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Original Research Article

A Stimulated FT–IR Biospectroscopic Study of Ritonavir Protective and Therapeutic Effect as a Potent Drug on *Coronavirus* Disease–2019 (*COVID–19*) Infection

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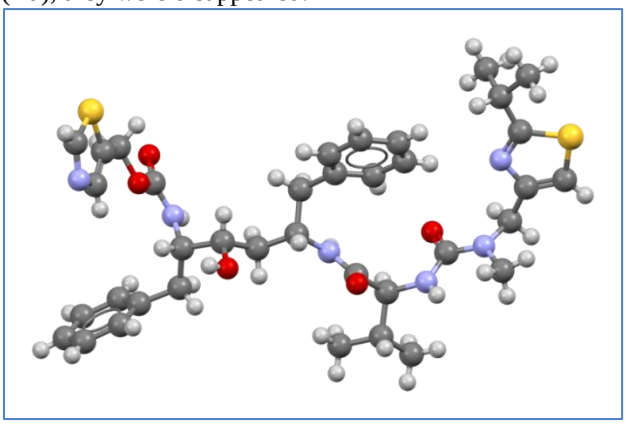
Abstract

Ritonavir is an antiretroviral of the protease inhibitor class. It is used against HIV infections as a fixed–dose combination with another protease inhibitor, ritonavir (lopinavir/ritonavir). In the current research, the stimulated FT–IR biospectroscopy of liquid sample of Ritonavir was investigated. The stimulated FT–IR diffractions emitted through focusing the second harmonic laser beam Nd:YAG into the sample were recorded by Echelle spectrometer and ICCD detector. Increasing the energy of laser beam from 2.6 (mJ) to 16 (mJ) was led to increase in stimulated FT–IR signal but after breakdown threshold of liquid sample, more increasing of energy was led to decrease in stimulate FT–IR signals and for energies higher than 20 (mJ), they were disappeared.



Ball–and–stick model of the ritonavir molecule, $C_{37}H_{48}N_6O_5S_2$, as found in the crystal structure reported in *Pharm. Res.* (2001) 18 859–866 (CSD Entry: YIGPIO02). Colour code: Carbon, C: grey Hydrogen, H: white Nitrogen, N: blue Oxygen, O: red Sulfur, S: yellow Model manipulated and image generated in CCDC Mercury 3.8.

Keywords: FT–IR Biospectroscopy, Stimulated FT–IR Biospectroscopy, Ritonavir, Breakdown, Coronavirus Disease–2019, COVID–19, Infection, Protective and Therapeutic Effect, Potent Drug.

INTRODUCTION

FT–IR biospectroscopy is a vibration biospectroscopy based on the influence of FT–IR [1–47]. The influence of FT–IR is elastically diffracting the electromagnetic ray due to rotational and vibrational transitions in molecules and its characteristic is changing the energy of diffracted beam photons compared to incident beam [48–95]. The difference between wavelength of incident beam light and diffracted light is related to molecular vibrations and is considered as exclusive "chemical finger print" of sample and can be used in identification of molecular compounds on a surface, into a liquid or into the air [96–142].

The stimulated FT–IR diffraction is a non–linear effect [143–189]. If the pumping intensity exceeds the threshold of this effect, it observes [190–

237]. The pumping threshold limit for stimulated FT–IR depends on FT–IR active material [238–285]. Regarding the spectral characteristics, stimulated FT–IR can be distinguished from normal FT–IR [286–333]. While the intensity of FT–IR bands are several times smaller than pumping laser intensity in normal FT–IR, the intensity of FT–IR bands in stimulated FT–IR can be similar to laser intensity and for most materials, only strongest FT–IR bands of material are intensified and are dominant in the recorded spectrum of material [334–377].

In the current research, the stimulated FT–IR spectrum are obtained through pumping the second harmonic beam laser Nd:YAG and it is performed by a spectrometer and detector. The resulted spectra and their characteristics are investigated here.

EXPERIMENTAL ARRANGEMENT

The experimental arrangement used in the current study is schematically shown in Figure (1). The first harmonic bicolor mirror reflects 1064 (nm) but passes the second harmonic one. As a result, the first harmonic removes from laser beam. The second harmonic laser Nd:YAG with wavelength of 532 (nm) and pulse width of 8 (ns) interacts with the sample after passing through bicolor mirror and lens with focal length of 3.5 (cm). The resulted emissions from this interaction filters by an optical system consisting some lens and optical fiber conducts to Eschelle spectrometer. The necessary time range for collecting spectra and its start time in ICCD detector controls by delayer device. Optical emissions of sample collects and intensifies from the striking moment of laser to sample until 5 (ms) after that moment. Test was repeated five times for each energy level for laser energy from 2.4 (mJ) to 29 (mJ).

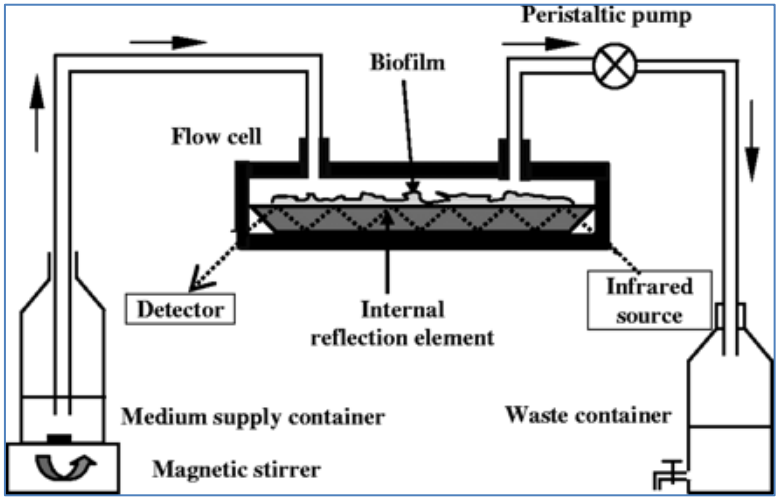


Fig-1: Schematic of stimulated FT–IR biospectroscopy test arrangement.

