G.U. Journal of Science
22(3): 117-121 (2009)

GUJS
www.gujs.org

REVIEW

Fourier Transform Infrared (FT-IR) Spectroscopy for Biological Studies

Dilek (YALÇIN) DUYGU[1♠], Tülay BAYKAL[2], İlkay AÇIKGÖZ[1], Kazım YILDIZ[1]

[1] *Gazi University, Faculty of Education, 06500, Ankara, Turkey*
[2] *Ahi Evran University, Faculty of Education, 40100, Kırşehir, Turkey*

Received: 07.12.2007 Revised: 23.01.2009 Accepted: 04.03.2009

ABSTRACT

In the recent years, vibrational spectroscopies (infrared spectroscopy) have been developed for all sorts of analysis in microbiology. Important features of these methods are the relative ease with which measurements can be performed. Fourier transform infrared spectroscopy (FT-IR) is a technique that has been used over the years in chemical analysis for the identification of substances and is one that may be applied to the characterization of microorganisms. Infrared spectroscopy is based on the measurement of the molecular bond vibration compounds, excited by radiation of a suitable frequency, when given the conditions for energy absorption by the molecules. Here, we review the potential application of vibrational spectroscopies for use in biology. It can be concluded that vibrational spectroscopy show high potential as novel methods.

Key Words: Fourier Transforms Infrared Spectroscopy, Microorganisms, Identification, Classification, Discrimination.

1. INTRODUCTION

Attempts to apply IR technology to biology began as early as the 1910s, when the use of IR spectroscopy for the analysis of biological samples was first suggested. By the late 1940s, the technique was being successfully explored for the study of biological materials and, in fact, IR spectroscopy has become an accepted tool for the characterization of biomolecules [1]. The earlier applications of FT-IR in microorganisms date back to the 1950s [2, 3, 4, 5]. The use of infrared (IR)-spectroscopy as a means of differentiating and identifying bacteria was extensively reported at nearly this year. A critical review on this subject published in 1959 summarized that, although bacteria definitely exhibit IR-spectra that are unique for individual strains, the identification of bacteria via IR-techniques cannot be regarded as a useful scheme as it is an impractical procedure [5, 6]. However, it was not until 1988 that Naumann et al. [7] reintroduced this technique as a useful tool in microbial identification [8].

The revival of IR-spectroscopy as a means for characterizing microbial samples was initiated after the development of modern interferometric IR-spectroscopy, the availability of low-cost mini-computers and powerful new algorithms for multivariate statistical analysis and pattern recognition methodologies [6]. In the past four decades it has been clearly demonstrated by several authors that infrared spectra from bacteria can be used for identification [9] and differentiation [10, 1]. In the last decade, FT-IR spectroscopy has been shown to be a powerful technique for the study of biological macromolecules [11] and of complex biological systems such as tissues and cells [12, 13, 14].

2. TECHNICAL PROPERTIES OF FT-IR

FT-IR spectroscopy is a form of vibrational spectroscopy, and the FT-IR spectrum reflects both molecular structure and molecular environment. In this technique, the sample is irradiated with infrared

♠Corresponding author, e-mail: dilek.yalcin@kosgeb.gov.tr

radiation from an infrared source, and the absorption of this radiation stimulates vibrational motions by depositing quanta of energy into vibrational modes [8, 15]. Therefore, a molecule, when exposed to radiation produced by the thermal emission of a hot source (a source of IR energy), absorbs only at frequencies corresponding to its molecular modes of vibration in the region of the electromagnetic spectrum between visible (red) and short waves (microwaves) [1]. These changes in vibrational motion give rise to bands in the vibrational spectrum; each spectral band is characterized by its frequency and amplitude [15].

