Fiber-optic Evanescent Wave Fourier Transform Infrared (FEW-FTIR) Spectroscopy of Polymer Surfaces and Living Tissue

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Summary: The new method of fiber-optic evanescent wave Fourier transform infrared (FEW-FTIR) spectroscopy has been applied to studies of polymer surfaces and the diagnostics of normal, precancerous and cancerous tissue. This technique using optical fibers and fiber-optic sensors operating in the attenuated total reflection (ATR) mode in the mid-infrared (IR) region of the spectrum (850 – 1850 cm^{-1}) has found recently application in the area of tissue diagnostics. The method is suitable for noninvasive and rapid (seconds) direct measurements of the spectra of normal and pathological tissues *in vitro, ex vivo,* and *in vivo.*

The FEW-FTIR technique is an ideal diagnostic tool for different types of soft, porous, foam, and rough polymer surfaces. Inhomogeneous coatings and defects on polymer surfaces as well as layer structures have also been detected by this method. It is convenient to apply this method to analyze large pieces of soft plastics and/or surfaces covered by plastics, since these types of surfaces are comparatively hard to analyze by traditional absorption spectroscopy. The FEW-FTIR technique is non-destructive, fast (15 seconds), and remote (up to a fiber length of 3m). In addition, it is sensitive enough to detect any changes in the vibrational spectra of a polymer surface, without heating and damaging it. The surfaces of polyethylene crumpled bags and rumpled films have been investigated in the range of 2000 – 1000 cm^{-1}. The distinct spectra of these surfaces as well as spectra of polytetrafluoroethylene have been recorded. The spectra of white and colored foams and different plastics have also been studied. Weak but distinct spectra have been recorded for carbon fibers (black, narrow fibers with a diameter of about 10 μm). Using the FEW-FTIR technique, measurements can be taken without preparing the sample. High quality spectra have also been obtained for the bulk and surfaces of apple, banana, grapefruit, and other food products. The method is expected to be further developed for geological and microelectronic applications.

Introduction

Fourier transform infrared (FTIR) spectroscopy has been used traditionally for the analysis of polymer structure and biomolecules[1)]. It has been widely applied for the diagnostics of tissues[4-14, 18-19)]. It is a very important to be able to diagnose the early stages of cancerous or precancerous conditions. The normal, precancerous, and cancerous tissues of different organs[8)]

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 CCC 1022-1360/99/$ 17.50+.50/0

have been investigated successfully by fluorescent[9] and autofluorescent[10] spectroscopy. Elastic and Raman[10] scattering and FTIR spectroscopy have been applied also for studying tissues *in vitro*, using microscopic and image formation technique[2,8]. The non-destructive methods of vibrational spectroscopy (IR and Raman) enable us to diagnose tumours and cancer at an early stage on a molecular level[5,6,10,12,14]. Recently, the applications for both methods of vibrational spectroscopy have been enhanced by using fiber-optic sensors[5,6,10-14]. Fiber-optic technology is relatively inexpensive and can be adapted easily to any commercially available table top compact Fourier transform (FT) spectrometer[13, 14].

The combination of fiber-optic sensors and FT spectrometers can be applied to many fields: e.g. (i) surface diagnostics of polymers and numerous materials, (ii) non-invasive medical diagnostics of cancer and other diseases *in vivo* and clinical application, (iii) remote monitoring of biochemical processes and environment, (iv) minimally invasive bulk diagnostics of tissues and materials, (v) characterization of the quality of food, pharmacological products, and cosmetics.

We have suggested[12, 14] that bare-core fibers should be used in the ATR mode with a different configuration of the "probe" for fast, remote, non-invasive and non-toxic diagnostics of cancer. We applied this technique and obtained measurements of cancer tissue from the kidney, stomach, and lung *in vitro,* breast *ex vivo* during the operation, and skin *in-* and *ex vivo* and in incisions. This minimally invasive technique could be used for endoscopic and catheter applications. The FEW-FTIR method is very suitable for the analysis of polymer surfaces as well as polymer blends and regions of inhomogeneity in any material.

