yields latent fingerprints that are overlaid on top of one another or that may contain trace evidence that needs to be distinguished from the print was described [46]. Because of the variation in the chemical composition distribution within the fingerprint, it was demonstrate by the authors, that linear unmixing applied to the spectral content of the data can be used to provide images that reveal superimposed fingerprints. Synchrotron-infrared microspectroscopy provides a non-destructive technique to investigate the chemical composition of latent fingerprints in situ [47]. The high spatial resolution and brightness of the synchrotron source also lends itself to the chemical characterisation of trace amounts of material on surfaces. Greatest loss of material appeared to occur during the first 3 months following deposition. However, no significant variation in lipid compo-

sition was detected over a 9-month period. Conventional Fourier transform infrared (FT-IR) spectroscopy and microscopy have been widely used in forensic science. New opportunities [48] exist to obtain chemical images and to enhance the spatial resolution using attenuated total reflection (ATR) FT-IR spectroscopy coupled with a focal-plane array (FPA) detector. In this paper, the sensitivity limits of FT-IR imaging using three different ATR crystals (Ge, ZnSe, and diamond) in three different optical arrangements for the detection of model particles were discussed.

The dating of historical manuscripts, which primarily means answering questions of ''is this manuscript authentic?'' and ''what is the true age of the manuscript?'', is frequently required by historians, literature historians, and forensic specialists.

For many centuries paper was the main material for recording cultural achievements all over the world. Paper is mostly made from cellulose with small amounts of organic and inorganic additives, which allow its identification and characterization and may also contribute to its degradation. There are some reviews that describe the application of infrared microscpectroscopy technique for paper characterization and conservation [22, 49]. Identification of thermal sensitive paper is of importance to forensic scientists in the document examination and file source identification. Spectral methods, including *infrared* spectroscopy, Raman spectroscopy, and energy dispersive spectroscopy have been proven to be accurate and rapid in the investigation of numerous compounds in many studies [50].

Fourier transform infrared spectroscopy was applied with good results to the characteization of red inks used to print one-penny stamps in Britain during the period 1841-1880 [51]. Micro- and macro-accessories were compared in transmission and reflection mode. Results were in good agreement with those obtained using scanning electron microscopy.

The presence of original and non-original prints in the art market is a problem, especially for contemporary artists. The characterization and discrimination of these prints is usually based on stylistic criteria and printing characteristics. These informations are sometimes not enough to differentiate between originals and non-originals. A new method [52] was proposed for discrimination by considering additional data, such as the chemical composition of the constituent materials involved in the production of the original series. Results indicate the capability of the approach because, following the procedure, it is possible to distinguish between black pigments attending to the presence of hydroxyapatite, silicate and some other specific bands, whereas black inks can be discriminated by paying attention to the inclusion in their composition of such pigments as bone black, Prussian blue, mars brown or other specific bands. Two methods of analysis of organic components of colour layers of art works have been tested: IR microspectroscopy of indigo, Cu-phthalocyanine, and Prussian blue, and MALDI-TOF-MS of proteinaceous binders and a protein-containing red dye [53]. The IR spectra distortion common for smooth outer surfaces and polished cross sections of colour layer of art works is suppressed by reflectance measurement of microtome slices. The detection limit of the three blue pigments examined is ~0.3 wt% in reference colour layers in linseed oil binder with calcite as extender and lead white as a drying agent. The sensitivity has been sufficient to identify Prussian blue in repaints on a Gothic painting.

In the field of FTIR spectroscopy, the far infrared (FIR) spectral region has been so far less investigated than the mid-infrared (MIR), even though it presents great advantages in the characterization of those inorganic compounds, which are inactive in the MIR, such as some art pigments, corrosion products, etc. In a new paper [54], ATR in the FIR region is proposed as an alternative method to transmission for the analyses of pigments. This methodology was selected in order to reduce the sample amount needed for analysis, which is a must when examining cultural heritage materials. The results showed that the ATR spectra were characterized by differences in both intensity and band shifts towards lower frequencies if compared with those recorded in transmission mode.

