USE OF THE FOURIER TRANSFORM INFRARED SPECTROSCOPY IN CHARACTERIZATION OF SPECIFIC SAMPLES

Gabriel-Lucian RADU[1], Georgiana-Ileana TRUICĂ[2], Ramona PENU[3], Veronica MOROEANU[4], Simona Carmen LITESCU[5]

Spectrometria în infraroşu cu transformata Fourier a devenit una dintre principalele tehnici utilizate atunci când este abordată analiza unor probe cu o specificitate deosebită, care necesită o analiză nedistructivă. Astfel de situaţii apar atunci când trebuie să fie analizate probe arheologice/de patrimoniu, ca şi atunci când trebuie caracterizate biomateriale specifice. În acest articol prezentăm unele rezultate experimentale care, prin argumentele aduse, justifică importanţa utilizării acestei tehnici în elucidarea unor aspecte critice privind caracteristicile structurale ale acestor tipuri de probe. Studiile noastre au fost efectuate pe probe de chihlimbar (arheologic si geologic) şi pe sisteme biomimetice obţinute prin imobilizare de biomolecule (enzime) pe nanoparticule funcţionalizate.

The Fourier Transform Infrared Spectrometry analysis became one of the main used techniques when peculiar topics are addressed, mainly when non-destructive analysis is needed. In this respect, according to our opinion, interesting analytical issues are raised, for example, when historic (archaeological)/patrimony materials have to be analyzed or when the analysis of highly- specific biomaterials have to be performed. In our paper data are supporting both potential specific application of Fourier Transform Infrared Spectrometry on provided samples. the studies have been performed on amber (archaeological and geological) and biomimetic systems obtained by immobilization of active biomolecules (enzymes) on functionalized nanoparticles.

Keywords: Fourier Transform Infrared Spectrometry, archaeological samples, functionalized supports analysis

1. Introduction

[1] Prof., Faculty of Applied Chemistry and Materials Sciences, University POLITEHNICA of Bucharest, Romania, gl_radu@chim.upb.ro
[2] PhD student, Faculty of Applied Chemistry and Materials Sciences, University POLITEHNICA of Bucharest, Romania
[3] PhD student, Faculty of Applied Chemistry and Materials Sciences, University POLITEHNICA of Bucharest, Romania
[4] PhD student, Faculty of Applied Chemistry and Materials Sciences, University POLITEHNICA of Bucharest, Romania
[5] Senior researcher, Centre of Bioanalysis, National Institute of Research and Development for Biological Sciences Bucharest, Romania

The Fourier Transform Infrared Spectrometry (FTIR) used in characterization of complex and specific samples it is not a trivial task to be fulfilled by chemists. The difficulty of FTIR characterization comes mainly from the high overlapping degree of the infrared absorption bands, making difficult the truthful ascription to certain functional groups, despite of the fact that up to date computer-searchable databases of spectra are currently available.

During recent years, analysts used mostly FTIR, mass spectrometry and pyrolysis-gas chromatography-mass spectrometry for the analytical study of geological or archaeological amber from different territories [1-5]. In 2005, Angelini and Bellintani [3] reviewed the analytical techniques used for the differentiation of amber types, dedicating special attention to the most suitable methods for archaeological materials (non-destructive methods).

Surfaces modifications in the view of using them for some applications have to be scientifically supported, both structurally and functionally. Surface analysis techniques such as Fourier transform infrared spectroscopy (FTIR) [6,7], scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS) [8] and atomic force microscopy (AFM) [9] can provide information on the structural organization of the deposed films, modifications occurred at the structural level of the solid supports and covalent bonds formation, and on the functionalization efficiency.

The surface modification is performed tacking into account the solid support nature. Usually materials as semiconductors (simple and doping SiO_2), conductors (metals, metal oxides (indium tin oxide (ITO), glassy carbon, etc.)) carbon paste, or functionalized nanoparticles (ITO, Au, Fe_3O_4, carbon nanotubes) are subsequently used as solid supports for immobilization. One of the major advantages of using functionalized nanoparticles is that of the formation of a controlled structure by the molecular density of the monolayer type found at interface.

