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*Dedicated to the memory of*
*Professor Eugen Segal (1933-2013)*

# MODIFICATION OF PROTEIN CONFORMATION CAN BE MONITORED BY FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY IN ONCOLOGICAL PATIENTS

Mioara DROBOTA,[a,b,*] Irena GRIEROSU,[c] Iulian RADU[c] and Cipriana STEFANESCU[c]

[a] "Petru Poni" Institute of Macromolecular Chemistry, 41A Gr. Ghica Voda Alley, 700487 Iaşi, Roumania
[b] University Politehnica of Bucharest, 313 Splaiul Independentei, 060042, Bucharest, Roumania
[c] Faculty of Medicine, University of Medicine and Pharmacy, "Gr. T. Popa", 16 University Street, 700115, Iaşi, Roumania

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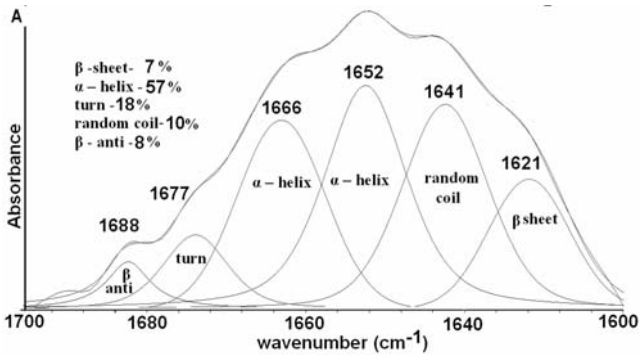
This article presents the experimental results concerning the physiological serum and in order to gain some insight about new understanding of the mechanism of radiotracers we studied some of them, focusing especially on albumin, by Fourier Transform Infrared (FTIR) spectroscopy. From FTIR data can be used as molecular signatures for physiological status once the spectral patterns are correlated with biological properties. FTIR spectroscopy with Fourier self-deconvolution technique was used to determine the protein secondary structure of human serum albumin (HSA, used as vector molecule in medicine) interaction by physiological serum and drug (ciprofloxacin) by Bruker Vertex 70 FTIR spectrometer, equipped with a diamond ATR device (Golden Gate, Bruker).




## INTRODUCTION

A radiotracer is a vector molecule binded with a radioisotope. The radiotracers are used in scintigraphy, mainly in oncologic diagnosis.

Fourier Transform Infrared (FTIR) data can be used as molecular signatures for physiological status once the spectral patterns are correlated with biological properties; therefore, FTIR spectroscopy is a unique resource to provide a global analysis of the organic contents of biological samples. An important analytical advantage of FTIR is that no sample content modification is required before analysis.

In medical sciences, FTIR spectroscopy could identify different strains of microorganisms from viruses, bacteria, fungi and parasites. Although it has wide applications, it is yet to be widely used in routine clinical diagnosis practice. FTIR spectroscopy can be used in clinical situations from inflammatory lesions to cancer diagnosis.

In this paper, our aimed is to evaluate some radiopharmaceuticals based on FTIR – ATR (attenuated total reflectance) to determine some interactions as the impact of nanoconjugates used in image diagnosis, the biological biocompatible and stability, the high selectivity for biological targets. These may be useful in the clinical

*Corresponding author: miamiara@icmpp.ro

presumptive image diagnosis. The distribution and metabolism of many biologically active compounds in the body whether drugs or natural products are correlated with their affinities toward serum albumin. Thus, the study of the interaction of such molecules with albumin is of imperative and fundamental importance. Extensive studies on different aspects of drug – HSA interactions are still in progress because of the clinical significance of the process.[1, 2]

Its physiological and pharmacological properties have been extensively studied over several decades. In addition to its ordinary clinical applications, many investigators have attempted to utilize HSA as a carrier to deliver various drugs to their specific targets. For many drugs, interaction to serum albumin is a critical determinant of their distribution and pharmacokinetics.[3]

This study will be limited to the mid-range infrared, which covers the frequency range from 600 to 4000 $cm^{-1}$. This wavelength region includes bands that arise from three conformational sensitive vibrations within the peptide backbone (Amides I, II and III). Among them amide I is the most widely used band because it can provide information on secondary structure composition and structural stability. [4-6]

Infrared spectroscopy provides measurements of molecular vibrations due to the specific absorption of infrared radiation by chemical bonds. It is known that the form and frequency of the Amide I band, which is assigned to the $\upsilon$(C=O) stretching vibration within the peptide bonds is very characteristic for the structure of the studied protein.[7, 8] From the bands of secondary structures, components of α-helix, β-sheets peaks can be derived and the analysis of these bands allows us to elucidate the conformational changes with high sensitivity.[9, 10]

## EXPERIMENTAL

Human serum albumin colloidal particles (HSA, vector molecule) 0.5 mg powder for injection for diagnosis use were purchased. Ciprofloxacin was purchased from Merck Chemical Company (Germany) Physiological serum from Sigma-Aldrich.