In this paper, we present IR spectra in the range of 2000 – 1000 cm^{-1} of soft polymer surfaces and fruit skin as well as living tissue obtained using the FEW-FTIR method. The main features in the spectra of polymer and tissue surfaces on a molecular level are discussed. This non-destructive, fast, and non-invasive technique should allow for the development of appropriate methods for quality control of numerous polymers and other materials as well as tissue and cancer diagnostics.

Materials

First, we non-invasively measured skin tissues *in vivo* (directly on patients) for the tumor and normal skin tissue[5,12-14,18]. After surgery the measurement procedure was repeated. Thereafter

the samples were cut at the center of the tumors to measure the different layers of tumor and normal tissue. The experiments have been performed in operating rooms, where fresh samples of tissue are provided from the patients and measured *ex vivo*[19]. While our main focus in this study has been human breast tissue, we have also obtained lung, stomach, and kidney tissues *ex vivo*. The results of these spectral measurements have been compared with available histological data

We recorded also spectra of numerous polymer surfaces without special preparation of these samples. Fruits and other food products were analyzed directly from their surfaces and/or in bulk from soft samples.

Method

We used polycrystalline $AgBr_xCl_{1-x}$ fibers (1 mm in diameter) in the spectral range 3-20 μm with low optical losses (0.1 to 0.5 Db/m in the region of 10 μm) and high flexibility ($R_{bending}$ > 10 to 100 fiber diameters) [13]. The optical scheme of the self-made accessory consists of optical fibers to input and output infrared radiation and a spherical mirror to focus light onto a nitrogen-cooled MCT detector. The optical scheme of the accessory was specifically designed to be used in any commercial Fourier transform (FT) spectrometer. The fiber has direct contact with the tissue in a manner analogous to a prism in the attenuated total reflection method. In particular we conducted a series of experiments as a function of the pressure applied to the tissue. In these studies the length of contact between the optical fiber and the tissue has been varied from one millimeter to a few millimeters. It is evident that a longer surface fiber contact corresponds to a more pronounced spectrum. An optimal number of scans was chosen taking into account the possible time of the patient's testing *in vivo* and the signal to noise ratio. In general the measurement time was a few seconds, with an optimal recording time of 15 seconds. However, when we tested very small human samples (at about 1 mm) of normal and malignant tissues the fiber had to be bent to a special angle, forming a tip probe for such testing[19] . Therefore, a few experiments were performed to optimize the fiber bending angle, resulting in a longer recording time. For measuring defects or points of inhomogeneity of polymers, we can use the tip probe in the same manner as it is utilized for biopsy and endoscopic applications. The special tips allow us to collect or scatter the light for different tissue examinations. The contact between the fiber and the tissue has been optimized. All the spectra were measured with resolution of 4 cm^{-1}.

The spectra were treated by a computer with a set of standard and specialized programs. The complicated absorption lines were deconvoluted into components and fitted with multiple

Lorentzian and Gaussian lineshape functions. The detailed studies into the position, intensity, and primarily, the shape of spectral bands highlight basic differences between the spectra of normal and malignant tissues within the range 850 –1850 cm^{-1}. All the absorption bands were interpreted, and the regularities of conversion from normal to malignant tissues are defined.

RESULTS AND DISCUSSIONS

Our first fiber-optic evanescent wave Fourier transform infrared (FEW-FTIR) spectra of skin tissue measured *in vitro*[12-14] enabled us to select the spectral ranges where the basic changes and processes of disordering in the protein, lipid, sugar systems, and hydrogen bonds are most pronounced[12, 14]. In these papers, attention was focused on the characteristic sections of the IR spectrum associated with malignant tumors[12-14, 18] in the range from 950 to 1500 cm^{-1}. After more detailed computer analysis we discerned small but characteristic shifts of the most intense bands in the mid-IR spectrum associated with vibrations of amide groups (amide I and amide II) in the spectra of normal and tumor tissues of the kidney, stomach and lung. We noted that the largest intensity variations and shifts were observed for the amide II bands in the region of 1530-1560 cm^{-1}. In many cases both amide bands decreased in intensity and broadened. In some cases, they completely disappeared. Furthermore the weak band in the region around 1740 cm^{-1} was found to have a tendency to disappear completely in the spectra of cancer tissue. These interesting observations have been confirmed in new studies of skin tissue *in vivo*. These new studies were undertaken when it became possible using this technique to test small samples of any surface *in vivo*, including tissues and soft polymers. Spectra of breast tissue[19] and small biopsy samples of stomach and lung tissues[12-14] were studied *ex vivo* in the region of the intense amide bands.