RESULTS AND DISCUSSION

Figure (2) shows the normal and stimulated FT–IR spectra. Normal FT–IR spectrum can be obtained when laser beam is not focused on the sample. When laser beam focuses on sample using a lens, non–linear effects stimulate and stronger bands of FT–IR spectrum intensify up to some levels of laser intensity.

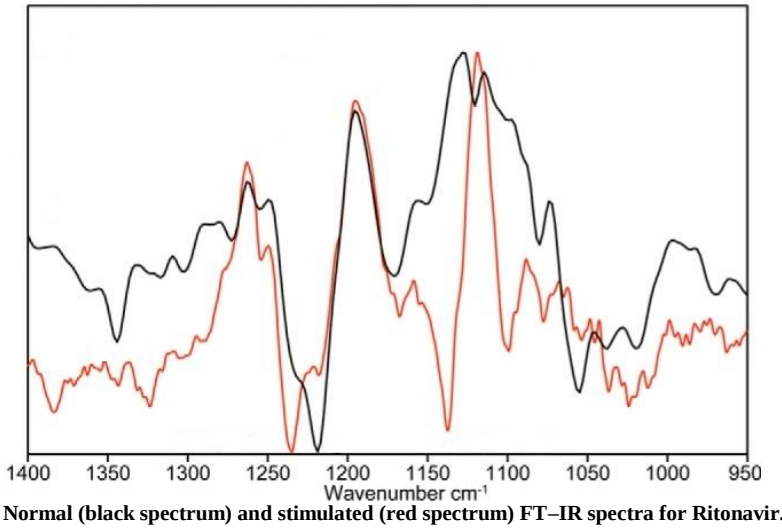


Fig-2: Normal (black spectrum) and stimulated (red spectrum) FT–IR spectra for Ritonavir.

By increasing the energy of laser beam, the intensity of main bands of 3123 (cm^{-1}) and 3444 (cm^{-1}) also are increased and for energy levels higher than 8 (mJ), anti–Stokes FT–IR band corresponding to 3123 (cm^{-1}) intensifies in the spectrum and can be observed at left hand side of laser line in FT–IR shift of –3123 (cm^{-1}). Recording the anti–Stokes band necessitates the occupation of corresponding vibration level through diffraction of Stokes FT–IR (Table 1).

By more increasing the energy level higher than 16 (mJ), all four graphs of Figure (3) shows reduction in intensity. The reason for this reduction is creation of spark in the Ritonavir liquid due to increase in energy of laser more than the breakdown threshold of liquid. As a result of this spark, which creates in the center of liquid, laser beam absorbs by liquid and some part of it diffracts and only this part plays a role in creation of stimulated FT–IR. By increasing the energy, beam has higher contribution in making the spark and the diffracted emission which reaches to detector decreases.

Table-1: FT–IR modes for Ritonavir

	FT–IR Shift (cm^{-1})	FT–IR Mode
1	900 (cm^{-1})	C–H Stretch
2	1327 (cm^{-1})	CH_2 Rocking
4	1685 (cm^{-1})	CH_2 Wagging
5	3123 (cm^{-1})	CH_2 Symmetric Stretch
7	3444 (cm^{-1})	C–H Asymmetric Stretch

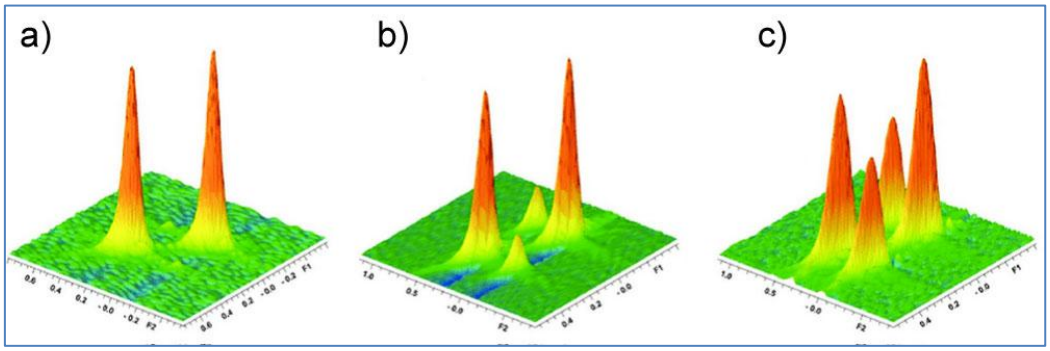


Fig-3: Peak intensity (a) band 3123 (cm^{-1}) (b) band 3444 (cm^{-1}) and (c) band –3123 (cm^{-1}) based on increase in energy level of beam focused on the liquid.

CONCLUSIONS AND SUMMARY

The stimulated FT–IR biospectroscopy test was performed for liquid sample of Ritonavir. The main band at 3123 (cm^{-1}) shows an intensity level comparable to pumping laser intensity. The intensity of stimulated FT–IR spectrum at 16 (mJ) energy level is the highest intensity in this test and more increasing the energy level reduces the intensity of spectrum. The reason for this reduction is creation of spark in the Ritonavir liquid due to increase in energy of laser more than the breakdown threshold of Ritonavir.

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