The IR region (1 to 100 μM) is subdivided in three zones, far-(100 to 25 μM), mid-(25 to 2.5μM), and near IR (2.5 to 1μM). The mid-IR depicts primary molecular vibrations and is the most common and widely employed region for the analysis of substances in chemistry and forensics. All molecules present characteristic absorbance peaks in a section of this region (1350 cm^{-1} to 1000 cm^{-1}; NB: by convention, the frequency is usually expressed as wave numbers using the reciprocal cm as its unit: 1μM=10^4 cm^{-1}); thus this physical property is considered as a molecular fingerprint. The far- and near IR are not frequently employed because only skeletal and secondary vibrations (overtones) occur in these regions producing spectra that are difficult to interpret [1].

Many of the infrared bands of biological interest occur in the frequency range between 4000 and 1000 cm^{-1}. The FT-IR spectrum of a cell will exhibit contributions from all cellular macromolecules, including protein, lipid, carbohydrates, and DNA. Although the spectra of macromolecules are complex, protein, lipids, and DNA provide characteristic, non-overlapping contributions to the FT-IR spectrum. These non-overlapping spectral contributions permit the determination of macromolecular concentration from the band's amplitude [15, 16].

To understand FT-IR spectra of biological samples, some fundamental knowledge on cell composition and the particular structures of the building blocks in biological samples is essential. It is important to recognize that infrared spectra of complex biological materials do not only describe the composition of cell, but also provide a number of specific bands that are sensitive to structural or conformational changes. It is also matter of fact that the physical state of the sample (hydration or aggregation state etc.) has a severe influence on FT-IR results. This makes it necessary to standardize sampling, preparation, and data acquisition procedures rigorously [16].

IR spectroscopy was greatly improved by the use of a new component: the interferometer and by the application of a mathematical transformation, the Fast Fourier Transform algorithm which allow for the simultaneous detection of all the transmitted energy. In general, for a system of identification of non-fastidious bacteria to be successful, growth conditions, such as the length of the growth period and the composition of the culture medium, should remain constant [1, 17].

An advantage of FT-IR spectroscopy is that this method can be applied to powdered, dehydrated, or aqueous samples. Also, the FT-IR spectrometer can be modified in order to make the study of very small samples, such as tissue sections and single colonies, a possibility. The technique is exquisitely sensitive [15].

The FT-IR technique has a remarkably far-reaching differentiation capacity in that it may provide results within a few minutes after obtaining single colonies of the pure culture, and may be uniformly applied to all microorganisms which can be grown in culture. Hierarchical clustering was applied to scrutinize spectral similarities and to achieve the classification of patterns. The results of cluster analysis are shown in the familiar form of minimal spanning tree, the so-called dendrogram. It is particularly interesting that the FT-IR technique is not restricted to the analysis of bacteria. Yeast, fungi and algae can also be analyzed [14, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26].

If cells are regarded as a complex mixture of bimolecular organized within different structures, every microbial cell will have a unique and characteristic spectrum stemming from the vibrational modes of all the molecules within it. The differences among taxons that are expressed as quantitative and qualitative differences in the composition and presence of the cell structures of each group could be translated into molecular differences and therefore into different spectral features. These spectral differences among groups could be used as a tool for identification [1, 27].

As all cell components depend on the expression of smaller or larger parts of genome, the FT-IR spectra of microorganisms display in a specific way a phonetic and a genetic fingerprint of the cells under study. This is why the specify of the technique is generally rather high, allowing differentiations at very different taxonomic levels, even down to the subspecies, strain and/or serotype level [16].

While it is a general prerequisite of the FT-IR technique to isolate pure cultures of the microorganisms in question, mixed cultures can be analyzed by measuring micro colonies (20 to 200 μm in diameter) which grow separately on solid agar media by means of a light microscope coupled to the FT-IR spectrometer. Small amounts of the microcolony spots suitable for FT-IR microscopic measurements are obtained by applying a special replica technique those spatially accurate transfers the first two to three cell layers of the microcolonies from the solid agar plates to infrared transparent plates (e.g. CaF_2, BaF_2 or ZnSe) [16].

