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Biomedical applications of FTIR and Raman spectroscopy

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The ability to retain spatial information and generate and manipulate two-dimensional (2D) and three-dimensional (3D) molecular images from the vast amounts of spectroscopic data produced in rapid time has enormous application in many fields, such as pathology and materials science. It is of particular relevance to cancer diagnosis where it is imperative to know the degree and penetration of tumour cells in order to determine surgical boundaries. We have developed a number of techniques for generating 3D FTIR images of multiple tissue sections by applying unsupervised hierarchical cluster analysis to the entire data set and generating 3D false colour maps based on spectral variance.[1] More recently we have applied neural networks to generate FTIR images of tissue sections.[2] Artificial neural networks have a number of advantages compared to multivariate methods. These include computational efficiency and the fact that these methods can model for non-linear relationships. More recently they have modified the Rmie scattering algorithm, which hitherto has only been applied to single spectra [3], to correct for dispersion in spectral image data.

High-speed low-cost computers, in combination with infrared imaging instruments based on Focal Plane Array (FPA) detectors, allow the image acquisition and reconstruction to be achieved within a reasonable time frame. Raman confocal microspectroscopy in biomedical research applications is also proving to be another important emerging molecular spectroscopic imaging technique because of its ability to optically section cells and tissues using Raman back scattered light. The presentation will cover a number of applications of FTIR and Raman confocal microscopy to disease diagnosis with particular relevance to imaging applications.

References

FTIR Spectroscopy Discriminates Early Differentiation Stages In Living Human Stem cells

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Human cells derived from stem cells destined for use in the clinic for regenerative medicine therapies will first need to be differentiated to specific differentiation or lineage commitment states for these treatments to be effective. Conversely, the transplantation of undifferentiated stem cells into the human body presents a danger as it may lead to the formation of cancerous tumours. In this context, we have been investigating using FTIR spectroscopy to define lineage commitment in human stem cells, and envisage spectroscopic approaches may be useful for quality control and selection of differentiated stem cells for use in the clinic. Recently, we have been successful in using FTIR spectroscopy to discriminate living, undifferentiated human embryonic stem cells from those differentiated to commitment stages equivalent to the earliest phases of embryonic development. These measurements used a specialised IR wet chamber and were acquired using the IR microspectroscopy beamline at the Australian Synchrotron [1]. Apart for the obvious practical need to analyse live cells in line with the aim to develop a new modality for cell selection for clinical practice, measurements using live cells had advantages compared to those from dried, fixed cells. Significantly, in terms of detecting changes in differentiation state, bands from DNA and RNA were observed in the FTIR spectra of live cells that were not detected at all or were much less prominent in the FTIR spectra from the dried cells [2]. Indeed, changes in bands assigned to nucleic acids, including the carbonyl stretching band from DNA at ~1720 cm\(^{-1}\), the anti-symmetric phosphodiester stretching band from DNA and RNA at ~1220 cm\(^{-1}\), and stretching bands from C-O groups in DNA and RNA sugars at ~1120 and 1050 cm\(^{-1}\), associated with the differentiation of cells from the stem cell progenitors, were observed in average spectra and loaded prominently in Partial Least Squares Discriminant Analysis (PLS-DA) models used to classify the spectra. Prominent changes in lipid absorbance were also observed as the live stem cells underwent differentiation, matching changes previously observed in the spectra of dried cells [3,4]. We will discuss these findings and the potential for FTIR spectroscopy as a quality control tool for cell selection in regenerative medicine practice.

References


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Recent developments in FTIR spectroscopic imaging

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FTIR spectroscopic imaging has emerged as a powerful tool for characterisation of materials and biomedical samples. An FTIR image can be acquired mainly in three different configurations: transmission, reflection or attenuated total reflection.[1]

This talk will first outline the research we are developing in this area with focus on ATR (Attenuated Total Reflection)-FTIR spectroscopic imaging.[2] Chemical visualisation with enhanced spatial resolution in micro ATR imaging mode broadens the range of samples (cross-sections of blood vessels or hair, surface of skin, single cells, etc.) amenable to study with FTIR imaging which were previously ruled out by the inadequate spatial resolution. Recent developments in macro ATR imaging with the use of inverted prism crystals show good potential with applications to protein crystallization,[3,4] imaging of flows in microfluidics and imaging of live cancer cells.[2] The opportunity to obtain chemical images of the same sample from different depths through the use of macro ATR-FTIR imaging with variable angles of incidence has also been demonstrated. The ability to make spatially resolved chemical snapshots as a function of time is the basis of dynamic chemical imaging offered by macro ATR imaging approach.[5]

While the micro ATR imaging approach provides the highest spatial resolution achieved with FTIR spectroscopic imaging, transmission mode still remains as the most common sampling method for imaging of biomedical samples, such as tissues and cells. However, when samples are sandwiched between infrared windows or placed underneath a layer of liquid for imaging in transmission, dispersion and refraction of infrared light occurs which result in different focal lengths for the different wavenumbers of the infrared light (chromatic aberration). We have recently introduced a “pseudo-hemisphere” approach which was applied to FTIR imaging in transmission mode, and demonstrated increased spatial resolution along with the removal of chromatic aberration and a reduction in scattering.[6] The further development of this powerful approach by using “pseudo-sphere” is beneficial for FTIR spectroscopic imaging in transmission for the study of live cells in aqueous solutions in microfabricated devices.[7] This approach is significant as spectroscopic imaging of live cells was achieved without the recourse to a synchrotron source of infrared radiation and that FTIR images of live cells have been measured in microfluidics in aqueous solutions and in droplets.

References


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A potential of FTIR spectroscopy in studies on blood components

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FTIR spectroscopy is a powerful tool which can be applied to investigate different biological systems (e.g., tissue, cells, body fluids) with their potential application in clinical practice. Its role in the diagnostic aspects involving body fluids is gaining importance in the last few years. Especially, blood and its components are ideal candidates for a screening test as they can easily, inexpensively and rapidly be isolated from the body. On the contrary to standard clinical chemistry tests, FTIR technique offers the free-reagent and non-destructive method of measurements providing the possibility of multi-component analysis. The aim of this presentation is to demonstrate advantages and limitations of various techniques of FTIR spectroscopy in searching spectral markers of a disease state such as diabetes, hypertension, arteriosclerosis and cancer metastasis. Blood samples were taken from animal models. In order to achieve health status prediction, it is also important to study and understand the blood components in terms of biomolecular changes using FTIR. Here, IR spectral signature of the major blood components isolated from controls are presented, including full blood, platelet–poor and –rich plasma, and WBC/RBC, to show their spectral similarities and differences. In addition, we present an effect of the anticoagulant on IR spectral profile of blood plasma. Although, it is well known that various types of anticoagulant influence on plasma biochemistry in clinical chemistry analysis, the evaluation of such an effect on IR spectrum has not been reported so far. In our work, we studied various types of heparins, citrate, EDTA, and aspisol that work by the binding of calcium ions or by the inhibition of thrombin.

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O5 DNA conformation and quantification using Fourier transform infrared spectroscopy

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Fourier transform infrared (FTIR) spectroscopy has often been described as having great potential in disease detection, monitoring and understanding. However, previous research using FTIR spectroscopy to investigate relevant biological samples has been reportedly hindered by non-Beer-Lambert interactions between infrared light and cell and tissue specimens. An example of this is the ‘Dark DNA’ effect [1]: a hypothesis that stemmed from the observation of absent or diminished DNA absorption peaks in cell spectra. This artifact was previously considered a non-Beer-Lambert absorption and was explained as a consequence of the infrared opacity of genomic DNA because of its high level of compaction. We have reconsidered this effect and demonstrated that it is not due to the condensation of DNA but rather the DNA conformation [2]. The conformation of the DNA is dependent on the hydration state of the sample [3]. Most previous FTIR research on cells has been conducted on dehydrated/fixed samples because of the ease with which such samples can be stored and their spectra acquired. We have shown that by working with fully hydrated samples the DNA is kept in its native B-form. By dehydrating these samples we have observed a transition to the A-form. If the sample is unfixed, the native B-DNA can be recovered by rehydration. Most recently we have demonstrated this reversible B-A-B DNA transition in live bacteria cells, demonstrating the biological significance of the A-DNA form. A bacterium undergoes the same B-A-B conformational change but unlike a eukaryotic cell is survives to undergo fission.