Optical microscopy, cross-section and fragment Micro-FTIR *spectroscopic* techniques along with microchemical tests were used for the identification of pigments in two different samples of an icon [55]. Representing the Last Judgement, and painted by the Greek master Ioannis from the village of Kapesovo in the year 1771, the kneeling desk icon under investigation is a noteworthy contribution to the study of materials in post-Byzantine visual arts. The main components found in the ground layer of both samples were gypsum, beeswax and a proteinaceous material. Cinnabar, Prussian blue and cerussite were identified on the paint layers. The binding medium on the paint layers was weddelite. The materials used in the painting and ground layers were characterized in order to clarify the painting technique.

Medical analysis. Relatively young if compared to the age of Infrared (IR) spectroscopy, the field of biological Infrared spectromicroscopy and imaging covers a wide range of fundamental issues and applied studies such as cell imaging or tissue imaging. Nowadays, characteristic biomedical analysis with IR microscopy regards pharmaceuticals, biomembranes, biopolymers, micro-biology, cancer diagnostics, etc…It is noteworthy to remember here that one of the first IR spectroscopy application has a strong biomedical background being devoted to the investigation of the IR absorption of neoplastic and normal tissues in the spectral regions 1–15 μm and 8–15 μm [56]. The biological applications of FTIR spectroscopy started with the analysis of simple biomolecules. In the last decade it was observed a continuous increase in the number of different FTIR microspectroscopy applications, such as: urinary calculi [57-59], and in particular cells and tissues: for prostate [60, 61], cancer [62] or other biological investigations [63, 64].

The qualitative and quantitative analysis of intact urinary calculi (stones) is challenging for several reasons, including the size and fragility of the calculi and the spatial domains of their sedimentary chemistry. Many modern methods of analysis destroy the structure of the calculi when the samples are prepared for introduction into the instrument. Maintaining the structural integrity of the calculi is important for the elucidation of the chemistry of formation and the etiology of the calculi in the urinary system. A facile method of analysis for isolated cross-sectioned urinary calculi was proposed

[57], either unmounted or stabilized in resin. A cross sectioned stone, regardless of thickness, can be analyzed using the reXectance mode of an IR microscope, yielding spectra that are qualitatively accurate and are similar in appearance to DRIFTS spectra. In addition to the qualitative IR microspectroscopic analysis of urinary calculi components, we also present a study of the eVects of surface roughness on IR reflectance spectra. Kidney stones can be a source of considerable morbidity for pregnant women. We performed a multi-institutional study to define the composition of renal calculi diagnosed during pregnancy [58]. Calcium oxalate calculi are the most common stone in non-pregnant women of a comparable age as our subjects. However, our present data suggest that stones detected during pregnancy are most commonly composed of calcium phosphate (hydroxyapatite). Indeed, it is the minority of stones that are composed of calcium oxalate. Although the reason for this unusual preponderance of calcium phosphate calculi is unclear, physiologic alterations that occur during pregnancy may be influential.

Distribution of chemical components in the mixed oxalatic, phosphatic and uratic urinary stones was analysed by means of infrared microspectroscopy [59]. The specular reflection from the surface of the cross-sectioned stones was used for the infrared spectroscopic imaging. It was found that the reflection spectra consist from specular and diffuse reflection components and the former one exhibit Reststrahlen bands which resemble first derivative of the spectral bands in the absorption spectrum.

Vibrational spectroscopy techniques can be applied to identify a susceptibility-to-adenocarcinoma biochemical signature [60]. A sevenfold difference in incidence of prostate adenocarcinoma (CaP) remains apparent amongst populations of low- (e.g. India) compared with high-risk (e.g. UK) regions, with migrant studies implicating environmental and/or lifestyle/dietary causative factors. Spectra were subsequently processed within the biochemical cell region ($1{,}800^{-1}$–500 cm^{-1}) employing principal component analysis (PCA) and linear discriminant analysis (LDA) to determine whether wavenumber–absorbance/intensity relationships might reveal biochemical differences associated with region-specific susceptibility to CaP.