Silanisation has been used as a process for modification of the nanoparticles surfaces. There are data proving that triethoxysilyl butyraldehyde (TESBA), a silane compound with terminal carboxyl functional group, is able to reduce the coating time [10]. In the same time aminosilanes such as (3-Aminopropyl)-triethoxysilane (APTES) are other compounds attractive for such applications. APTES, a common amino-silane coupling agent, has found its extensive applications in surface-decoration by forming a monolayer of amino-silane [11,12,13]. This film of amino-silane provides active platform for further bio-functionalization or linking organic molecules onto the nanoparticles: the active amino groups ($–NH_2$) facilitate the further functionalization and can covalently bond with other active groups, such as the carboxyl (–COOH) that can conveniently conjugate with enzyme, antibodies and other functional groups.

These are the reasons that make us choose TESBA, respectively APTES as functionalization reagents.

Considering the versatility of FTIR techniques (especially variable angle reflectance- FTIR abbreviated as FTIR-VAR and attenuated transmittance-FTIR abbreviated as ATR-FTIR) in solving the critical issues rose when characterisation of specific samples (as archaeological samples and biomimetic systems) is required, we provide in the present paper experimental data to sustain this assertion.

2. Experimental procedure

The present work is addressing, as case studies, both issues: the FTIR assessment of certain archaeological amber samples, respectively the FTIR assessment of a developed bio-mimetic system based on nanoparticles modified with biomolecules. In both cases the restricted amount of samples and the necessity of non-destructive analyses create constraints in the working procedure.

2.1. Materials

ITO-NP/ In_2O_3:Sn_2O_3; 3-(Aminopropyl)triethoxysilane (APTES); potassium bromide FT-IR (KBr); acetate buffer 10mM, pH=4,2; isopropanol; toluene, ethanol were purchased from Sigma-Aldrich. Triethoxysilyl butyraldehyde (TESBA) was provided by ABCR GmbH & Co. All the used reagents were of analytical grade.

2.2. FTIR-VAR method for characterization of archaeological amber samples

A large lot of samples from different controlled origins (Colti, Romania, Kaliningrad, Russia) and from archaeological sites (Noslac, Romania) were analysed by FTIR-VAR, in order to obtain a definite pattern to distinguish Romanian amber from the Baltic variety (the material was provided, mainly, from the National Geological Museum from Bucharest).

FTIR-VAR spectra of amber were recorded using a Bruker TENSOR 27 instrument. The spectral resolution was 4 cm^{-1}, with 96 scans, and an aperture of 4 mm. The optimum beam incidence angle was 45^o and all the spectra were acquired in a range between 4000 and 600 cm^{-1}.

2.3. FTIR transmittance method for characterization of nanoparticles modified with biomolecules samples

2.3.1. ITO-NP functionalization procedure using APTES

ITO nanoparticles were treated with 10% APTES in toluene solution under continuous stirring for 12 hours. Then the ITO-NP were rinsed with ethanol in order to remove the traces of toluene, and dried at 60^{o}C for 30 minutes.

2.3.2. ITO-NP functionalization procedure using TESBA

The functionalization mixture was obtained by mixing 150 µL TESBA with 150µL isopropanol and 150µL acetate buffer 10mmolL^{-1} (pH=4.2).

An amount of 10 mg ITO-NP was mixed with 150µL of functionalization mixture prepared as described previously and added to 1mL with 850µL isopropanol. The suspension obtained was stirred at room temperature for 15 minutes, than submitted to ultrasounds about 1 hour, washed with ethanol, centrifuged for 5 minutes at 3000 rpm, followed by washing, at the end the supernatant being removed. After supernatant removing, the previous procedure is repeated by adding a new volume (1mL) of diluted functionalization mixture. Subsequently the new suspension is submitted to ultrasounds for 15 minutes, followed by another period of immobilization under continuous stirring for 12 hours. After that, the mixture was centrifuged 5 minutes at 3000rpm and the supernatant was removed. The washing procedure continues with the addition of 1ml ethanol, centrifugation 5 minutes at 3000 rpm and supernatant removal. The suspension was dried at 60^{o}C for 30 minute.