By systematically identifying the characteristic B- and A-DNA absorptions we have also demonstrated that the absorption of DNA in cells is Beer-Lambert in nature and that the two different conformations display different molar absorption coefficients. This results in changes in band intensities upon changes to the hydration state of the sample. To further demonstrate the Beer-Lambert nature of these absorptions spectra of hydrated and dehydrated avian erythrocytes and extracted nuclei were collected of both single cells and populations. By applying Beer-Lambert’s Law, the weight percentage of nucleic acid form these samples was determined successfully (average experimental: 44.2±6.6% in nuclei, 12.8±4.3% in erythrocytes, actual: 44.3% in nuclei, 12.5% in erythrocytes) [3].

We have also used the DNA absorptions observed more clearly in the FTIR spectra of single hydrated cells to differentiate successfully between cells at different stages in the cell cycle. Using the multivariate statistical analysis Principal Component Analysis (PCA) cells spaced at two-hour intervals throughout the G1, S and G2 phases of the cell cycle were clustered successfully from triplicate trials. It is anticipated that the ability to consider DNA absorptions in FTIR spectra quantitatively and with such high sensitivity will lead to more straightforward chemometric analysis of samples for detection of changes in cell proliferation rate as well as aneuploidy. This is turn will allow for detection of cancerous and precancerous cells.

References


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Applications of chemometrics to spectroscopic data

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Chemometrics is a multivariate technique for analysing variance and correlation within large data matrices. There are three main chemometric approaches that can be applied to data analysis namely exploratory, regression and classification. The most commonly used exploratory method is Principal Component Analysis (PCA). PCA is designed to reduce large complex data sets into a series of optimized orthogonally related components of interpretable size. Each PC is a linear combination of the original variables projected on a new set of axis that has been rotated in multivariate space so that each successive PC is orthogonal to previous. The first PC accounts for the majority of variance in the data set; the second PC, which is orthogonal to the first, accounts for the second most variance in the data set and so on. The lower PCs (usually below PC 5 for spectroscopic data) usually account for noise only. PCA enables the generation of scores plots in 1, 2 or 3-dimensions, which can be used to look for clusters and relationships in a data set (s). The PCA loadings plots can then be used to determine what bands, in the case of spectroscopic data, are responsible for the clustering and show what bands are correlated and inversely correlated with one another. The regression techniques include Partial Least Squares regression (PLS) and Principal Component Regression (PCR). These tools use inexpensive variables e.g. spectra to predict expensive variables e.g. crystallographic data. Chemometric regression is extensively used in making decisions relating for quantification from a known set of standards. The techniques can also be used for simple “yes/no” decision making and are very useful in the pathological setting as will be demonstrated using FTIR and Raman spectroscopy applied to malaria diagnosis.

Classification techniques such as K-nearest neighbors (k-NN), Soft independent modeling by Class Analogy (SIMCA) and Unsupervised Hierarchical Cluster Analysis (UHCA) use predefined categories for samples and are used for predicting an unknown sample as belonging to one of several distinct groups. In this context a classification model is used to predict a sample's class based on its closest neighbors. The presentation will focus on the different chemometric techniques using spectroscopic data form a number of biospectroscopic applications.

Fig 1. Factor analysis performed on synchrotron FTIR mapping data of a MII mouse oocyte showing lipid deposits In blue.
Non-thermal effects of exposure to Near Infrared radiation in biological structures

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Introduction

At present, Near Infrared (NIR) radiation is widely used in biomedical engineering and medicine. The effect of NIR exposure on human body is the result of interactions at the cellular level. Light from this region can penetrate relatively deep into biological soft tissues and be easily absorbed by tissue components, like water, glucose, hemoglobin, amino acids, proteins, melanin, lipids and DNA \cite{1,2}. IR radiation absorbed by the biological structure is used to initiate photothermal and photochemical reactions. Understanding of these processes can serve better its use in medical therapy. Light irradiation from NIR region is able to induce non-thermal and nondestructive photobiological processes in tissues. This phenomenon has been used, \textit{inter alia}, in light therapy for faster healing of wounds \cite{3}. Despite their importance, there is little studies to attempt of explain the processes occuring at the molecular level. The primary mechanism of NIR action on living organisms has not yet been determined in details.

1. Results

In the present work, ATR-FTIR spectral investigations of the NIR influence on the example phenylalanine (Phe) amino acid were carried out. The changes in the spectral characteristics of the Phe exposed to a low-intensity laser radiation were analyzed using the NIR (range 700–2000 nm) absorption spectra. Spectral changes indicate decrease of energy of hydrogen bonds between water and polar groups of the amino acids (weakening of hydrogen bonds). Dehydration of amino acid surfaces enhances the hydrophobic interactions. The side chains (phenyl rings) can easily aggregate \cite{3}–\cite{5}. Aggregation of weakly soluble molecules in solution is known to lead to $pK_a$ shifts. Therefore, as a result $pK_a$ values of L-phe amino acid are shifted ($\Delta \text{INIR} (t) = +0.48$, $\Delta \text{NIR} (t) = -0.62$) \cite{5}. Process of dehydration under NIR exposure was confirmed on other amino acids and exampled proteins as well. Therefore, NIR radiation was used also to study other amino acids molecules (i.e. glycine, alanine), macromolecules (i.e. albumin, fibrinogen) and bigger structures such as blood and their components (i.e. plasma, erythrocytes).

2. Conclusion

The response of biological structures to the action of NIR range can give important information on the mechanism of interaction of NIR radiation with a living organism.

References

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**08 Raman and AFM imaging of biological samples in sub-micro scale**

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Due to its sub-micro lateral resolution, Raman imaging is an effective tool to track subtle biochemical alterations related to pathological changes in tissues and cells. AFM is a complementary method of surface analysis providing information about topography or mechanical properties of the sample, such as stiffness or adhesion. Additionally, Raman depth profiling enables collecting data from consecutive layers of the studied object being a basis for a reconstruction of 3D micro-images of the sample. Examples of Raman 3D imaging combined with AFM to analysis of biochemical changes in the tissues *ex vivo* are presented in this work.

![Raman and AFM images](image)

**Fig 1.** Raman distribution image of lipids in the vascular wall of db/db mice model of diabetes (right, 15x15 µm) and complementary AFM topography image from the same sample (left, 20x20 µm). Yellow dots in the Raman image denote lipid rafts.

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Monitoring UV induced damage in single cells using SR-FTIR and 3D confocal Raman imaging supplemented with multivariate statistical analysis

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Excessive exposure to ultraviolet (UV) radiation from the sun leads to DNA damage, which can result in skin cancer. DNA damage of cells caused by UV B radiation results in pyrimidine dimer formation and generates photoproducts, both of which distort the double-helical structure of DNA.\textsuperscript{[1]}

Live melanocytes and extracted nuclei were irradiated by using a combination of UV-A and UV-B light, to mimic sunlight and analyzed with synchrotron Radiation Fourier Transform Infrared (SR-FTIR) and Raman confocal imaging spectroscopy.

SR-FTIR in combination with Principal Component Analysis (PCA) was applied to investigate the influence of sunlight damage on a population of melanocytes and their extracted nuclei. The IR microspectroscopy beamline at the Australian Synchrotron was used to collect spectra of single living cells and single extracted cellular nuclei. DNA conformational changes were observed in cells exposed to radiation, as evidenced by a shift in the DNA asymmetric phosphodiester vibration from 1232 cm\(^{-1}\) to 1239 cm\(^{-1}\) in the case of the exposed cells.

Figure 1A shows a 3D scores plot (PC1 vs. PC2 vs. PC3), which clearly shows distinct clustering of cell spectra in response to radiation dose. The loading factors responsible for the separation will be discussed in terms of the chemical changes associated with UV exposure.

3D Raman confocal imaging in combination with k-means cluster analysis was applied to study the effect of radiation exposure on cellular nuclei. The chemical changes detected in cellular nuclei in response to UV radiation exposure occurring in the lipid membrane and cytoplasm will also be discussed. The 3D Raman imaging of a melanocyte nucleus generated by plotting the sum counts in spectral range 2500 cm\(^{-1}\) - 2900 cm\(^{-1}\) is presented in Figure 1B. This plot also proves that collecting just single spectra of cellular organelles such as nuclei which might be very complex could lead to unreliable results. To achieve comprehensive information on the sample, the “point by point” analysis at the entire structure is required.