We have investigated the normal skin tissue in several zones of the human body. Substantial differences have been seen in the IR spectra of cheek and hand tissue, for example. Such differences have also been observed in the spectra of the tissue from the palm and the back of the hand. We obtained the largest differences on the skin surface in the area of acupuncture points from numerous people.

In Fig.1 the spectrum of normal skin *in vivo* is shown in the range of 850-4000 cm^{-1}. In recording this spectrum for the first time we have used three meters of optical fiber for remote skin surface analysis of the hand tissue. The signal to noise ratio corresponds to high quality FTIR spectra. Full compensation is seen for the CO_2 absorption near 2340 cm^{-1}. We sub-divided up the entire remote spectrum into several parts for the convenience of the mathematical

treatment. This procedure allowed us to obtain more detailed information about vibrational modes of the hand tissue. Although not shown here, we have noted that when working with complicated overlapping bands it is sometimes beneficial to use the second derivative to locate the peak positions.

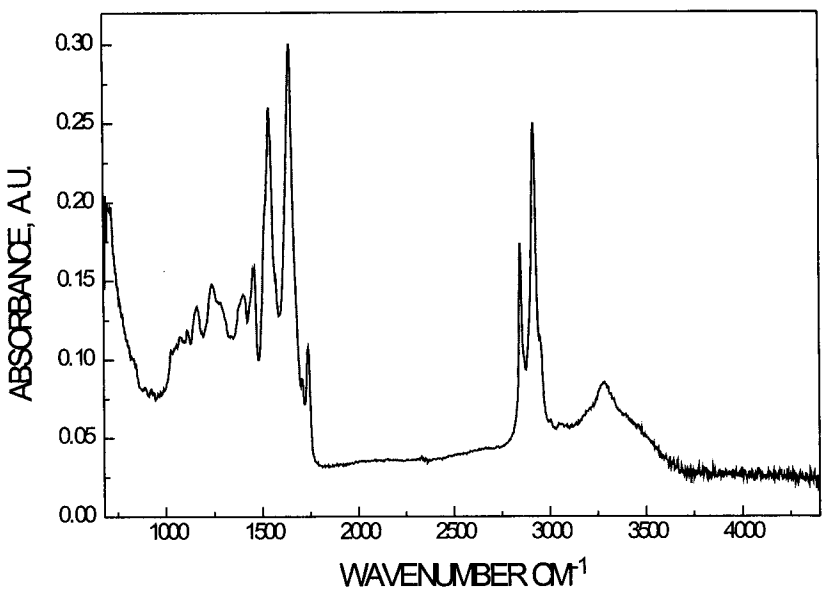

Fig.1: *In vivo* measurement of remote FTIR-FEW spectrum of the normal skin (hand) in the range 850-4000 cm^{-1}.

In the spectrum, we can see a complex asymmetrical band of amide A. This band consists of at least two main bands. The main peaks' positions are at 3279 and 3429 cm^{-1}. The band at 3429 cm^{-1} can be assigned to intermolecular hydrogen bond vibration in the amide A, similar to a polymeric system. The second component of this band with a distinctive maximum at 3279 cm^{-1} belongs to N-H stretching in the amide group. The amide A band is a complex system of hydrogen bonded amide stretching vibrations as well as vibrations of the hydrogen bonds in the tissue.

In the range of 2700-3100 cm^{-1}, two main peaks belong to C-H stretching vibration in the $-CH_2$ - groups (or R_2 $-CH_2$). The asymmetric stretching is located at 2923 cm^{-1}, while the

symmetric stretching mode is at 2850 cm^{-1}. The shoulder of the asymmetric stretching band belongs to the C-H mode in -CH_3 - groups. The weak structure at 2877 cm^{-1} is associated with symmetric C-H vibration of the same group. These features are associated with lipids found in skin tissue.

