VIBRATIONAL SPECTROSCOPIC STUDY OF INTERACTION BETWEEN HUMAN HEMOGLOBIN AND TWO NATURAL DYES

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Abstract: Vibrational spectroscopy has demonstrated itself to be an important contribution in the investigation of different fields of science, mainly because of the remarkable flexibility of examining strategies. Fourier transform infrared (FTIR) spectroscopy is a non-destructive procedure for structural characterization of proteins and poly peptides. Raman estimation gives the vibrational range of the analysis which can be processed as its "fingerprint", permits simple understanding and recognizable proof. Interactions between Human Hemoglobin and two natural dyes, namely, curcumin and gallic acid have been studied using FTIR and FT-Raman spectroscopy.

Keywords: Human Hemoglobin, natural dyes, FTIR, FT-Raman.

1.Introduction

Fourier Transform Infrared (FTIR) also Raman spectroscopy (RS) are amazing strategies of exploring the formation as well as sub-atomic composition of natural substances [1]. The two procedures depend on the recognition of sub-atomic vibrations giving a composition fingerprint of the extensive metabolome [2,3], that remembers exchanges for the formation or potentially oxidization disposition of macromolecules [4], interchanges in the ancillary structure of the proteins [5] also DNA compliance [6]. Lately, the improvement of FTIR and Raman based techniques in biological investigation has set off an extending area of study [7] handling by ongoing enhancements in instrumentation and progressed multicomponent information investigation systems [8,9]. Raman and FTIR both spectrometers can be coordinated within an optical magnifying lens empowering visualization of individual cells [10].

Infrared spectroscopy depends upon the retention of the FTIR light from vibrating nuclei when the wave number estimation of the occurrence radiation is equivalent to the vacillate nuclei or group transitional energy [11]. The Raman impact, then again, is seen subsequent to enlightening the illustrative with a Ultraviolet (UV), Visible (VIS) or near IR (NIR) and checking the inelastic dispersed light element [12].

The elemental variance in the instrumentation of both methods brings about uniqueness in the properties and highlights of every procedure. Example: I) IR absorption consequences from substitute in the dipole moment as the nuclei oscillate and hence skew and polar particles allow enduring IR activity contrasted with further symmetric non-polar atoms. Interestingly, Raman movement is identified with interchanges in the compatibility tensor of the functional group or particle and when all is said in done just symmetric modes and non-polar oscillating nuclei are powerful Raman scatterers. ii) The Raman effectiveness is very small contrasted and FTIR except if resonance or surface enhanced Raman dissipating components are included. In IR, practically the natural particles show impressive absorbance, the spectra are ordinarily made from numerous covering groups particularly when recording FTIR spectra of cells or tissues. iii) Raman can use visible laser light, which empowers a lateral resolution of under a large portion of a micron to be accomplished contrasted and the low resolution (around 2–10 mm relying upon the frequency) gave by the IR light. iv) Liquid water is a powerful absorber of IR light and producing a range of hydrated cells absence of a splendid IR source is for sure testing [13]. Conversely, solvent exhibition insignificant Raman scattering and therefore the procedure is famously appropriate for the investigation of active cells giving the laser light is of negligible capacity to evade cell harm. v) Focal plane array (FPA) FTIR imaging empowers the investigation of bigger and additional agent regions of cells or tissues in sensible time, though by RS just little territories must be broke down~20 × 20 μm except if under-inspecting is utilized.

The point of this research is to explore the adjustments in the structure of human hemoglobin with the expansion of natural dye like curcumin and gallic acid.

2. Materials and methods

Human hemoglobin, curcumin and gallic acid were purchased from Sigma Aldrich company, Bangalore. The above said synthetic compounds were bought and were utilized without further purification.

FTIR spectra were recorded utilizing AGILENT CARY 630 FTIR Spectrometer. FT-Raman spectra were recorded utilizing BRUKER RFS – 27 STAND alone FT – Raman Spectrometer.

3. Results and Discussion

Fourier Transform Infrared (FTIR) spectroscopy can be utilized to quantify the vibrational modes of functional groups of biomolecules. FTIR spectroscopy was completed for portraying the conformational changes in HHb upon binding to the natural dyes. FTIR procedures were utilized to contemplate the conformational changes in HHb since it has conformational intuitive spectral signature in the IR region [14]. Curcumin and gallic acid nanoparticles were utilizing ionic gelation technique. Figs 1a,1b and 1c

portray the FTIR spectra of hemoglobin, (hemoglobin + curcumin nanoparticles) complex and (hemoglobin + gallic acid nanoparticles) complex separately.