![Figure 1A: 3D scores plot](image1.png)  
![Figure 1B: 3D Raman imaging](image2.png)

Reference


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**O10** FTIR spectroscopy and microscopy of phytopathogenic fungi and wood material

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Numerous studies demonstrate the practical use of Fourier transform infrared (FTIR) and micro-IR spectroscopy for the classification of bacteria. In this presentation, examples for the discrimination of fungal strains by FTIR spectroscopy of mycelium will be given (Naumann 2009). Fungal cultures were grown in pure culture on solid medium. The huge potential variability of the chemical composition within fungal mycelium poses a challenge for classification. Next to discrimination of fungal species, the discrimination of fungal mycelium from host plant material is desirable. Visualisation of the mycelium distribution by FTIR imaging within plant material was possible (Naumann et al. 2005, Figure 1). Also changes of the chemical composition during fungal development and due to fungal decay of wood material can be monitored by FTIR spectroscopy (Naumann et al. 2012).

![Figure 1](image.png)  
**Fig. 1.** Light microscopic image of fungal mycelium in a beech wood section (A) and cluster analysis of FTIR spectra from a Focal Plane Array (FPA) dataset in the range 1188 to 922 cm\(^{-1}\) (B).

**References**


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Synchrotron X-ray and IR microbeam imaging in the research under epilepsy pathogenesis in electrical kindling rat model

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In recent decades, animal models of epileptogenesis have greatly enhanced our understanding of the processes leading to epilepsy and thus of potential targets for antiepileptogenic therapies. Many animal models of epilepsy are used in scientific research but only two groups of them are recommended through the National Institute of Neurological Disorders and Stroke. These are kindling and post status epilepticus models of temporal lobe epilepsy [1].

Our previous research used synchrotron radiation based techniques such as X-ray fluorescence microscopy and FTIR microspectroscopy showed that excitotoxicity, mossy fibers sprouting and affected creatine kinase activity may be responsible for neurodegenerative changes and spontaneous epileptic activity in pilocarpine model of temporal lobe epilepsy [2–4].

In the present study, for the first time, the results of elemental and biochemical investigation carried out for the other animal model of epilepsy will be presented. The subject of the examination will be the rat model of electrical kindling in which electrical excitatory initially induces subconvulsive or partial seizures but its multiple repetition results in progressive increase in the severity and duration of the seizures.

The topographic and quantitative elemental data obtained using X-ray fluorescence microscopy at the HASYLAB beamline L will be compared with the results of biochemical study done using FTIR microspectroscopy at SOLEIL beamline SMIS as well as data describing behavior of animals subjected to repetitive electrical stimulation.

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Combining vibrational spectroscopy with matrix isolation: A look into frosty molecules

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The matrix isolation method – using a cold inert environment to trap the studied system – was originally developed to freeze and isolate reactive species. Fortunately, and rather quickly, the method found its way into more diverse areas of scientific inquiry ranging from small atoms to molecular interactions and seemingly controlled chemical reactions. Today, the method of using cold solid environment to isolate the studied system is one of many approaches to prepare sample for elaborate investigations [1]. Thus, it is no surprise that vibrational spectroscopy has been connected to matrix isolation technique since the dawn of studies of frosty molecules [2].

The carboxylic acids are a notable group of molecules with functionality and interactivity that makes them important and ample in biological processes and systems. Moreover, to understand the chemistry these molecules engage into has prompted a plethora of research employing vibrational spectroscopy combined with matrix isolation methods all in order to understand the subtleties of the chemical properties of these molecules. Such studies involve structural characterizations [3] and isomerisation processes [4,5], molecular interactions [6-8], and photochemical processes [9,10].

Here, over 10-year-long history of research in low temperature matrices employing both IR and Raman spectroscopy is presented. The target is to give an insight into the chemical diversity of small carboxylic acids.

References:

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Correlation and identification analysis of Raman spectra of solid amino acids

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The simplest method of spectral analysis is peak identification directly from a graph. We identify peaks by taking their positions and intensities and checking in a related database if they fit to some peaks of the reference spectrum of a substance. If the number of substances in the database increases, we take into account more peaks to make the method more specific. The next step is to consider spectra as continuous functions and look in the database for a spectrum most similar to the analyzed one. As a standard measure of comparison for this task, one can choose the Pearson correlation coefficient, the Euclidean distance or the angle (dot product) between the spectra. It is obvious that this kind of analysis is well suited for computers, because doing it manually can be very tedious, especially when the number of substances in a database, together with the number of peaks to be considered, is growing. As a consequence, it is of no surprise that computers have been used in this domain for a long time. For instance, in [1] the authors identified single IR spectra by the Pearson correlation coefficient. They varied the wavenumber range and the number of data points to find the optimum values for this task. They also checked the stability of the method by shifting the wavenumber scale and by perturbing the intensities. These steps are necessary to account for the finite accuracy and reproducibility of the spectrometer, as well as for a possible presence of impurities in the sample.

In case of the spectrum of a mixture the identification is more complicated, even for non-interacting components, because in this simplest, ideal case the intensities are sums of component intensities. One may hope that for some spectral regions the intensities are due mostly to one component, i.e., this component is dominant in the mixture spectrum for a particular spectral interval. In case of treating spectra as continuous functions, one must define the width of a wavenumber interval to consider. The problem of appropriate interval choice for matching spectra with a correlation function was addressed in [2]. The authors presented a detailed analysis of simulated spectra. They varied peak positions and intensities and concluded that similarity functions, such as correlation coefficient and Euclidean cosine, together with their first derivative variations should be used in windows where their values are more pronounced, which means that the differences in spectra will be highlighted.

We present three variants of correlation analysis. The first is based on considering the number of common positions of some number of strongest peaks for two compared spectra, the second is based on considering relations of peak intensities and the third treats spectra as continuous functions with Pearson correlation coefficient as a measure of similarity. This analysis can be viewed as the first step towards identification of components in a non-interacting mixture by template spectra of single components and measured mixture spectrum. We present two methods of identification by reconstructing the measured mixture spectrum as a linear combination of spectra of single amino acids, and comparing the reconstructed and measured spectra with Pearson correlation coefficient and Canberra distance [3], respectively. The amino acids in our database are 20 most common ones coded by living organisms. We show some features of the spectra of these amino acids important for identification. Both methods were tested on 20 mixtures of 1 up to 8 components. We show the results of the tests.

References


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Statistics of single molecule SERRS spectra of porphycene

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The number of applications of Surface-Enhanced Raman Spectroscopy (SERS) is increasing rapidly owing to its high, even single-molecule sensitivity. The SERS spectra allow identification of compounds based on vibrational information they contain. Moreover, they are sensitive to conformation, orientation of molecule, isotopic substitution, substrate and excitation laser line used. Unfortunately, there are still problems with reproducibility, which may result from different hot spots structures and orientations of molecules.

So far, we succeeded in obtaining single-molecule spectra of porphycene, which was supported by several observations: blinking and bleaching of the signal, and its characteristic time evolution. Furthermore, the bi-analyte technique [1], in which we mapped 1:1 mixture of two porphycene isotopologues, gave us undeniable certainty of observing single molecules.

Since the spectra recorded on a single-molecule level occur from few or even one molecule, they possess much more information than spectra averaged over millions of molecules. We did some statistics on the recorded spectra, which included comparison between spectra of different porphycene molecules. The investigation of correlations between intensities of some bands may provide information on surface plasmon resonance in the hot spot and on the orientation of molecule. Moreover, the investigation of spectra time evolution may provide us with information on the behavior of a molecule in a hot spot.

Providing enormous enhancement factor, porphycene may serve as a probe in evaluation of the SERS-activity of a substrate. The statistical analysis may play an important role in getting knowledge about molecule itself and the substrate used.

References


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Chiral nature of selected bicyclic monoterpenes

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Introduction

Terpenes are hydrocarbons resulting from the combination of several isoprene (2-methyl-1,3-butadiene) building blocks. The modification of their structure by oxidation or rearrangement of the carbon skeleton leads to terpenoids, but some authors use the term terpene and terpenoids interchangeably.