The region of the main amide I and amide II bands mentioned has been investigated also. The peak position of the amide I band is located at 1645 cm^{-1} and is associated with the parallel-chain pleated sheet conformation[7, 17]. The amide II band position of 1545 cm^{-1} is associated with an α-helix structure. These two bands are sensitive to changes of conformation and they can differ for various proteins. The weak features appearing as side shoulders on these two bands indicate the presence of several nucleic acids. The asymmetry of the amide I band at 1688 cm^{-1} can be assigned to guanine residue[1, 7, 15]. This is a complicated mode of C=O stretching and NH_2 scissoring vibration[1, 7]. Usually two shoulders overlapping with the main structure of the amide II are present in the spectra. One of them is near 1585 cm^{-1}. This structure belongs to cytosine in-plane ring vibration[1, 7]. The second shoulder of the amide II band near 1515 cm^{-1} is assigned to tyrosine ring vibration[1]. Two bands of hydrogen bonded ester carbonyl groups in lipids are located at 1709 and 1741 cm^{-1}. This band belongs to the cyclic systems with hydrogen bonds[16]. A second band structure around 1709 cm^{-1} is, more likely, characterized by hydrogen bonds in noncyclic system[16].

The evaluation of the main structural parameters includes the number of bands within a definite range, their peak position, their half-width and the shape of the bands, and the parameter Δ (the distance between the peaks of the main amide bands). In all our investigations we analyzed intensity ratios for three characteristic band parts: R_I (I_{1642}/I_{1545}), R_{II} (I_{1642}/I_{1742}) and R_{III} (I_{1742}/I_{1710}). Average intensity ratios of the chosen bands corresponding to normal skin are in the limits of 1.4-1.5, 2.0-2.5, and 1.0-2.0 respectively[5]. In the spectra of benign tumor R_{II} changes dramatically. This intensity ratio is very sensitive to precancerous conditions. For example, this ratio decreases in the case of premelanoma by 1.5 times[5].

The distance between the peaks of the main amide bands (Δ) is important. In particular, the parameter Δ was useful in analyzing the changes taking place in the spectra of malignant tissues. The distance Δ between the bands at 1641 and ca. 1539 cm^{-1} mainly depends on the position of the 1539 cm^{-1} band structure. The distance (Δ) is 106 cm^{-1} for these normal tissues, but it is less then 100 cm^{-1} in the cases of precancerous and/or malignant tissue spectra.

The low frequency spectrum of the normal skin surface of the hand has been analyzed remotely for the first time. There are eight main bands present in the spectrum. The deconvolution of the spectral curve allows us to derive information on the structural parameters for this region of the spectrum for normal skin surface (hand).

This region of the IR spectrum of skin is very sensitive to any changes in the structure of proteins, lipids, phosphates and sugars[18]. The complicated bands observed contain several overlapping vibrational modes. Changes occur immediately in the band structure of the modes 1030, 1080, 1160, and 1240 cm^{-1} at the transformation from normal tissue to tumor or malignant tissue[18]. The protein system of the skin surface absorbing at 1300 cm^{-1} is sensitive also to these changes, as can be observed by splitting this band. The conformational changes in many side groups lead to the band structure over the entire IR range. Remote diagnostics should be able to detect any type of change in the structure of tissue via these spectra in seconds[18].

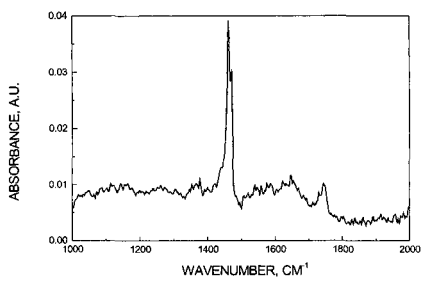

Fig.2: FEW-FTIR spectrum of the surface of a polyethylene bag (film) in the range 1000-2000 cm^{-1}.