Human serum albumin (1mg) was dissolved in physiological serum (1ml) obtained, this solution in interaction by Ciprofloxacin (1 mg) drug were analyzed. In this study, under normal physiological conditions, to determine the effect of temperature on the secondary structure of HSA used two solution (1) in physiological serum and (2) in physiological serum interaction by Ciprofloxacin drug.

Spectra were collected with FTIR spectrophotometer. The FTIR spectra were recorded on a Bruker Vertex 70 FTIR spectrophotometer, equipped with a diamond ATR device (Golden Gate, Bruker). Specac's High Temperature Golden Gate™ ATR Accessory is a high performance single reflection (45° angle) monolithic diamond ATR product offer for spectroscopic sample analysis of samples up to 200°C. The absorption was measured in a wavenumber range from 4000 to 600 $cm^{-1}$. For a spectrum 128 scans were taken, with a baseline correction.

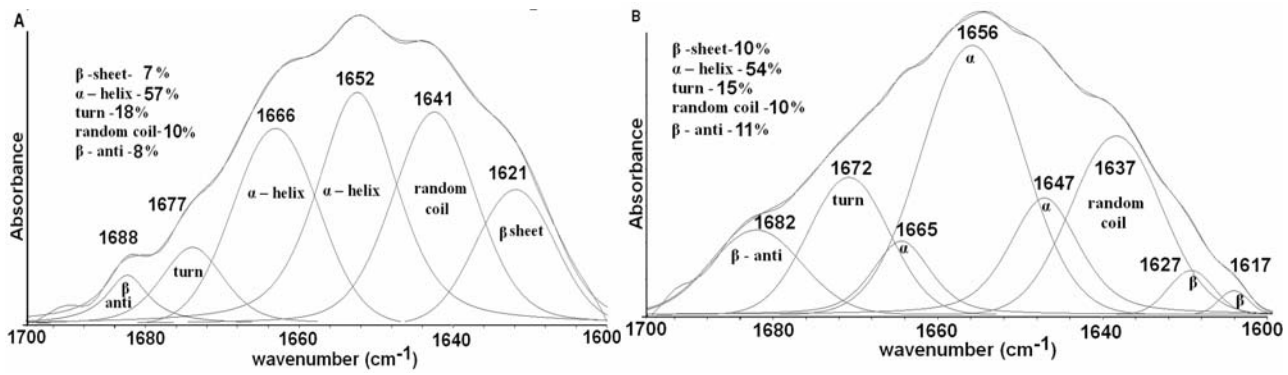
To study the effect of temperature on secondary structure of the solutions were heated in the FTIR-ATR from 28, 37 and 40 $^{o}$C. At each temperature, the inregistration was equilibrated for 5 min prior to recording of its spectrum. Fourier self deconvolution (FSD) was performed between 1800 and 1600 $cm^{-1}$. Spectra were collected of HSA in solutions at room temperature and measured in triplicate to eliminate errors. The infrared spectra of HSA, and Ciprofloxacin – HSA interaction were obtained in the region of 1000 –1800 $cm^{-1}$. The difference spectra [(HSA powder + physiological serum) − physiological serum] and [(HSA powder + physiological serum + ciprofloxacin) − physiological serum] were generated using the featureless region of the protein solution 1800 - 2200 $cm^{-1}$ as an internal standard.[10]

Baseline correction, normalization and peak areas calculations were performed for all the spectra by OPUS 6.5 software. The peak positions were determined using the second derivative of the spectra. Amide I is the most sensitive probe for detecting changes in the protein secondary structures. Its characteristic absorption band is located at 1600–1700 $cm^{-1}$. Finally, with use of the Gaussian/Lorentzian profile, each spectrum was deconvoluted. The contents of component bands for an aqueous HSA solution, as well as the peak positions, are consistent with those reported in the literature.[11] Percentages have been corrected previously obtained from total area. The resolution enhancement resulting from self-deconvolution and the second derivative was such that the number and the position of the bands to be fitted were determined. In order to quantify the area of the different components of amide I contour, revealed. [12]

To determine the effect of temperature on the secondary structure of HSA in serum solution and interaction by ciprofloxacin drug.

