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Study the Effects of Helium Neon (He-Ne) Laser Irradiation on Normal Human Blood in Vitro Using FTIR Techniques

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Abstract and Keywords: *Interaction of lasers with biomaterials such as blood, skin, and tissues is an important area of research. Laser tissue interactions can be understood by using spectroscopic and advanced microscopic techniques such as scanning electron microscope (SEM). In the present, work blood samples were collected from normal human subjects under standard laboratory conditions. Blood samples were irradiated by He-Ne laser (Wavelength λ = 632.8 nm, Power P = 3mW). The FTIR spectra for non-irradiated normal blood samples are compared with the FTIR spectra of irradiated blood samples. Significant changes are observed between the various bonds from the FTIR transmission spectra between C=O (Amide I), C-O (Anhydrides), N=O (Nitro), C-N (Amines) and C-H (Alkenes) etc. The significant results are obtained when He-Ne laser irradiation is incident on whole blood for 30 and 40 minutes and the transmittance decreases due to denaturation of proteins. The present work will provide more inside into laser irradiation - blood interactions and form a basic for therapeutic uses.*
Keywords: *FTIR; Helium Neon (He-Ne) laser,Normal human Blood.*

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I. Introduction

In photochemical interactions, light induces reactions and chemical effects within the tissue. Biostimulation and photodynamic therapy are essentially based on photochemical interactions [1]. The absorption spectra of whole blood, erythrocytes, and plasma to study photochemical reactions initiated by exposure of blood in vivo to UV radiation has been reported [2]. In biostimulation methods laser irradiation is used to treat some diseases [3]. He-Ne laser illumination of mitochondria isolated from rat liver showed increase in proton electrochemical potential and ATP synthesis [4]. For regenerating crust head rat facial nerves, best response was reported by 633 nm He-Ne laser [5]. Fourier transform spectroscopy is applied to biofluids and disease patterns have been identified. Structural and conformational changes of proteins, nucleic acids and lipids have been reported on the basis of FTIR. Complex structure of globular proteins and biomolecules in blood are identified on the basis of FTIR spectra [3]. The main objective of the present work is to study the changes in FTIR spectra of whole blood in vitro due to He-Ne laser irradiation (Wavelength λ = 632.8 nm, power P = 3 mW) and report the changes in various functional groups.

II. Experimental Work

He-Ne laser is incident on 2 ml blood samples for four cuvette for 10, 20, 30, and 40 minutes. FTIR spectra for normal and irradiated blood samples are recorded. The effect of He-Ne laser irradiation on normal and irradiated blood samples for various bonds is compared. From healthy human, 2 ml venous blood was extracted by sterile syringe using standard procedure for anticoagulation. Whole blood sample (2 ml) was taken using a micropipette in a cuvette and irradiated with a diode He-Ne laser (wavelength λ= 632.8 nm, power = 3 mW). Fourier Transform Infra Red Spectra (FTIR) is obtained using FTIR spectrophotometer (Jasco-6100) for control and He-Ne laser irradiated whole blood samples.

III. Results And Discussions

FTIR spectra of normal whole blood without laser irradiation shows the groups O-H, C=O, C-O and C-H in the region between the wave numbers 4000 to 400 cm^{-1}(Figure 1).

The wave number 3325.93 cm^{-1} indicate O- H bond peak. Amide-I is mainly associated with C-O, C=O, and C-H stretching vibrations and also related to the backbone conformation. The wave numbers 1639.18cm^{-1}, 1221.29 cm^{-1}, 772.18 cm^{-1} indicate C=O, C-O and C-H peaks respectively. The absorption peak in the region 1400cm^{-1} to 1200 cm^{-1} arises due to the C-H deformation methyl and methylene group of the proteins. The spectral region 3600 - 3000 cm^{-1} comprises of C-H, O-H and N-H stretching vibrations of the

proteins. The prominent absorption peak 3300 cm^{-1} is due to the N-H stretching mode (amide - A) of proteins. The asymmetric and symmetric stretching C-H vibrations of methyl and methylene group are found to be present around 2930 - 2875 cm^{-1}.The strong absorption band at 1650 cm^{-1} corresponds to C=O stretching vibrations (amide - I), whereas the vibration band at 1542 $cm^{-1}$is attributed to amide - II arising of N-H bending vibrations strongly coupled with C-N stretching of proteins.FTIR spectra of normal whole blood irradiated with He-Ne laser for10, 20, 30 and 40 min. duration are shown in Figures 2 to 5.

