

# Study of Stimulated Raman Biospectroscopy in Ritonavir as a Potent Drug against *Coronavirus Disease–2019 (COVID–19)* Infection

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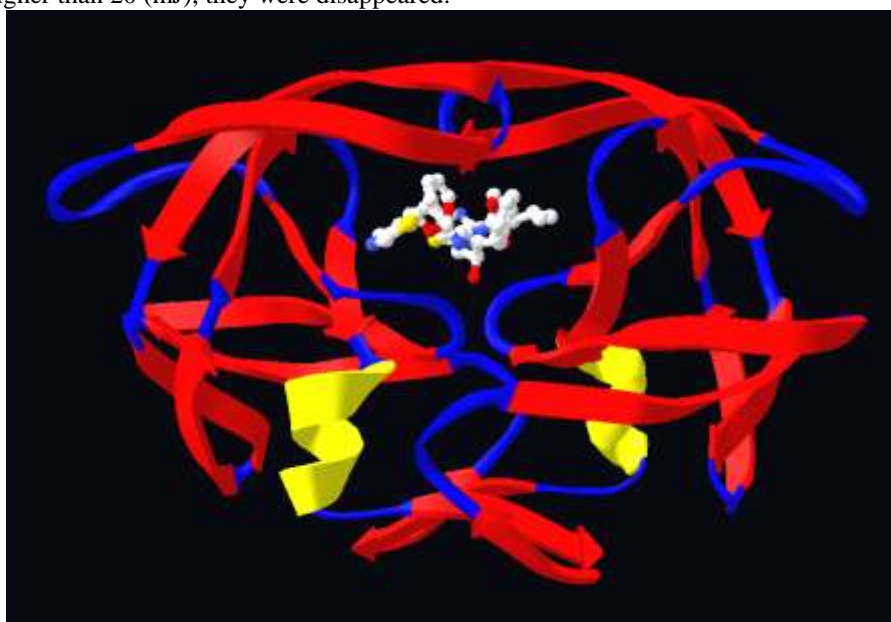
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## Abstract

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 Raman biospectroscopy of liquid sample of Ritonavir was investigated. The stimulated Raman 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 Raman signal but after breakdown threshold of liquid sample, more increasing of energy was led to decrease in stimulate Raman signals and for energies higher than 20 (mJ), they were disappeared.



Ritonavir (center) bound to the active site of HIV protease.

**Keywords:** Raman Biospectroscopy, Stimulated Raman Biospectroscopy, Ritonavir, Breakdown, Coronavirus Disease–2019, COVID–19, Infection.

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## INTRODUCTION

Raman biospectroscopy is a vibration biospectroscopy based on the influence of Raman [1–47]. The influence of Raman 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 Raman 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 Raman depends on Raman active material [238–285]. Regarding the spectral characteristics, stimulated Raman can be distinguished from normal Raman [286–333]. While the intensity of Raman bands are several times smaller than pumping laser intensity in normal Raman, the intensity of Raman bands in stimulated Raman can be similar to laser intensity and for most materials, only strongest Raman bands of material are intensified and are dominant in the recorded spectrum of material [334–377].

In the current research, the stimulated Raman 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).

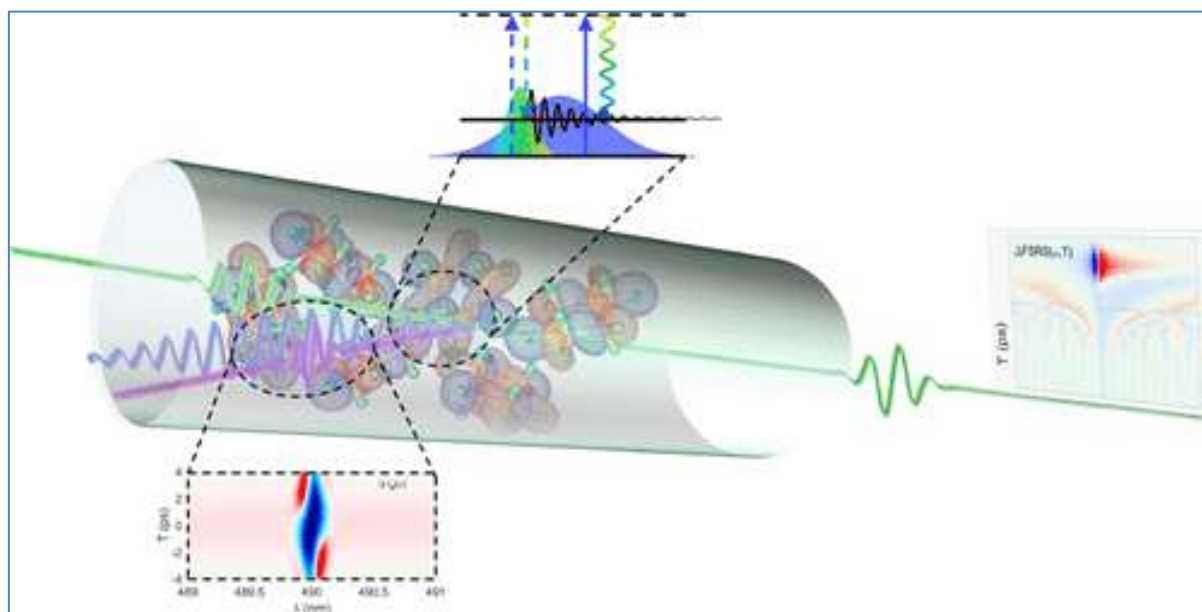
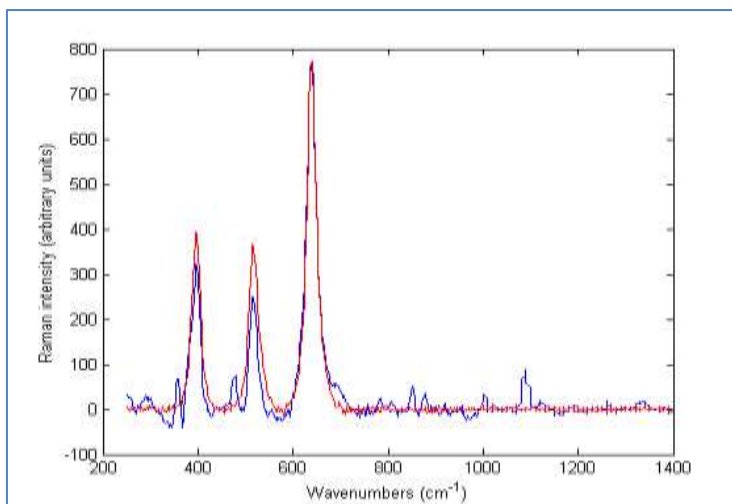