Terpenes are a large and diverse class of natural products, produced by varied plants, microorganisms, insects and animals. They are very interesting from the pharmacological point of view because of their various and valuable properties like antifungal, antibacterial, antioxidant, anti-inflammatory, anticancer or anti-spasmodic [1,2].

Heading

In this work we provide solid bases for the understanding of the molecular structure of enantiomers of three bicyclic monoterpenes: bornyl acetate, carene and fenchone and their in situ detection in pichtae, pine and fennel essential oils.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{structures.png}
\caption{The structures of bornyl acetate (A), carene (B) and fenchone (C).}
\end{figure}

The chiral nature of selected terpenes was studied by using Raman optical activity (ROA) technique and DFT calculations. These investigations allowed us to provide reliable determination of absolute configuration together with conformational details of molecules of bornyl acetate, carene and fenchone. Additionally, insightful assignment of the experimental Raman and ROA spectra of abovementioned terpenes was crucial in detection of their specific enantiomer present in the essential oils samples.

Acknowledgment

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References


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Substituent effect in VCD Spectra

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Vibrational Circular Dichroism spectroscopy is gaining more and more popularity and significance in chemistry, especially in pharmaceutical industry. The advent of commercially available spectrometers made it possible to use VCD measurements as a routine tool in absolute configuration assignment, assessing enantiomeric purity or monitoring of chemical reactions. There are however still some untackled basic problems requiring deeper or pioneering exploration.

For example, a careful literature query allows to find only single and superficial attempts at characterisation of substituent effect on spectra parameters - an issue well-known, understood and thoroughly explored for other spectroscopic methods like IR or NMR.

In our research we try to uncover the possible impact of ring-substitution on VCD spectra of chiral compounds. To this end, we use the sEDA and pEDA substituent effect descriptors\textsuperscript{1,3} developed previously and theoretical calculations of the VCD spectra. Several model compounds chosen for the analysis are chiral mono-substituted 1-phenyloethanols and 2-benzo-3-fluorofuran-1-ones (3-fluorophthalides). The optimization, conformational analysis and spectra calculations are performed at B3LYP/aug-cc-pvdz in gas phase. The calculated VCD intensities of several modes are then correlated with the proposed descriptors.

No meaningful correlations could be found for Boltzmann-averaged intensities in 1-phenyloethanols, however inspecting conformer series in separation, we discovered that certain positions of the ring which allow overlapping of benzene π orbitals with free electron pair of –OH group can give rise to substituent effect. For example, excellent correlations of intensities and pEDA descriptor are found for stretching C-H and O-H in chiral center.

References

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**017 Raman Optical Activity analysis of cinchona alkaloids**

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**Introduction**

Cinchona alkaloids, i.e. quinine, quinidine, cinchonine, and cinchonidine (Fig. 1), are known as antimalarial compounds and probably have been used for medical purpose since the 5th century. They occur naturally in the bark of the Cinchona tree and other related species [1]. Quinine, the active ingredient of antimalarial pharmaceuticals, was isolated in the 19th century and was the first drug produced on the industrial scale. Moreover, quinidine is an antiarrhythmic drug and shows antimuscarinic and alpha-adrenoceptor blocking properties. All cinchona alkaloids are also used in asymmetric synthesis in organic chemistry.

![Fig. 1. Structures of cinchona alkaloids.](image)

**ROA analysis of cinchona alkaloids**

Cinchona alkaloids have been already studied by chromatographic and fluorescence methods due to their bioactive properties and applications in medicine [2]. However, only a few reports can be found concerning vibrational spectroscopy, and in particular optical activity, of these alkaloids [3]. In our study, we demonstrate Raman Optical Activity (ROA) spectra of aqueous solutions (pH=1) of cinchona alkaloids compared with Raman spectra collected with excitation at 512 and 1064 nm (for aqueous solution and the solid state) to show the influence of nitrogen protonation on spectral properties of alkaloids. ROA spectra are used for an identification of the individual isomers based on the characteristic bands. Additionally, theoretical calculations were performed to interpret experimental frequencies and to explain the spectral ROA pattern.

**Acknowledgements**

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**References**


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**P1** CNTs coatings for medical therapy and diagnostic

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Carbon nanotubes (CNTs) are gaining increasing attention for biomedical applications due to their unique physical, chemical and biological properties. These nanometric carbon materials are becoming more and more popular in many fields of applications, including medicine. Carbon nanotubes were found to be a useful material for the construction of biosensors, neural electrodes, nano-drug carriers and as a modifier of scaffolds for regenerative medicine (1,2,3,4).

The aim of this work is the manufacture of CNTs coatings on titanium surface. The nanotubes were deposited on the metal surface by means of electrophoretic deposition (EPD). This method provides significant possibilities to control parameters responsible for chemical and structural properties of the obtained CNTs coatings. The two kinds of CNTs layers manufactured under different conditions of EPD process were characterized using SEM microscopy, contact angle wettability, Raman spectroscopy and biological tests in in vitro condition.

Our research shows that a crucial parameter for medical applications is the degree of crystallinity of carbon. Raman spectroscopy enables to determine precisely the content of graphite-like and amorphous phases. The intensity of G peak (graphite) indicates the level of crystallinity, i.e., broad range of ordering in a material, whereas the D band (disordered) is connected with carbon phase of a low level of ordering. Our results indicate that the EPD method is very useful for obtaining a carbon layer displaying physical and biological characteristics suitable for medical applications.

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Application of Raman and FTIR spectroscopy in discrimination of biological material

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Introduction

Rapid detection and discrimination of dangerous biological materials such as bacteria and their spores has become a security aim of considerable importance. Various analytical methods, including FTIR and Raman spectroscopy combined with statistical analysis have been used to identify biological specimens. The present work discusses the application of different spectroscopy techniques to the discrimination of biological materials.

1. Material and methods.

1.1 Methods

- FTIR spectra were obtained using Perkin-Elmer Spectrum GX Optica FTIR spectrometer. Measurement range was 4000 - 650 cm⁻¹ (2.5 μm – 12.5 μm) with 4 cm⁻¹ resolution. Three methods of measurement were applied: HATR, KBr, DRIFT.
- Raman spectra were obtained using Confocal micro-Raman system (Renishaw): equipped with lasers He-Ne (633 nm), diode (785 nm,) objectives 10x, 20x LWD, 50x, 100x, notch filter, CCD detector.
- For constructing of PCA model the software SIMCA-P 11 (UMETRICS) was used. HCA analyses were performed using the STATISTICA 7 software by StatSoft.

1.2 Material

Examined materials: amino acids, proteins, pollens, fungi, bacteria (spores and vegetative).

It has been demonstrated that each kind of biological material have unique infrared and Raman signatures in the spectrum. It can be used to identify the kinds of materials and to distinguish biological specimens from background interferants. Chemometric techniques (HCA and PCA) has been used to confirm the ability to reliable discrimination of different biological materials.

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Raman microimaging of chemical components co-occurring with calcific deposits in stenotic aortic valves

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Aortic valve stenosis is the third of the most common heart disease in Europe and North America, after the arterial hypertension and the myocardial ischaemia [1]. Recent studies have revealed some similarities with atherosclerosis, including mechanisms and risk factors. Ones of the most essential factors are those connected with higher concentration of lipids, especially hyperlipidemia and hypercholesterolemia [2]. Calcific process of valve leaflets is also known to have many features similar to bone formation that is manifested by expression of osteopontin [3].

Raman microimaging was used to investigate occurrence and distribution of various lipids, such as fatty acids, cholesterol and cholesterol esters, in close vicinity of calcium phosphate salts in stenotic human valves. Fig. 1 presents Raman distribution images illustrating spatial colocalization of cholesterol and hydroxyapatite in a stenotic valve.

Fig. 1. Raman distribution images obtained by integration of characteristic bands for cholesterol at ca. 702 cm⁻¹ (left) and for hydroxyapatite at ca. 965 cm⁻¹ (right), respectively. Yellow color denotes high concentration of a studied component.

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References


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**Application of SR-FTIR microspectroscopy for the preliminary biochemical study of the adrenal gland tumor**

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**Introduction**

The adrenal glands are the endocrine organs located at the bottom pole of each kidney. Anatomically they are divided into the adrenal cortex (outer part) and the medulla (inner part). Both of them differ each structurally and functionally. Hence the studied tumor tissues were supposed to differ biochemically depends on the region of gland from which they originated. Investigations which were done concern two types of tumors: adrenal cortical adenoma - derives from adrenal cortex, and pheochromocytoma - comes from chromaffin cells of the adrenal medulla.