2.3.3. Biomolecules immobilization

Laccase immobilization on ITO-NP surface consisted of the dispersion of a quantity of 50 mg ITO-NP into a polyphenol oxidase solution that was prepared in 0.1 molL^{-1} phosphate buffer pH 4.5. The mixture was stirred for 12 hours at 16ºC. The modified ITO-NP were rinsed with distilled water and then dried at 4^{o}C for 24 hours. They were stored at -18^{o}C.

2.3.4. FTIR Methods

FTIR spectra were recorded with the Bruker-Tensor 27 Fourier Transform spectrometer equipped with a RT-DLaTGS detector. Each measurement was recorded in transmitance (ratio 1:100, sample: KBr) in the range 4000–400 cm^{-1} and average value of 128 co-added scans, with 4 cm^{-1} resolution and 4 mm aperture, at room temperature. The spectra were registered and ascribed using Opus software, version 6.0.

3. Results and Discussions

3.1. Assesment of amber origin based on FTIR spectra

Taking into account the transformation occurring on amber samples during historical ages strongly influenced by the storing conditions in the sites where were discovered, the analysis started not directly with archaeological samples but first, with geological amber, of controlled and certified origin, both from Baltic sources and from Romanian sources in order to establish definite FTIR criteria to be useful for amber origin assignment.

Spectra were analysed on three wave-numbers domains for amber, those between 3600-2500 cm^{-1}, 1800-1000 cm^{-1} and the 1000-600 cm^{-1} regions, which correlate with the presence of hydroxyl groups, carboxyl groups, carbonyl groups.

The FTIR spectrum of geological amber samples presents a large number of peaks between 3600-3400 cm^{-1}, more or less intense, due to the OH stretching bands of alcohols and/or carboxylic acids.

The bands corresponding to the alkyl stretching show three characteristic peaks. The assignment is the following: the CH3 asymmetric stretching vibration occurs at 2961 cm^{-1} and may be distinguished from the CH2 absorption at around 2926 cm^{-1} while the symmetric stretching absorption band of the methylene group occur at 2859 cm^{-1} (Fig1).

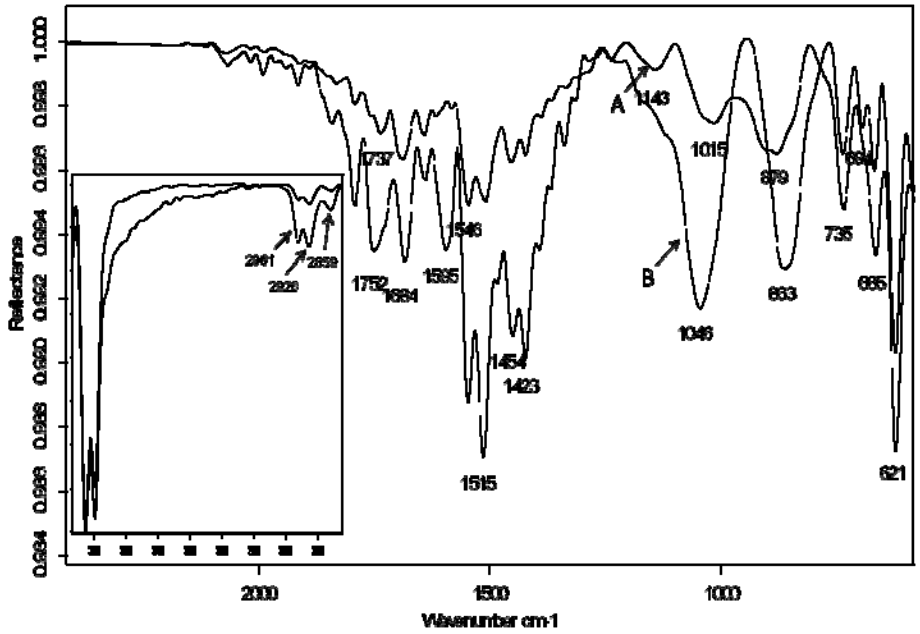

Fig. 1. FTIR-VAR (45°) spectra comparison, regions 2000-600 cm^{-1} and 3700-2800 cm^{-1} (inset), for Baltic amber (A) and Romanite amber (B).