For microbiological identification, two basic approaches can be distinguished in the chemometric techniques used at the moment. The first is based upon non-supervised or objective classification methods, analyzing naturally occurring groups in the data set and requiring no a priori knowledge of the sample identity; examples are factor analysis, principal component analysis (PCA) and hierarchical cluster analysis. Based on objective criteria of group membership, unknown samples are assigned to naturally occurring groups in

the data set. Inclusion of well-characterized samples in the analysis scheme allows groups to be identified on the basis of the properties/identities of these reference samples. The second group of chemometric techniques used in microbial identification is supervised techniques, i.e. requiring a priori knowledge of the sample identity. With a set of well-characterized samples, a model can be trained so that it can predict the identity of unknown samples. Examples of supervised methods are linear discriminant analysis (LDA) and artificial neural networks (ANNs) [6].

3. APPLICATIONS OF FT-IR SPECTROSCOPY

FT-IR spectra of intact microbial cells are highly specific, fingerprint-like signature which can be used to (i) discriminate between diverse microbial species and strains, (ii) detect *in situ* intracellular components or structures such as inclusion bodies, storage materials or endospores, (iii) detect and quantify metabolically released CO_2 in response to various different substrates and (iv) characterize growth-dependent phenomena and cell-drug interactions. The characteristic information is extracted from the spectral contours by applying resolution enhancement techniques, difference spectroscopy, and pattern recognition methods such as factor, cluster, linear discriminant analysis, and artificial neural networks. Particularly interesting applications arise by means of a light microscope coupled to the spectrometer [16].

FT-IR spectroscopy of the whole cells is a valuable tool for the identification of microorganisms and is used, e.g. in strain collections, in medical applications, in the pharmaceutical industry and in drinking water control [28].

Microbial determination is a primary consideration with respect to food safety and quality in a processing, retail, or production environment. Conventional methods for production and identification of microorganisms depend on biochemical and serological tests that usually involve incubating the culture in selective agar media or broth for up to several days and then performing a specific test to determine the presence of a certain species of bacteria. The risk of such a lag in determination could potentially deter timely intervention and appropriate remedial measures to countered food contamination. Therefore, rapid and direct screening of microorganisms on food products could be an essential and a valuable tool to the food industry. With the improvement in analytical instrumentation FT-IR spectroscopy has been applied to chemically differentiate intact microbial cells nondestructively, by producing complex, yet distinct and reproducible biochemical fingerprints of bacteria [29].

An extensive investigation of bacterial cells of different species and strains has been undertaken by Naumann and his co-workers [30, 16]. These authors have shown that FT-IR absorption spectra are highly specific fingerprints of microbial cells and that, when multivariate statistical methods are employed to analyze the spectra, they make microbial characterization possible down to subspecies level. FT-IR spectroscopy not only provides a competitive and rapid identification method, but, since it allows the study of microorganisms in their intact state, it appears to be a very promising tool also for the study of microbial metabolism, antibiotic susceptibility and other interactions with drugs [31].

FT-IR can also be used to detect and identify particular cell constituents such as capsules, endospores or storage materials. Already in formerly published studies, detection of intracellular polysaccharides, poly-β-hidroxybutyric acid (PHB) granules, and endospores was reported by authors using dispersive infrared spectroscopy [32].

4. CONCLUSIONS

The prospect of FT-IR microscopy for microbiological characterizations is very promising. The main advantages of FT-IR which constitute its attractiveness are extreme rapidity compared to conventional techniques, uniform applicability to very diverse microorganisms and a high specificity that allows differentiations even down to subspecies level. The strength of the FT-IR technique is its ability to conduct epidemiological case studies and large screening experiments very quickly. Additional fields of applications are the detection of infection chains and the control of therapy, the maintenance of strain collection and the differentiation of microorganisms from the environment for which established systems are not yet available. In the food, water and pharmaceutical industries FT-IR may also contribute to improve microbiological quality control. For the control of biotechnological processes it might also be an alternative or additional to already existing analytical tools. This new technology may help to scale down the number of cells to less than 10^3, to analyze mixed cultures and to detect light-microscopic and spectroscopic features of microorganisms simultaneously. Perceptively, the development of a fully automated system of microbiological analysis that combines detection, enumeration, and identification of microorganisms can be addressed [16].