The presented studies were performed to investigate the possibility of Synchrotron Radiation Fourier Transform Infrared (SR-FTIR) microspectroscopy application for the analysis of adrenal gland tumors’ biomolecular composition. Due to high resolution, this method gives the chance of insight to molecular and submolecular level of studied tissue samples and better structural characterization. In future the results might help in understanding of pathological processes promoting adrenal tumor growth. Regarding preliminary character of performed studies it was desirable to set the optimal conditions which cover both – sample preparation and measurements requirements.

**Materials and Methods**

The studied tissue samples were taken intraoperatively from patients with different types of adrenal gland tumors. The specimens were frozen and cut into section of 10 μm or 6 μm thick in a cryo-microtome. The slices were mounted on silver coated sample support (Low-e MirrIR, Kevely Technologies) and freeze-dried at -80°C.

The researches were carried out at the beamline B22 (MIRIAM) at Diamond Light Source. The measurements were performed using Brooker Vertex 80V Vacuum-FTIR interferometer coupled with the Brooker Hyperion 3000 microscope (fully automated microscope with MCT detector liquid nitrogen cooled). The spectra were collected in reflection mode for the wavenumber range from 400 to 4000 cm⁻¹ with a spectral resolution of 4 cm⁻¹.

**Results**

The performed investigations enabled to set the optimal conditions for the future SR-FTIR measurements. One of the conclusions is that the tissue sample thickness might be reduced from 10 μm to 6 μm what affects the quality of spectra. Moreover the preliminary researches show that the level of lipids is higher in the adenoma samples comparing with pheochromocytoma cases. What is more it seems that the level of proteins in pheochromocytoma samples is higher than in adenoma cases. For more reliable results the further analysis is needed.
Physicochemical and micro-tomographic characterization of inorganic deposits in tissues

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Introduction

A main problem of aortic stenosis is massive mineralization of the aortic valves (AVs). A similar feature occurs in case of yellow ligaments (YLs). The mechanism of the process is still not elucidated [1]. The aim of the studies was to characterize the chemical composition and morphology of inorganic deposits from surgically excised AVs and YLs and to look for similarities to minerals in bones and teeth.

Materials and Methods

Deposits from 30 surgically excised AVs were examined. The control group consisted of autopsy samples (aortic valves, vertebral bodies) as well as teeth obtained after extractions. Additionally, 10 samples of YLs, excised due to the spinal stenosis and 9 control samples, excised of the patients suffering after accidents were investigated. The micro-computed tomography (µCT) was used to describe the morphology and density of minerals (Fig. 1). The X-ray fluorescence method and FTIR were applied to determine the chemical composition.

Fig. 1. Micro-CT images representing morphology of mineral deposits in: a) (left) aortic valve, b) (right) yellow ligament.

Results

The results confirm that a poorly crystalline, carbonate-containing hydroxyapatite constitutes the mineral phase of all aortic valve cusps [2] and majority of YLs. The main phase of the two YLs was Ca₅P₂O₇. The elemental composition of minerals in AVs, YLs and bone/tooth does not differ markedly except Mg concentration in AVs (~4 times higher in AVs). The AV deposits are irregular in shape and they occupy ~40% of the cusp volume while in YLs does not exceed 9%.

References


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ATR-FTIR vibrational spectroscopy as a tool to study of *Chlorophytum comosum*

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**Introduction**

Vibrational spectroscopy is a technique that permits to study structure and intermolecular interactions. IR and Raman spectroscopy are techniques of vibrational spectroscopy [1]. They enable to study objects at the tissue level; therefore, it is possible to receive information about their chemical composition. The advantages of spectroscopic techniques include a small amount of material required to experiment, short time to obtain spectra, high sensitivity, and in the case of tissue it is not necessary to do preparation of samples, consolidation of material and use biochemical markers [2]. These result in reduced preparation time of samples and the costs of experiments.

Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) is one of methods, which permits to do research of biological systems. It is a sensitive method, which is possible to reveal changes in biochemical properties of samples, which are studied at the molecular level [3].

**Materials and methods**

Leaves of *Chlorophytum comosum* were experimental material. It is evergreen plant, which belongs to perennial plants. It has narrow leaves, which can be 45cm long [4]. In order to do the experiment, 1 cm samples from this plant were collected. Then ATR-FTIR spectra of plants were registered using Thermo Scientific Nicolet iN10 FT-IR Microscopy (USA). Samples of plants were placed on glass slide; additionally ATR accessory with germanium crystal was applied.

In preliminary analysis the specific vibrations of functional groups were assigned to the bands and spectral parameters were determined. These alternations suggest that the water content changes in different parts of plant leaves.

**Results**

Spectroscopic methods (i.e. ATR-FTIR) can be used to study of quantitative research of water loss in plant tissues. We expected that properties and functions of tissue depend on presence of neighbouring water, what have an impact on the ATR-FTIR spectra. For measuring absorbance of the OH vibrations at 3242 cm\(^{-1}\) and the hydrophobic CH\(_3\) mode at 2916 cm\(^{-1}\), it is showed that the content of water in tissue is decreasing. It is very important to do research in short time after samples were collected. Storage time can influence on the final performance and conclusions.

**References**


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**P7 Micro-IR imaging of yew needles as an analysis tool in extraction processes**

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European Yew (Taxus baccata) is an evergreen tree growing predominantly in western and southern Europe, northwest Africa and southwest Asia. During the past three decades the metabolic profiles of various yew species have been screened. As a result especially taxol and related taxanes were found to be responsible for the observed anticancer activity. These plant substances act as mitotic inhibitors and are usually applied as a chemothrapeutic infusion. Beside taxol also its precursors such as 10-deacetylbaccatin are of interest for the pharmaceutical industry and are therefore extracted from the needles of various Taxus species. In order to reduce the costs for extract processes, it is essential to collect information about the raw material and the distribution of valuable compounds in the plant matrix\(^1\). It has been shown that Raman- and IR-Imaging of medicinal plants, resultant extracts and tablets can visualize the individual steps of the extraction processes and the final quality control of phytopharmaceuticals\(^2\). In this study micro IR-imaging of microscopic sections (8 µm) from yew needles was used to characterize the changes of the metabolic profile (e.g. water, lipids and waxes, proteins, carbohydrates) occurring during the exposure to methanol (Fig. 1).

![Visual image of a transversal section (8 µm) of a yew needle (left), micro IR images presenting the carbohydrate distribution before (middle) and after (right) methanol extraction. Light areas show high and dark areas low concentration of carbohydrates in the plant matrix.](image)

**Fig. 1.** Visual image of a transversal section (8 µm) of a yew needle (left), micro IR images presenting the carbohydrate distribution before (middle) and after (right) methanol extraction. Light areas show high and dark areas low concentration of carbohydrates in the plant matrix.

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**References**


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Comparison of surface-enhanced Raman spectroscopy and fluorescence for endothelial cells studies

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Introduction

Endothelium form the barrier between blood and vascular wall, and plays a crucial role in many biological processes including inflammation, hemostasis, thrombosis, and angiogenesis [1]. Mechanical damage of the endothelium, or loss of its functional integrity, disturbs homeostasis of microenvironment, leading to the development of pathological states, such as hypertension, or atherothrombosis.

Surface-enhanced Raman spectroscopy (SERS) has dynamically developed, especially in the field of bioanalysis, such as studying of cells, because of its ultrasensitive detection limits [2]. As the native constituents are present in cells at very low concentrations, it is challenging to find a method which can be easily applicable for the cellular studies. However, it is possible to collect informative signals at cellular level within seconds with SERS spectroscopy. To carry out SERS experiment on the cells, it is crucial to synthesize appropriate SERS labels i.e. metal nanoparticles conjugated to Raman reporters (mainly dyes with a large cross section for Raman scattering). These nanoparticles can be introduced into the cells to give information about intracellular environment along with SERS signal of a reporter [2].

Highly sensitive fluorescence imaging technology, using metal nanoprobes and a fluorescence scanning microscope, has been widely applied to cellular imaging and biomedical diagnostics. Development of multiwavelength lasers and sensitive detectors has contributed to its utility. However, fluorescence-based imaging techniques often lack the sensitivity and selectivity to environmental conditions of biological samples [3].