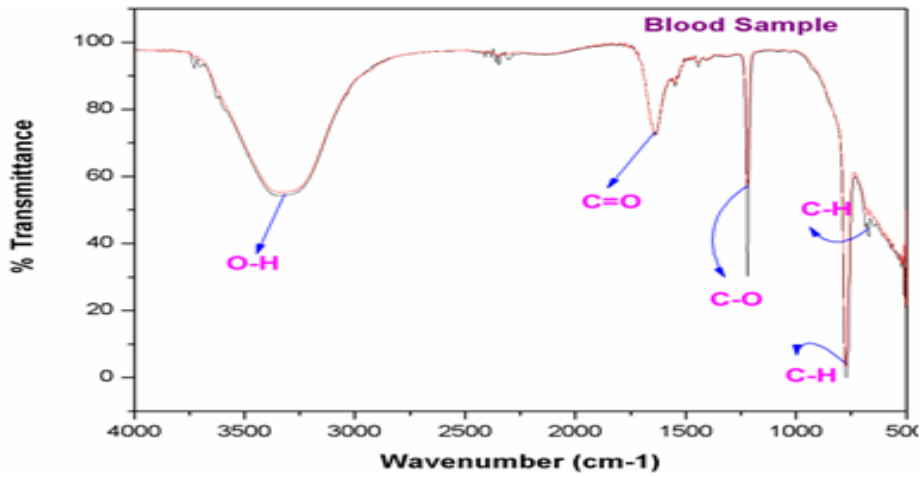
Whole blood samples in vitro is exposed to He-Ne laser irradiation for 10 to 20 min. and FTIR spectra shows the groups O-H and C=O and other groups C-O and C-H are absent. It shows the most intense bands in the IR spectra of blood are due to absorption by polypeptides with a large number of amino acid residues.

The IR bands of the peptide groups, assigned to stretching and bending vibrational bands of the NH, CN, and C=O bonds contain information about the conformational structure of the globular protein molecules. When blood is exposed to low therapeutic doses of light, the spectral changes in the regions of the IR spectrum where the absorption bands of peptide groups appear to be more sensitive to breaking due to weak intermolecular bonds. FTIR spectra of irradiated whole blood with He-Ne laser for 30 and 40 min. show the groups O-H,C=O, C-O, C-H, C-N and N=O.But transmittance decreases for 40 minutes due to denaturation of proteins i.e. it breaks the polypeptide bonds due to conformational changes of proteins polypeptide bonds due to conformational changes of proteins.