The spectral region 1820-1690 cm^{-1} presents the predominant absorptions bands that have been assigned to ester, ketone and carboxylic acid groups. The carbonyl range shows a principal band around 1752 cm^{-1} of esters and acids and a strong band at 1143 cm^{-1} that can be ascribed to the C–O simple bond stretching of esters.

Baltic amber shows this characteristic broad band at 1155 cm^{-1}, followed by two absorption peaks, which reaches maximum intensity at 1015, and 879 cm^{-1}, attributed to C-O stretching, respectively C-H out-of-plane bending. Two bands at 1046 and 863 cm^{-1} are always observed in Romanite amber spectra with the same attribution but slightly shifted due to intermolecular/intramolecular bonds. Differences in FTIR-VAR spectra appear also in the region 900-600 cm^{-1} from were the two species could be differentiated, depending on the age of the polymer.

In archaeological material the region 3600-3400 cm^{-1} generally presents a less intense signal, especially for Romanite amber, due to climatic condition of the storage environment. In addition, the fingerprint zone is different in most of archaeological samples in comparison with geological ones. This region presents adsorption bands of lower intensity for the majority of archaeological samples and some shifts of the specific wave number.

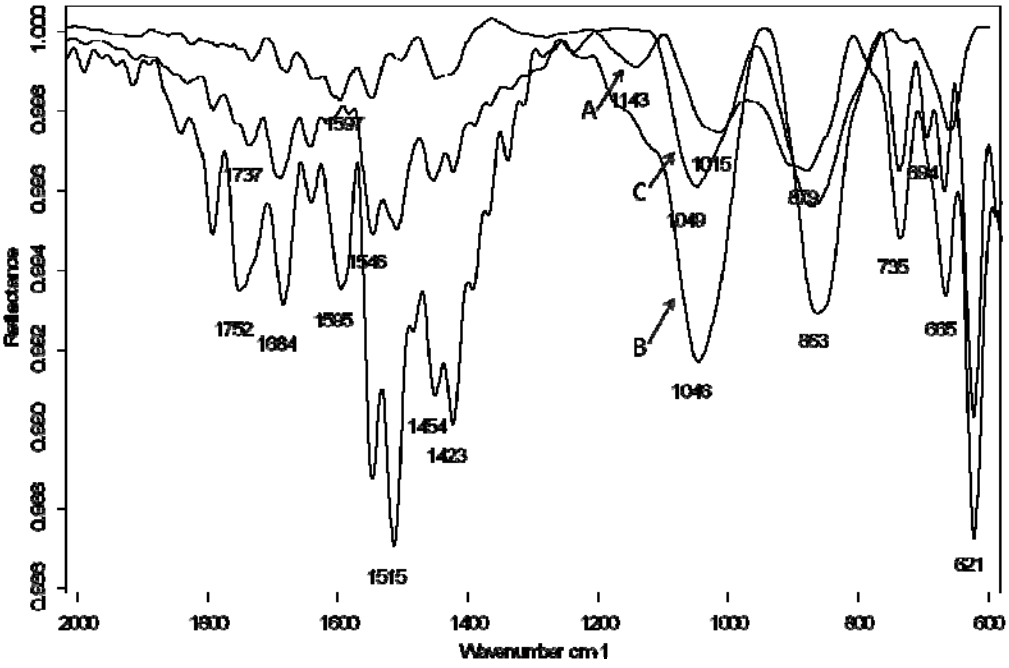

Fig. 2. FTIR-VAR spectra comparison, regions 2000-600 cm^{-1}, for Baltic(1) and Romanite amber (2) with archaeological sample from Noslac (3)

The notable differences appear in "Baltic shoulder" region, 1250 - 1060 cm^{-1}, and in 1640-1580 cm^{-1} region. All reference spectra of Romanite amber present a characteristic band at 1595 cm-1 assign to C=C aromatic stretch and/or CO_2^-stretch of carboxylic acid salts, as it could be noticed from figure 2.