Here we reviewed and discussed the opportunities of vibrational spectroscopies to study microorganisms. The techniques can be applied, but are not restricted to the fields of general microbiology, routine identifications, and specific clinical applications. A wide variety of methodologies or measurement schemes are available nowadays, allowing spectroscopists to define the most convenient approaches. Combined with the ever-expanding realm of chemometric analysis procedures, it is difficult to predict what exactly the niche for FT-IR spectroscopy will be in the microbiological toolbox.

REFERENCES

[1] Margarita P., Quinteiro R., "Fourier Transform Infrared (FT-IR) Technology for the Identification of Organisms", ***Clinical Microbiology Newsletter***, (22), No.8 (2000).

[2] Greensteet, J.E., Norris, K.P., "The existence of differences between the infrared absorption spectra of bacteria", ***Spectrochim. Acta***, 9: 177-182 (1957).

[3] Goulden, J.D.S., Sharpe, M.E., "The infrared absorption spectra of lactobacilli", ***J.Gen.Microbiol.***, 19: 76-86 (1958).

[4] Jackson, M., Mantsch, H., "Biomedical infrared spectroscopy", In: H.H. Mantsch and D. Chapman (eds.), ***Infrared spectroscopy of biomolecules***, Wiley-Liss, NY (1996).

[5] Riddle, J.W. et al., "Bacterial identification by infrared spectrophotometry", ***J.Bacteriol***, 72: 593-603 (1956).

[6] Maquelin, K., Kirschner, C., "Identification of medically relevant microorganisms by vibrational spectroscopy", ***Journal of Microbiological Methods***, 51: 255-271 (2002).

[7] Naumann, D., Fijala, V., Labischinski, H., "The rapid differentiation and identification of pathogenic bacteria using Fourier Transform Infrared spectroscopic and multivariate statistical analysis", ***J.Mol.Struct***., 174: 165-170 (1988).

[8] Gomez, M.A., Perez, M.A.B., Gil, F.J.M., "Identification of species of *Brucella* using Fourier transform infrared spectroscopy", ***Journal of Microbiological Methods***, 55: 121-131 (2003).

[9] Lipkus, A.H., Chittur, K.K., Vesper, S.J., "Evolution of infrared spectroscopy as a bacterial identification method", ***J. Ind.Microbiol***., 6: 71-75 (1990).

[10] Curk, M.C., Peledan, F., Hubert, J.C., "Fourier Transform infrared (FT-IR) spectroscopy for identifying Lactobacillus species", ***FEMS Microbiol. Lett***., 123: 241-248 (1994).

[11] Siebert, F., "Infrared spectroscopy applied to biochemical and biological problems", In: Sauer, K. (Ed), Biochemical Spectroscopy, ***Methods in Enzymology***, 246: 501-526 (1995).

[12] Jackson, M., Sowa, M.G., "Infrared spectroscopy: a new frontier in medicine", ***Biophys. Chem.***, 68: 109-125 (1997).

[13] Diem, M., Boydston-White, "Infrared spectroscopy of cells and tissues: shining light onto a novel subject", ***Appl. Sepctrosc***., 53: 148-161 (1999).

[14] Wenning, M., Seiler, H., Scherer, S., "Fourier-transform infrared microspectroscopy, a novel and rapid tool for identification of yeast", ***Applied and Environmental Microbiology***, 68(10): 4717-4721 (2002).

[15] Sacksteder, C., Barry, B.A., "Fourier Transform Infrared Spectroscopy: A Molecular Approach to an Organismal Question", ***Journal of Phycology***, 37: 197-199 (2001).