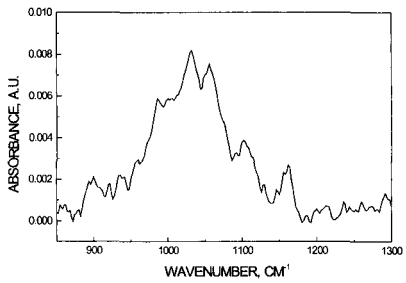

Fig.3: FEW-FTIR spectrum of the surface of soft white filter paper in the range 850 -1300 cm^{-1}.

The non-toxic and non-invasive (or minimally invasive) non-destructive spectroscopic device method promises fast, direct diagnostics of tissues on the molecular level. In addition we suggest it should be possible to detect rapidly several diseases (for example, basal-cell carcinoma) directly from the skin surface[5, 18].

We have measured also small samples of polymers. In particular, FEW-FTIR spectra of the surfaces of several polymer-packaging materials have been studied. In figure 2, a FEW-FTIR spectrum of a polyethylene bag (film) is shown. A distinct doublet band at 1462 and 1471 cm^{-1} can be seen in this spectrum. The spectrum was recorded with 16 scans and a resolution of 4 cm^{-1}. Absorption although weak was detectable by this method.

In figure 3, a FEW-FTIR spectrum from the surface of soft white filter paper is shown. The diffuse scattering from this sample was considerable. The spectrum is extremely weak. Thus the spectra required 64 scans at a resolution of 4 cm^{-1}. (The spectral curves are shown in Figs. 1-9 without any computer smoothing procedure.)

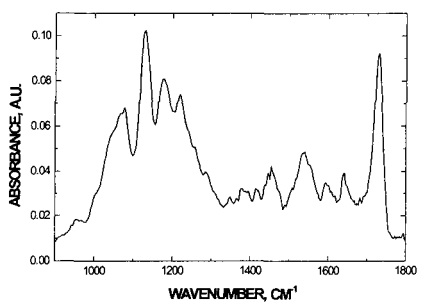

Fig.4: FEW-FTIR spectrum of the surface of white foam rubber in the range 900-1800 cm^{-1}.

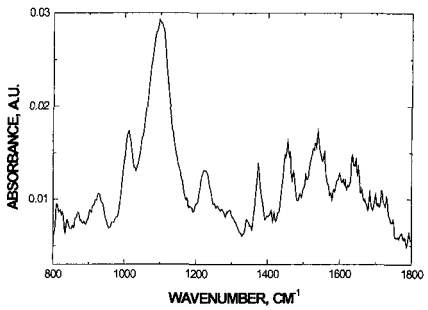

Fig.5: FEW-FTIR spectrum of the surface of a green foam rubber cube (15 cm^3) in the range 800-1800 cm^{-1}.

In figures 4 and 5, the spectra of two different soft elastic foam rubber materials are shown. The spectra of a colored foam is pictured in figure 5. In the range 1000-1200 cm^{-1}, distinct IR bands have been recorded: doublet at 1098 and 1010 cm^{-1} in figure 5 and a triplet in figure 4. In addition, the intense C=O band at 1730 cm^{-1} is seen in the spectra in figure 4. The similarities and differences in these two spectra allow us to make inferences about the structure elements of both these polymers.

In figures 6 and 7, the spectra of two packaging materials (styrofoam) in the region 800-1800 cm^{-1} are shown. Several strong bands allow for the identification of the spectra. Signal/noise ratio is appropriate for this type of evanescent spectra. Both spectra have been obtained with 64 scans and a resolution of 4 cm^{-1}.

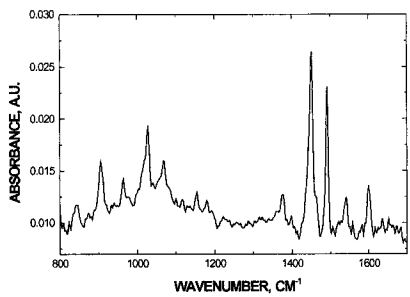

Fig.6: FEW-FTIR spectrum of the surface of styrofoam (packaging material) in the range 800-1700 cm^{-1}.