This band could also be observed in majority of archaeological amber samples, around 1597 cm^{-1}, as well as the similarity in the region 1050-800 cm^{-1}.

Based on comparative association with the geological reference material (and according to the FTIR bands assignment of archaeological samples from the region 1800-1000 cm^{-1} and 900-600 cm^{-1}), the most probable classification of archaeological material would be Romanite amber.

3.2. Functionalised nanoparticles characterisation

Chemical modification of ITO-NP was performed in order to generate functional groups on their surface, groups that are able to further interact with biological components that will be immobilized.

The functionalization was performed in order to increase the number of active -OH, -COOH, -C=O groups on the solid support surface of which bind covalently or by forming H bond using -OH, -SH, -NH groups from biological components (in our case – laccase).

These functionalization methods are based on the reaction between the same terminal functional groups of the protein and reactive groups found on the solid surface of the solid support.

Several procedures that use APTES and TESBA as functionalization agents for the functionalization of ITO-NP were used, and based on the FTIR characterisation the best procedure will be chosen.

3.2.1 APTES-ITO-NP FTIR characterisation

Analysing the spectra of uncoated ITO- NP, coated ITO-NP with APTES and pure APTES (Fig.3), it was noticed that two absorption peaks at 2928 and 2873 cm^{-1}, ascribable to asymmetric and symmetric CH_2 stretching, respectively, appeared in the APTES-ITO-NP spectrum (spectrum B), while the bands at 2979 and 2928 cm^{-1} in APTES spectrum (spectrum C) are assigned to the asymmetric and symmetric CH_3 stretching. The ethoxy group of APTES is absent in B spectrum, therefore the ITO-NP coating proves the APTES bond to solid supports.

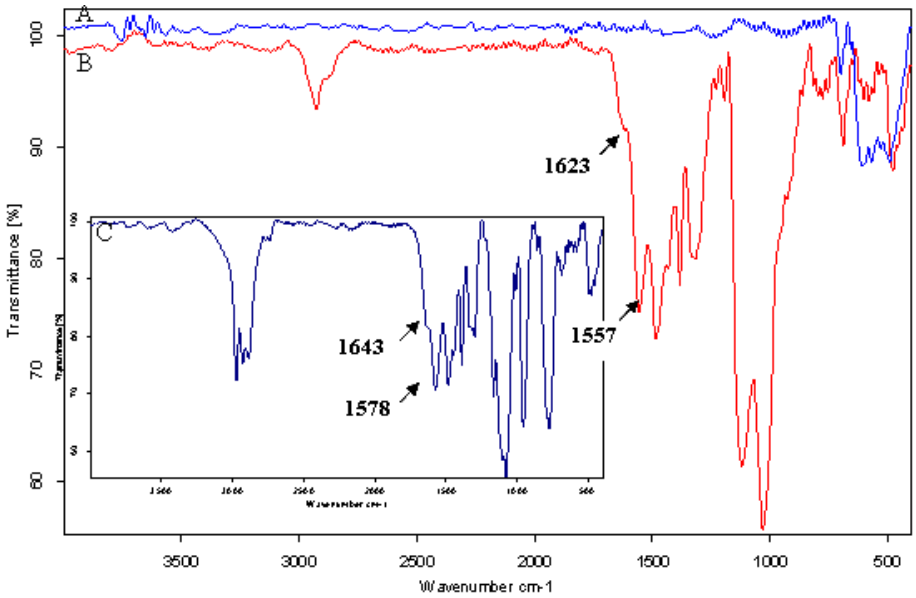

Fig. 3. Spectra of uncoated ITO-NP (A), coated ITO-NP with APTES (B) and APTES (C)