Fig. 1 shows the changes in the secondary structures, the values by comparing the spectra of HSA both with and without the presence of ciprofloxacin in amide I region. Information about the different types of secondary structures such as α-helix, β-sheets, turns and random coil can be obtained. The measurements at 28 $^{o}$C temperature are reflecting those changes on the albumin molecule which do not yet have any influence on the helix content.

Fig. 1 shows the fitting analysis of amide I profile in FTIR spectrum HSA (Fig. 1(a)) and in spectrum HSA – ciprofloxacin (Fig. 1(b)). In FTIR spectrum, it contains five major components. They are located at 1610-1627 $cm^{-1}$ (β-sheets), 1627 - 1643 $cm^{-1}$ (random coils), 1643-1672 $cm^{-1}$ (α-helix), 1672-1687 $cm^{-1}$ (turns) and 1687-1700 $cm^{-1}$ (β-sheets antiparallel).[11-13] Based on the above assignments, the percentages of each secondary structure of HSA were calculated by integrated areas of the component bands in amide I, then summed and divided by the total area. The obtained number is taken as the proportion of secondary structures chain in that conformation.




(a) (b)

Fig. 1 – Curve-fitted amide I (1,700 – 1,600 $cm^{-1}$) region in physiological serum at 28 $^oC$ of (a) free HSA and (b) HSA– ciprofloxacin complex.

## RESULTS AND DISCUSSION

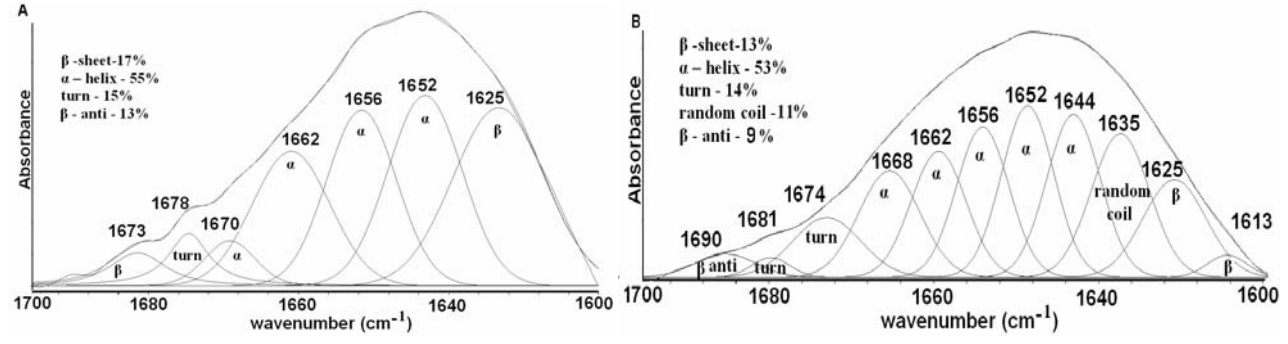
The changes of these peak positions and peak shapes implied that the secondary structures of HSA had been changed by the interaction of ciprofloxacin with HSA. The minor changes in peak positions can be attributed to the effect of the newly formal H-bonding between drug molecules with the albumin.

The effect of temperature on the conformational stability of HSA (Figs. 2 and 3) indicated the structural transformation from α-helix to random coil due to the shifting of the predominant peak from 1652 $cm^{-1}$ to 1647 $cm^{-1}$. The appearance of lower frequency band suggested the formation of an intermolecular hydrogen-bonded antiparallel β-sheet structure to cause thermally-induced protein aggregation.[14-16] This phenomena was observed in both free HSA and HSA-ciprofloxacin mixture. These results can be explained by the location of α-helices as well as the hydrogen bonding between its residues are longer than their corresponding ones in both types of β-sheets.[16] The hydrogen bonds are more susceptible to be broken by both temperature and ciprofloxacin interactions in α-helices than hydrogen bonds in both types of β-sheets. The relative decrease in α-helices molecular content percentage cause the relative increase in β-sheets molecular. The changes in conformation from 37 $^oC$ (temperature typical human body) to 40 $^oC$ are not significant.