Fig-1: Schematic of stimulated Raman biospectroscopy test arrangement.

## RESULTS AND DISCUSSION

Figure (2) shows the normal and stimulated Raman spectra. Normal Raman 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 Raman spectrum intensify up to some levels of laser intensity.



**Fig-2: Normal (blue spectrum) and stimulated (red spectrum) Raman spectra for Ritonavir.**

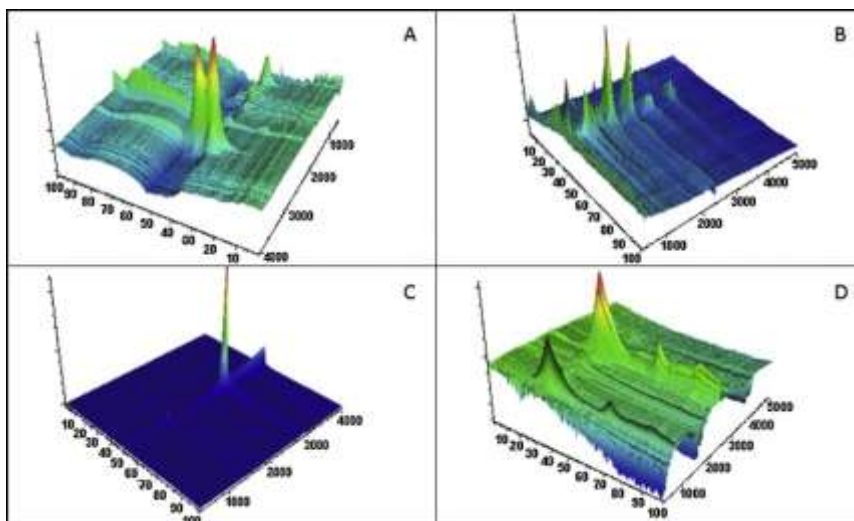
By increasing the energy of laser beam, the intensity of main bands of 2852 (cm<sup>-1</sup>) and 2938 (cm<sup>-1</sup>) also are increased and for energy levels higher than 8 (mJ), anti-Stokes Raman band corresponding to 2852 (cm<sup>-1</sup>) intensifies in the spectrum and can be observed at left hand side of laser line in Raman shift of -2852 (cm<sup>-1</sup>). Recording the anti-Stokes band necessitates the occupation of corresponding vibration level through diffraction of Stokes Raman (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 Raman. By increasing the energy, beam has higher contribution in making the spark and the diffracted emission which reaches to detector decreases.

**Table-1: Raman modes for Ritonavir**

	Raman Shift (cm <sup>-1</sup> )	Raman Mode
1	800 (cm <sup>-1</sup> )	C-H Stretch
2	1028 (cm <sup>-1</sup> )	CH <sub>2</sub> Rocking
4	1400 (cm <sup>-1</sup> )	CH <sub>2</sub> Wagging
5	2852 (cm <sup>-1</sup> )	CH <sub>2</sub> Symmetric Stretch
7	2938 (cm <sup>-1</sup> )	C-H Asymmetric Stretch



**Fig-3: Peak intensity (a) band 2852 (cm<sup>-1</sup>) (b) band 2938 (cm<sup>-1</sup>), (c) band -2852 (cm<sup>-1</sup>) and (d) laser based on increase in energy level of beam focused on the liquid.**

## CONCLUSIONS AND SUMMARY

The stimulated Raman biospectroscopy test was performed for liquid sample of Ritonavir. The main band at 2852 ( $\text{cm}^{-1}$ ) shows an intensity level comparable to pumping laser intensity. The intensity of stimulated Raman 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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