The most important structural information in terms of APTES adsorption was observed in the range 1700-650 cm^{-1} where APTES (spectrum C) and APTES-ITO-NP spectra are compared. Two characteristic bands of $–NH_2$ terminal groups occurring at 1643 and 1578 cm^{-1} in APTES are present even in the ITO-NP APTES coated spectra, slightly shifted at 1623 and 1557 cm^{-1}, respectively, assigned to (-N-H) stretching and bending vibration. Data are in good agreement with those reported in literature [13]. Other characteristic bands at 1123 and 1031 cm^{-1}, ascribable to Si-O stretching of the silane group, are present in both cases (APTES and modified ITO-NP) and confirm the adsorption of the silane polymer on the nanoparticles surface.

3.2.2 TESBA-ITO-NP FTIR Characterisation

In order to provide evidence on coating ITO-NP with TESBA, the recorded spectra for TESBA-ITO-NP and pure TESBA were compared (Fig.4).

Both samples show broad bands at 3632 and 3697 cm^{-1} related to the hydroxyl deformation vibration of the silanol group present on TESBA-ITO-NP. The band from 1056 cm^{-1} the Si-O-Si functional group asymmetric stretching vibration can be ascribed. Two other bands at 682 and 444 cm^{-1} confirmed Si-O-Si functional group presence on functionalized ITO-NP.

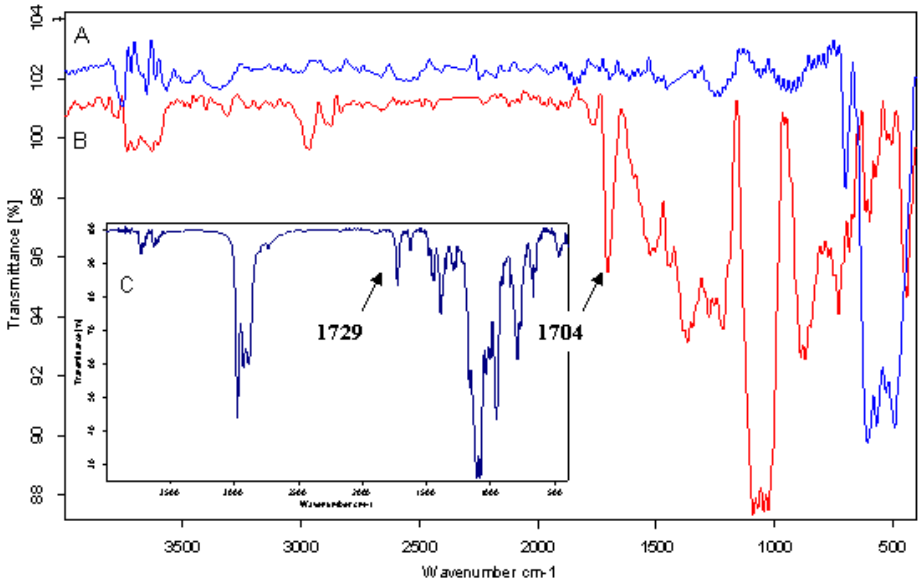

Fig. 4. Spectra of uncoated ITO-NP (A), coated ITO-NP with TESBA (B) and TESBA (C)

Compared with the uncoated ITO-NP, all the coated ITO-NP proved absorption bands at 2969 and 2873 cm^{-1} due to stretching vibration of C-H bond. The increasing number of Si-OH functional groups on the nanoparticles surface confirms the NP modification with TESBA. In addition, the band at 1704 cm^{-1} ascribable to stretching vibration of C=O bond proves the TESBA binding to ITO surface.

At this point it may be emphasized that it was proven the FTIR utility in providing data on effective NP functionalization.

Further, starting from the functionalized NP, biomimetic systems based on laccase were obtained and characterized considering the requirement of non-destructive analysis, by FTIR.

3.2.3 Laccase APTES-ITO-NP FTIR Characterisation

The functionalized nanoparticles (APTES-ITO-NP / TESBA-ITO-NP) were used for laccase immobilization.