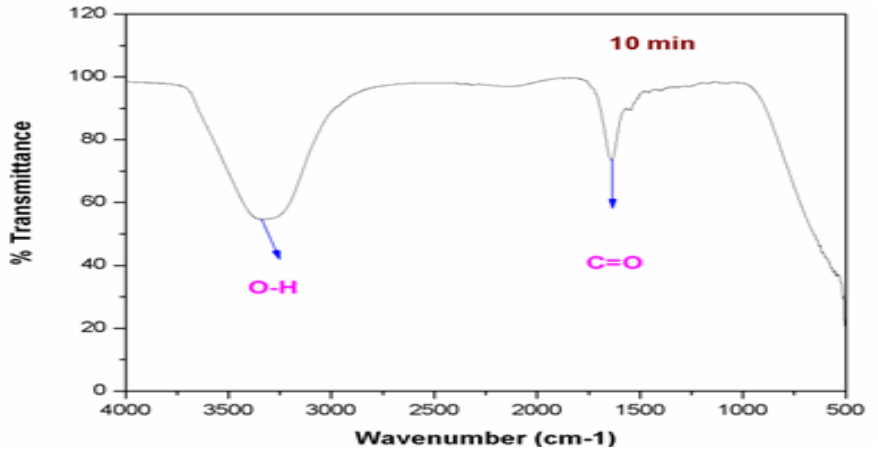
IV. Table And Figure

Table no1:FTIR Spectra Analysis

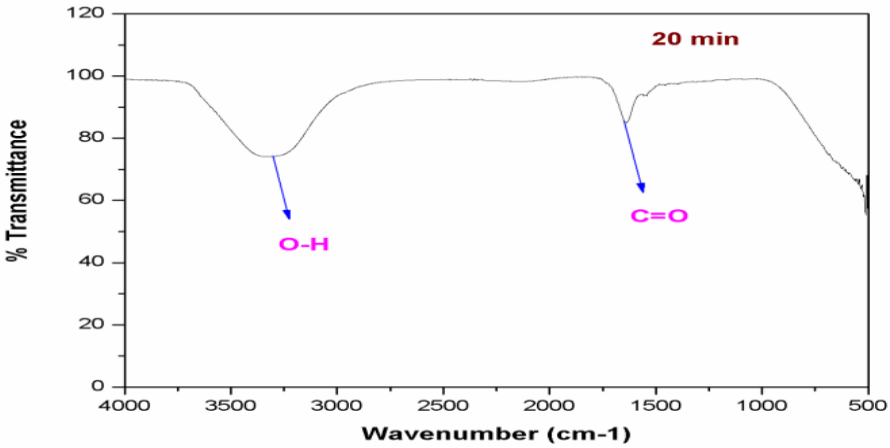
Sr. No.	Wave number cm^{-1}	Group	0/0 T	Irradiated Time (minute)	Wave number cm^{-1}	Group	0/0 T
1.	3325.93	O-H (free group)	54	10	3302.72	O-H (free group)	55.33
2.	1639.18	C=0 (Amide)	72.98		1637.58	C=O (Amide)	75.76
3.	1221.29	C-0 (Anhydrides)	30.72		-	Absent C-O	-
4.	772.18	C-H (Alkenes)	0.79		-	Absent C-H	-
5.	671.317	C-H (Alkenes)	43.90		-	Absent C-H	-
6.	3325.93	O-H (free group)	54	20	3302.98	O-H (free group)	73.80
7.	1639.18	C=0 (Amide)	72.98		1647.98	C=0(Amide)	86.43
8.	1221.29	C-0 (Anhydrides)	30.72		-	Absent C-O	
9.	772.18	C-H (Alkenes)	0.79		-	Absent C-H	-
10.	671.317	C-H (Alkenes)	43.90		-	Absent C-H	-
11.	3325.93	O-H (free group)	54	30	3342.59	O-H (free group)	48.77
12.	1639.18	C=0 (Amide)	72.98		3016.70	C-H (Alkenes)	75.17
13.	1221.29	C-0 (Anhydrides)	30.72		1731.52	C=0 (Aldehyde)	27.38
14.	772.18	C-H (Alkenes)	0.79		1629.03	C=0 (Amide)	36.94
15.	671.317	C-H (Alkenes)	43.90		1363.18	N=O (Nitro)	24.20
16.	-	Absent C-N	-		1218.25	C-N (Amine)	21.48
17.	3325.93	O-H (free group)	54	40	3316.97	O-H (free group)	15.40
18.	1639.18	C=0(Amide)	72.98		1740.33	C=0 (Aldehyde)	14.18
19.	1221.29	C-0 (Anhydrides)	30.72		1645.84	C=0 (Amide)	14.81
20.	772.18	C-H (Alkenes)	79		1363.18	N=O(Nitro)	13.22
21.	671.317	C-H (Alkenes)	43.90		1218.25	C-N (Amine)	12.06