Results

Here we present the SERS and fluorescence studies of EA.hy.926 endothelial cells. Gold nanoparticles have been conjugated to rhodamine 6G and introduced to cells by fluid phase uptake during 2h With both methods it is possible to study 3D distribution of the Raman reporter within the cell, however, fluorescence only enables investigation of the fluorescence dye and SERS provides additional information about biological environment of the cells (e.g., bands from lipids or proteins in the SERS spectrum).

References


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A comparative study of carbonate bands from nanocrystalline carbonated hydroxyapatites and bone animal using FT-IR spectroscopy in the transmission and photoacoustic modes

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Nanocrystalline carbonated apatites (CHA) of the mixed type AB, containing ca. 1 wt\% of the A-type carbonates (substituted for OH\textsuperscript{-}) and ca. 5-8 wt\% of the B-type carbonates (substituted for PO\textsubscript{4}\textsuperscript{3-}) and hydroxyapatite derivated from animal bone were studied using the IR spectroscopy in the transmission (TRANS) and photoacoustic (PAS) modes.

The respective $v_2$ and $v_3$ carbonate spectral regions were subjected to peak fittings and the obtained TRANS and PAS bands were compared. We found that the A-type carbonates were located mainly in the interior of the crystals, while the B-type carbonates were prevailing on the crystal surface in CHA. Two different types of the A-type carbonates were found, competing with each other for the position in the channels, normally occupied by hydroxyl ions. A considerable amount of labile carbonates was found in the water layer on the crystal surface. The HPO\textsubscript{4}\textsuperscript{2-} ions were mostly located in the crystal lattice. A new band was discovered in the PAS spectrum at 884 cm\textsuperscript{-1} and it was assigned to carbonates from the crystal surface. It was shown that semi-quantitative conclusions can be drawn from the PAS IR spectra of apatites, if appropriate conditions are fulfilled. In particular, the comparison of the TRANS and PAS IR spectra gives valuable information on distribution of carbonate ions between the crystal interior and the crystal surface.

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**P10** Characterization of inorganic matter in meteorites by use of Raman spectroscopy

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Introduction

The investigation of minerals by Raman spectroscopy is a suitable method to typify minerals within planetary material. Raman spectra provide information on compositional variations of pyroxene, olivine and Fe–Ti–Cr oxides and modal proportions of the rock [1]. Chemical identification may be made by matching the spectrum with a database of standard spectra, or the individual bands in the Raman spectrum may be used to infer the presence of particular bonds in the sample by reference to tables of characteristic bond vibration frequencies. As an analytical technique, Raman spectroscopy has the attractive feature that it may be applied to substances, without prior sample preparation, and it is usually non-destructive of the sample.

The present study was undertaken to examine the general characteristics of the various pyroxene and olivine groups in meteorite. Chondrites are a mix of materials formed under different conditions in different environments. With some understanding of the star-forming process, we can begin to discern what these environments were [2]. Our results were supported by AAS (Atomic Absorption Spectrometry) analysis of meteorite sample.

![Raman spectrum of the major crystalline mineral olivine, and its distribution on the measurement surface.](image)

References


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**P11** Diet–induced changes in liver lipid profile of mouse model of atherosclerosis: Raman nad infrared spectroscopic studies

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Arteriosclerotic vascular disease (ASVD) is among the most dangerous diseases of modern civilization, which continue to increase in importance [1]. It arises as a result of abnormal accumulation of fat such as cholesterol and triglyceride in arteries, which is why it is obvious that it affects most strongly artery walls. However, liver is also closely linked with ASVD due to the key role of this organ in fat metabolism. It has been suggested that atherosclerosis may therefore be associated with other liver diseases, like Non-Alcoholic Fatty Liver [2]. Moreover, liver may not only be a marker of cardiovascular diseases, but also can be involved in their pathogenesis. Existence of a well–defined relationship between these diseases and pathology of the liver suggests that a comprehensive investigation of hepatic tissue alterations might provide new, considerably important information in this research filed and shed new light on the pathogenesis.

While the effect of High–Fat diet on atherosclerosis development is well established, the influence of Low–Carbohydrate/High–Protein (LCHP) diet is still investigated. Based on previous studies it seems that LCHP diet substantially promotes the development of atherosclerosis [3].

Both spectroscopic techniques combined with 2D imaging/mapping represent powerful tools for investigation of tissue components, including various lipids (cholesterol and its esters, triglycerides, etc.) [4,5]. The use of confocal Raman microspectroscopy enables visualization of lipid droplets in liver tissues [6]. Moreover, determination of total degree of unsaturation is also possible (using the ratio of intensity of bands at 1266 cm\(^{-1}\) to 1300 cm\(^{-1}\) or 1444 cm\(^{-1}\) to 1656 cm\(^{-1}\)) [5,7]. By combining the results obtained with Raman mapping and infrared imaging it is possible to gain complementary information, that guarantees a comprehensive knowledge about the biochemical composition. Additionally, the simultaneous application of both methods allows performing study on macro and micro scale.

The aim of this work was to (1) characterize the influence Low Carbohydrate High Protein (LCHP) diet on the liver fat content in mouse model of atherosclerosis (ApoE/LDLr\(^{-/-}\)) by means of vibrational spectroscopic imaging techniques combined with multivariate data analysis (KMC, HCA). We found significant differences in both, total lipid content of the large tissue fragments, as well as the composition (degree of unsaturation) in arising microsteatosis. Additionally, we have (2) investigated the effectiveness of nicotinic acid inhibitory effect on the development of steatosis in the liver.

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**References**


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P12 Hyperspectral Raman imaging for assessment of biochemical composition of endothelial cells

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Raman microspectroscopy is a noninvasive optical method, which allows obtaining detailed information about the molecular composition of the biological material with a high spatial resolution. Raman spectroscopy combined with confocal microscopy can be used for early detection of biochemical changes in cells upon stress or disease, so can be considered as a new trend in analytical spectroscopy. Based on Raman spectrum, which gives a unique chemical signature of a specimen and using multivariate statistical and chemometric approaches, Raman maps of a single cell can be produced. The use of advanced data analysis methods allows for observation of changes in the content of many compounds simultaneously. The multivariate methods of data analysis create spectral correlations and maps by including not just one intensity or frequency point of a spectrum, but by utilizing the entire spectral information [1, 2], which poses a huge diagnostic potential.

In this work the Confocal Raman Imaging was used as a nondestructive tool to illustrate the variety at the subcellular level. Such measurements provide information about the distribution and composition of selected cellular structures, e.g. nucleoli, nucleus, cytoplasm, endoplasmic reticulum, small organelle and peripheral membrane. Raman mapping was done with a Confocal Raman Imaging system Witec alpha 300 with the application of a 60× water immersion objective (Nikon Fluor, NA=1). Data analysis of the Raman spectral hypercubes was performed by methods of multivariate statistics, since spectral differences from the subcellular organelles are quite subtle, and cannot be perceived by visual examination of the spectra. These methods include such approaches as Principal Component Analysis (PCA), and different methods of Cluster Analysis (hierarchical cluster analysis (HCA), fuzzy c-means cluster analysis, vertex component analysis (VCA) and k-means cluster analysis (KMC)). Results of KMC of a single cell Raman imaging is shown in Figure 1.

![Fig. 1. Averaged spectra of selected classes assigned to the cellular structures.](image)

Acknowledgement

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References


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**P13 Overtone-initiated isomerization and unimolecular decomposition reactions of oxalic acid in low-temperature solids**

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Unimolecular chemical reactions taking place on a ground electronic state, especially decomposition and isomerization induced by high-overtone excitation, are very important from the atmospheric chemistry point of view. \cite{1-3} They are initiated by promoting a molecule to chemically relevant energies in high vibrational excited states through the thermal excitation or direct absorption of visible or near-infrared radiation. Unlike the termally induced processes, the reactions initiated by vibrational overtone-pumping of ground electronic state molecule through direct absorption of photon can be treated without the need to consider the collisional activation processes. In general, it is required that the energy is deposited into the initially excited vibrational state and subsequently transferred by intramolecular vibrational redistribution (IVR) to other modes of the molecule including the reaction coordinate. \cite{4,5}

Here we focus our attention on the reactions observed upon excitation by visible radiation of high vibrational states of oxalic acid isolated in low-temperature argon matrix. For this system high-overtone induced isomerization and decomposition processes are successfully observed.