The immobilization procedure was supposed to be based on the covalent bonding of the biological component (laccase) on the ITO-NP surface by the means of carbonyl and amino groups which were generated in the functionalization step. These groups are characteristic to the structure of TESBA and, respectively, APTES compounds. Changes on these groups absorption bands intensities were considered significant to prove the efficient enzyme binding to NP supports.

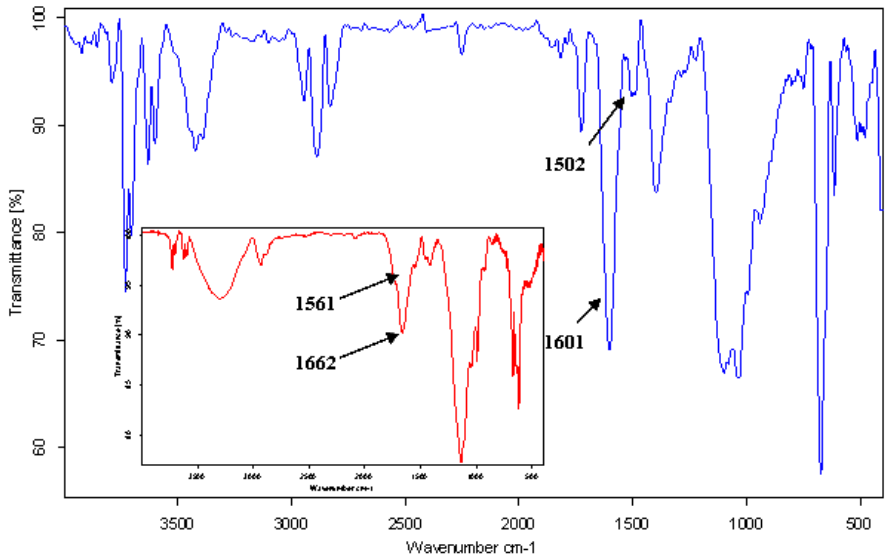

Fig. 5. Spectra of laccase modified APTES-ITO-NP. Inset laccase spectrum

FTIR studies were performed in order to obtain information about the immobilized enzyme structural organization. The recorded spectra of laccase modified APTES-ITO-NP were compared to those of free laccase (Fig. 5). The FTIR spectrum of free laccase (Fig 5, inset) showed two absorption bands, at 1662 and 1561 cm^{-1} corresponding to amide I and amide II. The 1662 cm^{-1} peak was assigned to the stretching vibration of the C=O amide bond, the 1561 cm^{-1} peak was assigned to the bending vibration of the N-H bond and stretching vibration of the C-N bond. The corresponding absorption peaks in laccase-ITO-NP occurred at 1601 and 1502 cm^{-1}, suggesting that laccase was immobilized on solid support. The observed slight shift of peaks wavenumbers to the right and increasing of absorption bands indicated the covalent interaction between APTES-ITO-NP and laccase.

4. Conclusions

The use of FTIR, as variable angle reflectance technique or transmittance mode, proved to be of significant importance in two types of applications where the critical issues are the importance to preserve the sample integrity and the limited amount of available sample.

FTIR-VAR technique used as non-destructive way to analyze archaeological artifacts, based on comparison between reference spectra of fossil resin-amber and spectra of archaeological material and the assignment of specific

adsorption bands, was fit for the purpose of ascribing the origin of samples found in archaeological sites.

In the same time, FTIR spectroscopy was used to confirm that APTES, TESBA and Laccase were successfully deposited on the ITO-NP.

FTIR analysis of APTES and TESBA coated ITO-NP proved the presence of amino and carbonyl functional groups on their surface, functional groups available for binding with biological compounds. The nanoparticles so functionalized were further used for enzyme immobilization.

Presented data justify the usefulness of the technique when the analysis is dealing with valuable samples, sometimes of patrimony value, as it was the amber samples case study, or when the analyses have to provide arguments on specific structural changes, as it was the case of modified surfaces study.