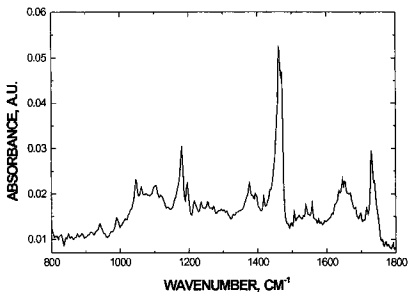

Fig.7: FEW-FTIR spectrum of the surface of pink styrofoam (packaging material) in the range 800-1800 cm^{-1}

The spectra of soft and rigid polymer surfaces presented in this paper have shown that the FEW-FTIR method is suitable for different industrial applications. For the production of both small and large size products this quality control method has potential. The method does not require any special sample preparation. It could be used in any conditions. The surfaces of block materials and/or any plastic surfaces could be analyzed using this technique. In addition to polymers, it is also possible to measure the surfaces and bulk of food products.

In figures 8 and 9, FEW-FTIR spectra of the internal surfaces of banana and grapefruit skin are shown. These soft, diffuse, scattering surfaces are difficult to investigate by any other method. These spectra demonstrate yet other potential applications of evanescent wave IR spectroscopy to the analysis of food, cosmetic, and pharmaceutical products. Related agricultural applications might also be developed for this powerful diagnostic tool. This non-destructive, "non-invasive," direct, fast, and remote method of quality control has been demonstrated for the mid-IR "fingerprint" region for several materials.

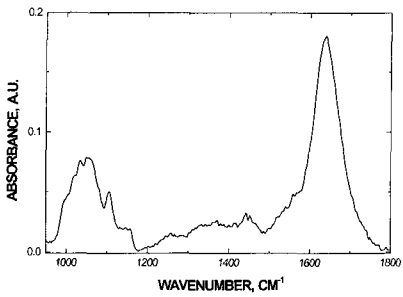

Fig.8: FEW-FTIR spectrum of the internal surface of a banana peel in the range 950-1800 cm^{-1}.

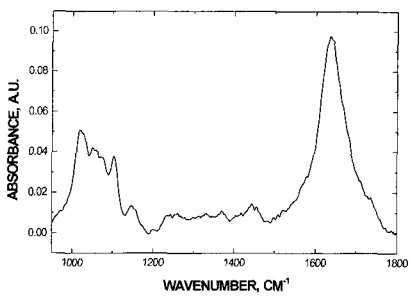

Fig.9: FEW-FTIR spectrum of the internal surface of a grapefruit peel in the range 950-1800 cm^{-1}.

CONCLUSION

Remote FTIR- FEW spectra have been obtained over the wide IR region from 850 to 4000 cm^{-1} for the first time. The main changes in the spectra from the remote testing of the skin surface of normal and malignant tissue have been discussed. It is convenient to detect changes in the skin tissue from the surface directly. The spectra in the range of 950-4000 cm^{-1} measured at the different points of healthy skin were found to be in good agreement with each other in position, intensity ratios of the main spectral bands, and in the shape of the bands. A preliminary interpretation of the normal skin tissue spectra in the spectral range of 950-1500 cm^{-1} is suggested. Spectral methods are being developed for diagnostics by the remote FTIR-FEW technique of the skin surface. Statistical data concerning various normal and pathological tissue are being accumulated. Some results presented here can be considered as the first attempt to test polymer material and tissue *in vivo* by the remote FTIR-FEW spectroscopic technique and as a result identification by their IR absorption spectra was carried out.

ACKNOWLEDGMENTS

I would like to thank very much my young colleagues Sergei Kolyakov (Institute of Spectroscopy, Russian Academy of Sciences), Vladimir Makhine (University of Nevada-Reno), Angelique Brooks (University of Nevada-Reno), and Slava Artjushenko and his colleagues for their patience, enthusiasm, and efforts to develop with me this new combination of Fourier

transform spectroscopy and fiber-optic technique. I wish to thank the Moscow (Russia) and United States departments of Perkin-Elmer Inc. for providing us with the FTIR spectrometers.

I greatly appreciate the help of Prof. Dr. Reinhard Bruch from the University of Nevada-Reno and his permanent interest in my work.

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