The experimental setup used for our studies allows the same laser source for simultaneous pumping and Raman excitation. Therefore it can be applied concominantly to induce and track the unimolecular processes. The interpretation of experimental results was supported by high-level harmonic and anharmonic ab initio calculations.

References


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Oxidation of plasma isolated lipids – ATR-FTIR spectroscopic study

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Introduction
Blood components are exposed to oxidative stress, which is consequence of immunological system response, during extracorporeal circulation or hemodialysis. Complications may arise primarily from the morphological damage of blood components upon contact with biological material not compatible with cells. For example, activated neutrophils secrete to plasma reactive oxygen species: hydrogen peroxide and superoxide anion. ATR-FTIR spectroscopy was used for study of in vitro oxidation of plasma-extracted lipids.

Materials and methods
Plasma lipids were extracted following to Folch method [1]. After drying, lipids were dissolved in distilled water to concentration 0.5 mg/ml. Specified volumes of hydrogen peroxide was added to lipid suspension. After 30 min incubation in 37°C lipids were extracted and dried. Dried lipids were again dissolved in 50 μl of chloroform and put on diamond crystal of ATR accessory. FTIR spectra were collected after disappearance of the 776 cm⁻¹ band, which corresponds to chloroform presence in the sample.

Results
Deconvolution of the ATR-FTIR spectra of dried lipids in the 3040–2760 cm⁻¹ and 1760–1680 cm⁻¹ range has been performed. Next, area under fit curve of following bands: ν(C=O) 1737 cm⁻¹, νs(CH₂) 2850 cm⁻¹, νs(CH₃) 2870 cm⁻¹, and νas(CH₃) 2954 cm⁻¹ were measured and ratios of absorbance area (AA) of the proper bands were calculated. The plot of the ratio of the AA of the 1737 cm⁻¹ band to the AA of the 2954 cm⁻¹ component as a function of hydrogen peroxide concentration was drawn. In the same way, the ratio of the AA of the 2916 cm⁻¹ band to the AA of the 2954 cm⁻¹ band versus the H₂O₂ concentration was determined. It was observed that both ratios exponentially increase with the increase of the oxidant concentration. Above certain critical concentration (2.5 mM) of hydrogen peroxide both processes stabilizes. It means that the further addition of more oxidants does not cause higher oxidative stress in the extracted lipids.

Conclusions
On the basis of our experiment can be concluded that increase in absorbance area ratios of mentioned-above bands can be proposed markers of lipids oxidation, what is confirmed by other studies [2, 3]. ATR-FTIR spectroscopy can be used for studying processes of lipids oxidation.

References

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P15 Biochemical differences in a vascular wall of diabetes model investigated by using Raman microspectroscopy

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Long standing diabetes leads to structural and functional changes in both the micro- and the macrovasculature system. The most vulnerable part of the blood vessel is the endothelium, a single layer of cells lining the aorta and sitting on a basement membrane [1]. The endothelium is not only a physical blood-tissue barrier, but takes part in the biochemical and morphological response during a pathological state of the organism such as diabetes. The next inner layer of the blood vessel is a three-dimensional network built mainly of collagen and elastin fibers with supportive perivascular cells (pericytes or smooth muscle cells) [2].

The aim of the study was to analyze the condition of the cross-section of the aorta of control and diabetic mice (db/db model). The db/db mice represent a genetically-modified model (leptin receptor–deficient mice) of non-insulin-dependent diabetes mellitus (diabetes mellitus of type II) [3]. Raman microimaging allowed finding subtle changes between healthy and diabetic subjects. The difference in the spectra can be seen in two spectral regions: 2850-3000 cm\textsuperscript{-1} attributed to the stretching C-H vibration and in the region of 1200-1400 cm\textsuperscript{-1}.

Experimental techniques were supported by chemometrics, e.g. a hierarchical cluster analysis. Simultaneously, atomic force microscopy (AFM) measurements were performed to examine the topography of the cross-sectional aorta in exactly the same areas where the Raman images were recorded. This approach provides a comprehensive information about the structure of vascular wall and differences that exist in the vascular wall of diabetic mice and the control ones.

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**P16** Prediction of L-Methionine Conformation, VCD and IR Spectra in the Gas Phase and Water Solution

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**Introduction**

The primary importance of amino acids is recognized in their role as building blocks of peptides and proteins fundamental for every living creature. For their importance, the amino acids are extensively studied. Amino acids adopt their neutral form in the gas phase and in low-temperature inert matrices, while the zwitterionic forms dominate in solution. So far, conformational landscape and vibrational spectroscopy of L-methionine have not been systematically studied. The aim of this study was two-fold: (i) to characterize the conformational landscape of neutral and zwitterionic L-methionine in the gas phase and in water solution, and (ii) to predict the L-methionine IR and VCD spectra in the two environments.

**Results**

As a result of systematic conformational search of neutral methionine we found 104 conformers to be stable at the B3LYP/aug-cc-pVDZ level. To simulate their IR and VCD spectra, the conformer population was based on the △G values calculated at the MP2/aug-cc-pVDZ level. It was shown that none of the methionine conformers dominates in the gas phase despite two stabilizing intramolecular H-bond interactions: OH···NH\(_2\) and NH···O=C for trans and cis configuration of the COOH group, respectively. The most abundant conformer at 298K (12%) is stabilized by OH···NH\(_2\) H-bond. The calculated anharmonic IR and VCD spectra show that two groups of conformers with different H-bond interaction can be identified. Based on the △(OH) range in the IR spectrum, two gaseous methionine OH···NH\(_2\) conformers can be unequivocally distinguished, whereas two conformers, one stabilized by OH···NH\(_2\) and the second by NH···O=C interactions, can be recognized in the VCD spectrum.

Out of 23 methionine zwitterion structures, ten were surrounded by five H\(_2\)O molecules and their IR and VCD spectra were calculated assuming the explicit first solvation sphere embedded in the polarized dielectric continuum. Three MET-zwi-(H\(_2\)O)\(_5\) structures with populations higher than 5% were obtained and the predicted VCD spectra suggest that recognition of these three conformers in the mixture is possible.

The influence of the solvation model and the number of water molecules in the hydration sphere on the methionine VCD spectra was also checked. In general, the simulated VCD spectra in the 4000-1300 cm\(^{-1}\) and 800-0 cm\(^{-1}\) spectral ranges suggested strong dependence of the spectra on implicit vs. explicit model applied and presence of the sixth water molecule locked between the COO\(^-\) and the NH\(_3^+\). However, in the 1300-800 cm\(^{-1}\) region, where mainly bands of the zwitterion skeleton are present, the way of solvent treatment and number of “microsolvating” water molecules do not significantly affect the VCD spectrum.

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The multi-method approach to physico-chemical imaging of the “en face” aorta in liquid and air

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Raman imaging spectroscopy and Atomic Force Microscopy (AFM) were used to study the physical and chemical properties of the tissue sample. The Raman chemical images were correlated with AFM topographic information taken from the same sample area. The combination of both methods provides the complementary data about the properties of the studied biological system.

The research was carried out for the mouse vascular wall in “en face” preparation mode \cite{1}. The presented maps were recorded using WITec alpha 300 system. The main technical problem of the study was the way of fixation of the biological sample. The efforts are paid to preserve the physico-chemical features of the tissue. Here, the analysis of the mouse vascular wall was carried out for fixed sample (formalin) in air and for non-fixed sample in the phosphate buffered saline (PBS) solution. Both approaches enable to obtain different information, which can be correlated with the chemical changes in the sample undergoing upon fixation and drying.

Presented here multimodal Raman imaging combined with AFM offers a great potential in biomedical studies to be explored further.

![Fig. 1. The AFM topography image (left) together with Raman maps of the distribution of proteins and lipids (middle) and DNA (right) obtained by integration of in the 2800 – 3100cm\textsuperscript{-1} and 775–800cm\textsuperscript{-1} range, respectively. The tissue was obtained from the control mouse C57BL/6J, fixed in formalin.](image)

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\textbf{References}


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Raman spectroscopy in analysis of rabbit bone with novel bone substitute material

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Introduction

Raman microscopy is a non-destructive technique requiring minimal sample preparation that can be used to measure the chemical properties of the mineral and collagen parts of bone simultaneously [1]. This method has been used for a wide variety of bone studies including mineralization, ageing, the variation of composition within a bone. Microcharacterization of biominerals allows a better understanding of the pathophysiological processes that occur in classified tissues and synthetic biomaterials [2]. Raman spectroscopy has been successfully demonstrated as an effective tool for tissue characterization and diagnosis.