Acknowledgements

Georgiana-Ileana Truica acknowledges the Sectoral Operational Programme Human Resources Development 2007-2013 of the Romanian Ministry of Labour, Family and Social Protection through the Financial Agreement POSDRU/88/1.5/S/61178 and Ramona Penu acknowledges CNCS-UEFISCDI, project number PN II-RU TE-100/2010.

R E F E R E N C E S

1. *C.W. Beck,* ''Spectroscopic investigations of amber'' in Applied Spectroscopy Review, **vol. 22,** 1986, 57–200.
2. *A.M. Shedrinsky, T.P. Wampler and K.V. Chugunov* , ''The examination of amber beads from the collection of the state hermitage museum found in Arzhan-2 burial memorial site'' in Journal of Analytical and Applied Pyrolysis, **vol. 71**, no. 1, 2004, 69–81.
3. *I. Angelini and P. Bellintani*, ''Archaeological ambers from northern Italy: an FTIR–DRIFT study of provenance by comparison with the geological amber database'' in Archaeometry, **vol**. **47**, no.2, 2005, 441-454.
4. *M. Guiliano, L. Asia, G. Onoratini and G. Mille*, ''Applications of diamond crystal ATR FTIR spectroscopy to the characterization of ambers'' in Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, **vol. 67**, no. 5, 2007, 1407-1411.
5. *I. Pakutinskiene, J. Kiuberis, P. Bezdicka, J. Senvaitiene and A. Kareiva*, ''Analytical characterization of Baltic amber by FTIR, XRD and SEM'' in Canadian Journal of Analytical Science Spectroscopy, **vol. 52**, 2007, 287- 294.
6. *Y. Mei, L. Miller, W. Gao, R.A. Gross,* ''Imaging the Distribution and Secondary Structure of Immobilized Enzymes Using Infrared Microspectroscopy" in BioMacromolecules, **vol. 4**, 2003, 70-74.
7. *L.A. Forato, R. Bernardes-Filho, L.A. Colnago,* ''Protein structure in KBr pellets by infrared spectroscopy" in Analytical Biochemistry, **vol. 259**, 1998, 136-141.
8. *J. Wang, G. Liang, W. Zhao, Z. Zhang,* ''Enzymatic surface modification of PBO fibres'', in Surface and Coating Technology", **vol. 201**, 2007, 4800-4804.

9. *L.S. Shlyakhtenko, A.A. Gall, J.J. Weimer, D.D. Hawn, Y.L. Lyubchenko,* ''Atomic Force Microscopy Imaging of DNA Covalently Immobilized on a Functionalized Mica Substrate", in Biophysical Journal, **vol. 77**, 1999, 568–576.
10. *M. Diaconu, A. Tache, S. A-M. V. Eremia, F. Gatea, S. Litescu, G.L. Radu*, ''Structural caracterization of chitosan coated silicon nanoparticles- a FT-IR approach" in U.P.B. Scientific Bulletin, **vol. 72**, 2010, 115-122.
11. *K.M.K. Selim, Y. Ha, S. Kim, Y. Chang, T. Kim, G. Lee, I. Kang,* ''Surface modification of magnetite nanoparticles using lactobionic acid and their interaction with hepatocytes*"*in Biomaterials, **vol. 28**, 2007, 710-716.
12. *K. Herve´, L. Douziech-Eyrolles, E. Munnier, S. Cohen-Jonathan, M. Souce´, H.Marchais, P. Limelette, F. Warmont, M.L. Saboungi, P. Dubois, I. Chourpa,* ''The development of stable aqueous suspensions of PEGylated SPIONs for biomedical applications" in Nanotechnology, **vol. 19**, 2008, 465608 – 465701.
13. *G. Arslan, M. Ozmen, B.Gunduz, X. Zhang, M. Ersoz,* ''Surface Modification of Glass Beads with an Aminosilane Monolayer", in Turkish Journal of Chemistry, **vol. 30,** 2006, 203 -210.