The relative intensity increases- for the antiparallel β-sheets while it remarkably decreases for α-helix and the rate of increase for antiparallel β-sheets remarkably faster than the parallel β-sheets.[17]

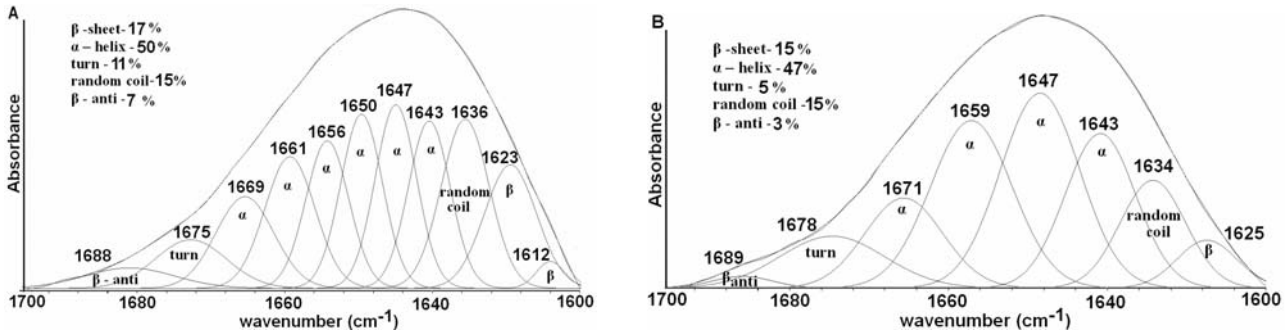
The difference in behavior for the two types of β-sheets can be explained by the different amino acids and their preferred secondary structural arrangements in these β-sheets. It has been reported that the two forms of β-sheets have different thermo-dynamic propensities scale.[15]

Furthermore, the content of α-helix structure of HSA decreased but the heating process made no major alteration for α-helix. It is well known that the denaturation of protein is only partially reversible in many cases, but in our cases this decrease is not so pronounced.




(a) (b)

Fig. 2 – Curve-fitted amide I (1,700 – 1,600 $cm^{-1}$) region in physiological serum at 37 $^oC$ of (a) free HSA and (b) HSA– ciprofloxacin complex.




(a) (b)

Fig. 3 – Curve-fitted amide I (1,700 – 1,600 $cm^{-1}$) region in physiological serum at 40 $^oC$ of (a) free HSA and (b) HSA– ciprofloxacin complex.

Thus, the hydrogen bond system is disturbed in both cyclic and noncyclic fragments of the main chain and the aminoacid. It is suggested that, the shift to a higher frequency for the major peak in amide I region (1656-1658 $cm^{-1}$) came as a result of stabilization by hydrogen bonding.[18]

The shifts in peak positions and shape of HSA amides after ciprofloxacin mixing with HSA come from the changes in protein secondary structure. The minor changes in peak positions can be attributed to the effect of the newly imposed H-bonding between the ciprofloxacin molecules and the protein.

## CONCLUSIONS

The interaction of physiological serum to HSA has been proved by FTIR spectroscopy. Analysis of the FTIR spectra reveals that HSA – physiological serum interaction results are not major protein secondary structural changes in the compositions of α-helix to β-sheets. Reflected in the decrease of relative percentage of α - helices parallel with an increase in the relative percentage of β-sheets in HSA secondary structure composition.[19] Normal doses of ciprofloxacin do not have major effect on the HSA secondary structure composition at 37 $^oC$, however have a mild effect on the HSA optical properties at 40$^oC$. Ciprofloxacin mixing with HSA at physiological and pathological temperatures and therefore not altering its binding properties, did not heavily influence the conformation of which could be significant interference in drug and complications.[20, 21] That means that the patients that took drugs ciprofloxacin as current medication and need to do scintigraphy with nanocolloids (HSA) have not major interaction that can change the oncological diagnosis and images.

In future we intended to continue our researches areas. The FTIR technique needs, however, to improve in sensitivity and accuracy compared with histology. The aim of future investigations with FTIR spectroscopy is to detect cancer in vivo using intra-operative or pre-operative diagnosis to avoid unnecessary dissection to minimize surgical trauma.

The mean FTIR spectra for parathyroid adenoma, hyperplasia, thyroid tissue and lymph nodes plotted with deviation range were shown for each pathology group. FTIR spectra measured from standard can distinguish between the four pathology groups despite the variability observed in the measured spectra. Biological tissue is essentially made up of proteins, nucleic acids, carbohydrates and lipids each of which have their unique IR spectra.

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