The aim of this work was to determine chemical changes of novel bone substitute material and its biocompatible properties for bone repair.

The composite is a novel bone substitute material with apatite-forming ability. Ca-P precipitation on the surface of biomedical materials plays an important role in orthopedic surgery due to very good biocompatibility of implants when the apatite is in contact with the bone tissue. Rabbit bone samples were tested with an implanted bioactive material for a period of 1, 3 and 6 months.

The obtained results of analysis novel biomaterial were compared with a sample of the composite prior to implantation into the living animal. Results of Raman spectroscopy were compared with SEM-EDX analyses which show changes in surface morphology and composition.

Conclusions

The obtained results confirmed biointegration of the novel bone substitute material. We indicate bioactive and biocompatible properties of this composite for bone repair.

References


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P19 Fourier transform infrared spectroscopy as a tool in searching plasma markers of civilization diseases

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With the progress of the civilization development, the number of people suffering from diabetes or hypertension has significantly increased. Despite the fact that their symptoms are clearly identified, their etiology is often associated with pathology of vascular endothelium. Thus, a reliable and fast diagnosis of endothelium dysfunction has become an urgent need. Currently, endothelium pathology has been diagnosed with the use of expensive and time-consuming clinical tests.

On the other hand, infrared microspectroscopy (FTIR) has been employed in the analysis of biological material such as cells, tissues or body fluids towards medical diagnosis for several years [1-4]. Spectral profiles of biological samples give an insight into general information on their biochemical composition, which is delivered from bands positions and shapes originating from proteins, lipids, nucleic acids, and carbohydrates. It is also possible to analyze them for the quantitative and qualitative purpose.

The aim of this presentation is to demonstrate the application of FTIR technique in the diagnostics of diabetic and hypertension in blood plasma in terms of endothelium dysfunction. Plasma samples were collected from the two animal models: diabetic (transgenic mouse db/db) and hypertension (induced by L-N\textsuperscript{G}-nitroarginine methyl ester, L-N\textsuperscript{G}-NAME), and then spectral response of the diseases were compared with spectra of control plasma (C57/Bl6J mouse). For the diabetic model, FTIR spectra exhibit a significant increase in intensities of bands in the range of \textsuperscript{1}1100–1000 cm\textsuperscript{-1}, while the hypertension model is mainly characterized by shifting of amide I and II bands and an intensity decrease of the spectral region below 1300 cm\textsuperscript{-1}. The common spectral features of both the models are associated with alternation of the content and structure of proteins. Additionally, we correlate the observed changes with clinical chemistry tests.

Acknowledgements

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Evaluation of variability of biomolecular components in substantia nigra tissue of human senile brains - by means of FTIR microspectroscopy

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Introduction

One of the major challenges of modern neurobiology is to understand the main pathophysiological mechanisms connected with “natural aging”\textsuperscript{[1]}. There is a growing evidence that the most prevalent neurodegenerative diseases indicate some biochemical similarities with these ones occurring as a result of aging, such as: deposition of misfolded proteins (alpha synuclein, beta-amyloid, neurofibrillary tangles) and neuronal loss (i.e. in cortex, hippocampus, substantia nigra). Particularly, in senile brains there is observed an age-related neuronal loss of neuromelanin pigmented substantia nigra neurons and formation of intra-neuronal structures termed Lewy Bodies (similarity of age related changes to Parkinson’s disease) \textsuperscript{[2]}. Interestingly, an age related formation of neurofibrillary tangles and senile plaques occur also (putative similarity of age related processes to Alzheimer’s disease) \textsuperscript{[2]}. Influence of genetic and environmental factors onto human phenotype main promote oxidative stress and alterations of ubiquitin-preoteasome \textsuperscript{[1]}. This may indicate changes in protein’s and lipid’s content within tissue \textsuperscript{[1]}. 

Material and Methods

The Fourier Transform Infrared Microspectroscopy (FTIRM) was applied to analyze biomolecular composition of thin tissue slices. Both normal aged and neurodegerative diseased neuromelanin-pigmented substantia nigra neurons were studied.

Results and Conclusions

There was analyzed a dependence of content of main biomolecules (such as proteins, lipids) and variation in proteins secondary structure with age. Overall results were compared with these ones obtained for diseased cases. The studies allowed for getting an insight into the main mechanisms involved in age-related pathophysiological process (lipids peroxidation, formation misfolded proteins deposits).

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**P21 Spatial distribution of polysaccharides in plant cell wall of vegetables and fruits**

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**Introduction**

The plant cell wall is kind of the cellular skeleton that controls cell shape and determines the relationship between turgor pressure and cell volume. The cell wall is composite of many different natural polymers, mainly cellulose, xylglucan, pectins and also lignin for secondary cell wall that forms after cell growth. Proteins, lipids, enzymes, aromatic compounds and water are another components of this part of plant cell [1].

It is thought that percentage of components of plant cell wall has an important influence on mechanical properties of fruits and vegetables. Therefore research on content and spatial distribution of each component of these part of cells are extremely important in studies of quality of fruits and vegetables [2]. So far many analytical and microscopic methods of investigation of plant cell wall was developed. Nevertheless, none of these methods gives data relating to accurate distribution and amount of individual substances in micro-scale. Raman microscopy can resolve this problem without necessity of staining section of plant tissues.

Briefly, Raman microscope is connection of microscope and Raman spectroscopy. It allows collecting spectra at each points of sample. In this way map of spatial distribution of sample’s components can be obtained.

In this work, we would like to discuss the methodology of measurement using Raman microscopy and present Raman images obtained for cell walls of several plant tissues. Examples of spatial distributions of main cell wall compounds will be depicted.

**Results**

The chosen plant materials were tissues obtained from common fruits and vegetables. The cross sections (100-150 µm thick) of plant material were placed on microscope glass, dried and subjected to Raman measurement. Fig.1. presents obtained results for carrot parenchyma.

Due to choosing specific band location, we were able to localize and indentify pectins (856 cm$^{-1}$ α-glycosidic bonds in pectin), hemicellulose (1735 cm$^{-1}$) or lignin (1600 cm$^{-1}$ phenyl groups in lignin).

![Fig. 1. Raman maps obtained for carrot](image)

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P22 Semi-quantitative analysis of diet (LCHP) induced changes in atherosclerotic plaque of ApoE/LDLR−/− using FT-IR and Raman imaging

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Introduction

The main goal of this research was to recognize and image (in situ) the characteristic compounds and their changes inside aortic plaques of an apolipoprotein E and LDL receptor double knockout (ApoE/LDLR−/−) mouse models – one on a normal AIN-93G diet and second on a Low Carbohydrate High Protein (LCHP) diet, which is more likely to form vulnerable plaque.

Results

Fig. 1. Distribution of cholesteryl esters in the whole cross-sections of aortas (image size of 700x700 μm²) in control (AIN-93G) and LCHP diet samples based on FT-IR measurements (left panel). Intensity scale correlated to total amount: 0-0.9 in AIN-93G samples and 0-1.9 in LCHP samples. Distribution of cholesteryl esters and pure cholesterol in smaller parts of selected aortas (image size approx. 130x130 μm²) based on Raman measurements (right panel).

A total of 5 mice per group were measured, with 8-12 sections from each mouse. We have located and studied the distribution of lipids and proteins with the aid of both IR and Raman spectrosopies [1-3]. Analysis of images was aided by chemometric techniques such as HCA in order to give better insight into the composition of the plaque. Chemically different sub-classes of the plaque were found and protein to lipid ratio seems to play a vital role in plaque stability along with cholesteryl ester amount.

Additionally, Raman spectroscopy allows detecting not only the cholesterol crystals and calcium mineralization but also such morphologic features as fibrous cap, neurotic core/foam cells, ceroid presence, hemoglobin (Hb), β-carotene and thrombus on the surface of a plaque which makes this imaging technique a very powerful method in detection of unstable plaque.

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References


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