[16] Naumann, D., "Infrared and NIR Raman spectroscopy in medical microbiology", In: Manch, H.H., Jackson, M. (Eds.), Infrared Spectroscopy: New Tool in Medicine, ***SPIE Proceeding Series (3257)***, SPIE, Bellingham, WA, 245-257 (1998).

[17] Naumann, D., et al., "The characterization of microorganisms by Fourier-transform infrared spectroscopy (FT-IR)", In W.H. Nelson (ed.), ***Modern techniques for rapid microbilogical analysis***, VCH, NY (1991).

[18] Montechaiaro, F., Hirschmugl, C.J., Raven, J.A., Giordano, M., "Homeostasis of cell composition during prolonged darkness", ***Plant, Cell and Environment***, 29: 2198-2204 (2006).

[19] Sekkal, M., Legrand, P., Huvenne, J.P., Verdus, M.C., "The use of FTIR microspectormetry as a tool for the identification in situ of polygalactanes in red seaweeds", ***Journal of Molecular Structure***, 294: 227-230 (1993).

[20] Benning, L.G., Phoenix, V.R., Yee, N., Tobin, M.J., "Molecular characterization of cyanobacterial silification using synchrotron infrared micro-spectroscopy", ***Geochimica et Cosmochimica Acta***, 68(4): 729-741 (2004).

[21] Giordano, M., Kansiz, M., Heraud, P., Beardall, J., Wood, B., McNaughton, D., "Fourier transform infrared spectroscopy as a novel tool to investigate changes in intracellular macromolecular pools in the marine microalgae *Chaetoceros mullerii* (Bacillariophyceae)", ***J.Phycol***., 37: 271-279 (2001).

[22] Dean, A.P., Sigee, D.C., "Molecular heterogeneity in Aphanizomenon flos-aquae and Anabaena flos-aquae (Cyanophyta): a synchrotron-based fourier-transform infrared study of lake micropopulations", ***Eur.J.Phycol.***, 41(2): 201-212 (2006).

[23] Haag, H., et al., "Characterization and identification of *Actinomycetes* by FTIR spectroscopy", ***J. Microbiol. Methods***, 27: 157-163 (1996).

[24] Kos, G., Lohninger, H., Krska, R., "Fourier transform mid-infrared spectroscopy with attenuated total (FT-IR\ATR) as a tool for the detection of *Fusarium* fungi on maize", ***Vibrational Spectroscopy***, 29: 115-119 (2002).

[25] Fischer, G., et al, "FT-IR spectroscopy as a tool for rapid identification and intra-species characterization of airborne filamentous fungi", ***Journal of Microbiological Methods***, 64: 63-77 (2006).

[26] Oust, A., et al., "FT-IR spectroscopy for identification of closely related lactobacilli", ***Journal of Microbiological Methods***, 59: 149-162 (2004).

[27] Naumann, D., et al., "What can infrared spectroscopy tell us about the structure and composition of intact bacterial cells?", In H.H. Mantsch and D. Chapman (eds.), ***Infrared spectroscopy of biomolecules***, Wiley-Liss, NY (1996).

[28] Schuster, K.C., Mertens, F., Gapes, J.R., "FT-IR spectroscopy applied to bacterial cells as a novel method for monitoring complex biotechnological processes", ***Vibrational Spectroscopy***, 19: 467-477 (1999).

[29] Irudayaraj, J., Yang, H., Sakhamuri, S., "Differentiation and detection of microorganisms using Fourier transform infrared photoacoustic spectroscopy", ***Journal of Molecular Structure***, 606: 181-188 (2002).

[30] Naumann, D., Helm, D., "Microbiological characterizations by Fourier transform infrared spectroscopy", ***Nature***, 351: 81-82 (1991).

[31] Orsini, F., Ami, D., Villa, A.M., "FT-IR microspectroscopy for microbiological studies", ***Journal of Microbiological Methods***, 42: 17-24 (2000).

[32] Helm, D., Naumann D., "Identification of some bacterial cell components by FT-IR spectroscopy", ***FEMS Microbiology Letters***, 126: 75-80 (1995).