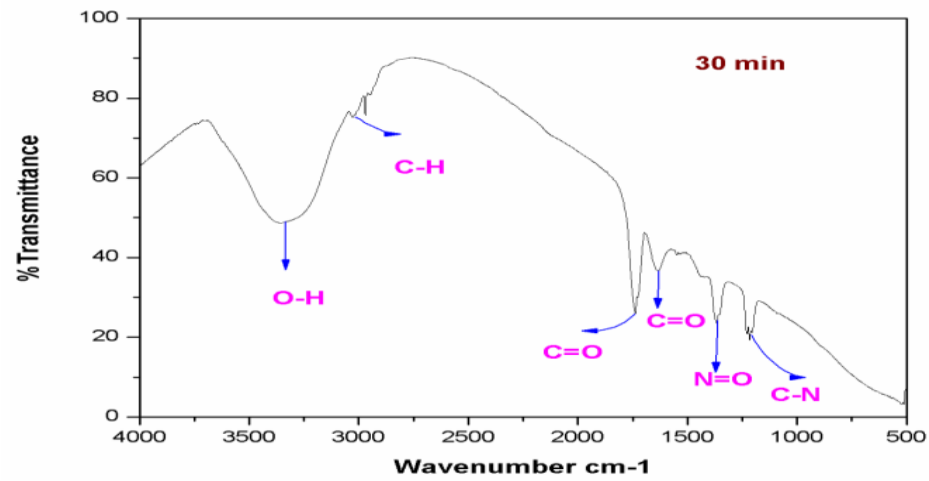
"**Fig"1:** FTIR spectrum for two normal blood samples (control)



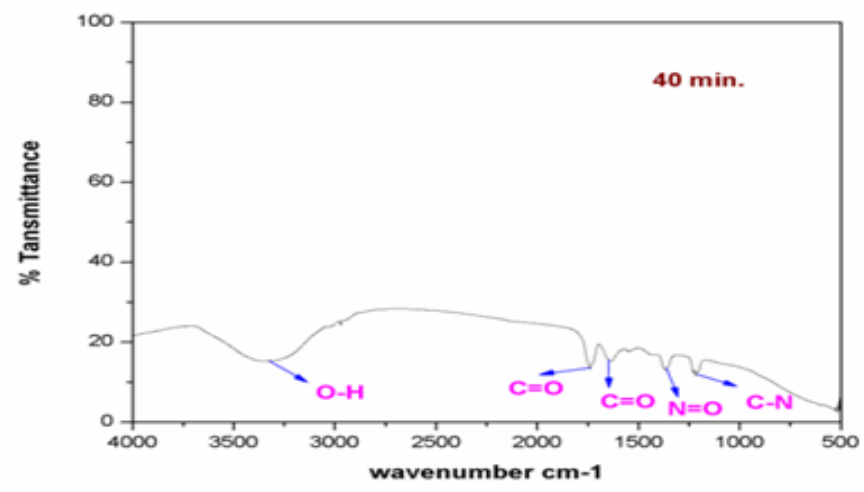
"**Fig" 2:** FTIR spectrum for irradiated blood with He-Ne laser (10 min).



"Fig" 3: FTIR spectrum for irradiated blood with He-Ne laser (20 min).



"Fig" 4: FTIR spectrum for irradiated blood with He-Ne laser (30 min).



"Fig" 5: FTIR spectrum for irradiated blood with He-Ne laser (40 min).

V. Conclusion

In present investigation, study the of changes of normal blood, before and after He-Ne laser irradiation by using Fourier transform infrared spectroscopy is presented. FTIR spectrum of normal blood laser irradiation indicates the groups O-H, C=O, C-O and C-H.As exposure time of He-Ne laser irradiation on whole blood is increased then photon dose also increases.For 40 minutes of laser irradiation on whole blood shows transmittance decreases 30% to all bonds due to denaturation of proteins i.e. it breaks the polypeptide bonds due to conformational changes of proteins. To study the molecular level changes in whole blood and plasma due to laser radiation, the FTIR analysis is one of the good tool.

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