Awareness of the chemical composition of prostatic calculi is of great importance for pathogenesis of prostatic lithiasis, the feasibility of FTIR microspectroscopic mapping system used for rapidly screening and detecting the real composited components of prostatic calculi in a short time was initially evaluated [61].

Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) microspectroscopy was applied for detection of colon cancer according to the spectral features of colon tissues [62]. Major spectral differences were observed in 1,740–900 cm^{-1} spectral region. Several chemometric methods such as analysis of variance (ANOVA), cluster analysis (CA) and linear discriminate analysis (LDA) were applied for classification of IR spectra. Using LDA technique, the spectra were classified into cancer and normal tissue classes with an accuracy of 95.8%.

IR spectroscopy is a powerful technique to investigate biological systems and offers many new imaging opportunities. The field of infrared biological imaging covers a wide range of fundamental issues and applied researches such as cell imaging or tissue imaging [63]. Moreover, changes of the protein structure are detectable in an IR spectrum and cellular molecular markers can be identified and used to recognize a pathological status of a tissue. Molecular structure and functions are strongly correlated and this aspect is particularly relevant for imaging.

Practical applications of microspectroscopic mid-IR and near-IR imaging are presented in a review [64]. The two imaging techniques complement each other having reciprocal strengths and limitations. The mid-IR, although readily interpreted based on six decades of serious well documented classical IR investigations, have the practical limitation of thin specimen path length of 6–8 μm and difficult sample preparation. In contrast, near-IR imaging data is more readily obtained with minimal sample preparation. It provides deeper sample penetration applicable to bulk analysis. However, interpretation usually requires statistical multivariate data treatment because broader overlapping near-IR absorption bands are the norm.

Synchrotron radiation (SR) infrared emission was used, for a deeper understanding, in the field of cultural heritage analytical science [65]. The principal motivation for synchrotron-based IR micro-spectroscopy is to achieve significantly greater lateral resolution (typically close to the diffraction limit) in combination with superior signal-to-noise ratio without resorting to prohibitively long acquisition times. This technique can be applied to nearly all kind of materials found in museum objects, going from hard materials, like metals, to soft materials, like paper, and passing through hybrid materials such as paintings and bones.

The state of conservation/degradation of mummy human remains can be inferred from their biochemical composition, as well as the secondary structures of proteins. Infrared microscopy is a very appropriate technique for addressing such issues [66]. The external layer of mummy skin appears to be more preserved as evidenced by the presence of characteristic biological vibrational features (in particular amide bands) in spectra collected within this region. Adipocere and calcium oxalate are the main degradation products identified in the mummy skin.

An analytical approach based on X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and gas chromatography/mass spectrometry (GC/MS) was used to characterize Egyptian embalming materials from the University of Florence's (Italy) Natural History Museum [67]. FTIR was used as a fast fingerprinting tool due to its ability to characterize inorganic compounds and to highlight different classes of organic materials. The results show that the embalming materials consisted of complex mixtures of natural products as well as transformation products due both to natural ageing and human interventions. FTIR analyses, along with evidence of organic materials, enabled the authors to identify the presence of calcium oxalates, phosphates and carbonates, while XRD patterns confirmed the crystalline nature of both phosphates and oxalates. The combination of results allowed the identification of the organic and inorganic composition of the residue of embalming material sampled from the skulls to be achieved and also permitted to

exclude the presence of possible products used during past restoration cure.

4. CONCLUSION

Throughout this paper we have attempted to demonstrate the broad applicability and versatility of IR microspectroscopy in many disciplines and over enormous variations in physical scale. It is a powerful tool for chemical analysis because it simultaneously reveals the type, distribution, and relative abundances of chemical components within a particular system.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

Declared none.

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Received: April 04, 2012 Revised: May 20, 2012 Accepted: May 25, 2012