The main vibrational band in Hemoglobin is the amide I band (1700 - 1600 cm^{-1}) which emerges because of C=O and C-N extending vibrations and is related with the backbone conformation of Hemoglobin. The amide I band is not shifted from 1648 cm^{-1} in free hemoglobin to Hemoglobin + Natural dye complexes. Yet, the band at 1325 cm^{-1} is moved to 1326 cm^{-1} in (Human hemoglobin + curcumin nanoparticles) complex and 1328 cm^{-1} in (Human hemoglobin + gallic acid nanoparticles) complex.

This move to soaring wave number is an aftereffect of the association among hemoglobin and the natural dyes and the complexation marvel was perhaps determined hydrogen bonds, hydrophobic and hydrophilic collaborations [15]. Moreover, a conformational change in HHb from the helical structure to β-sheet like amassed structure may likewise represent the little shifting in the wave number [16].

Raman spectroscopy illustrations solid potential for giving intrusiveness data out of different specimen significant in science and medication [17]. Various procedures (for example ROA, UVRR) remained effectively pragmatic for drug examines containing protein - protein interactivity, protein accumulation then compliance [18]. Raman spectroscopy is particularly valuable while the investigation can likewise be founded on the spectra estimated as of secluded materials.

Figs 2a, 2b and 2c show the FT-Raman spectra of human hemoglobin, human hemoglobin + curcumin intricate and human hemoglobin + gallic acid complex individually.

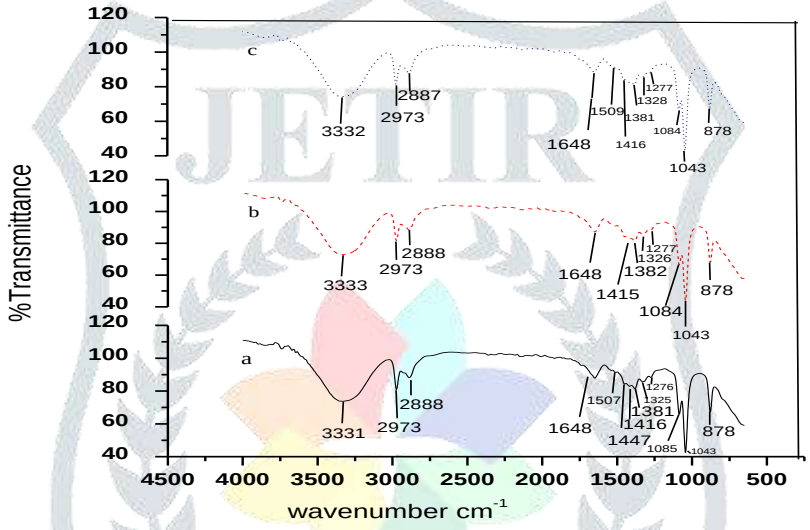

Fig 1: FTIR spectra of a) free hemoglobin, b) hemoglobin + curcumin nanoparticles complex and c) hemoglobin + gallic acid nanoparticles complex

General Raman characteristics related through the α-helix composition protein human hemoglobin, have been concentrated on the replica compounds, for example poly-L-lysine [19,20]. Diagnostic areas are generally amide I (1650–1680 cm^{-1}) and amide III (1230–1280 cm^{-1}). The utmost predictable difference of the helix contrasted with both the β-sheet and indiscriminate coil is the nonexistence of spectral intensity at 1235–1240 cm^{-1}.

Table 1: FTIR table for free hemoglobin, hemoglobin and natural dyes complexes

Frequency cm^{-1}			Assessment
Hemoglobin	**Hemoglobin + curcumin nanoparticles**	**Hemoglobin + gallic acid nanoparticles**	
3331.826	3333.683	3332.681	O-H Stretching
2973.513	2973.492	2973.189	C-H Stretching
2888.135	2888.504	2887.673	C-H Stretching
1648.746	1648.617	1648.943	C=N Stretching
1507.803	-	-	C-C Stretching of aromatic ring
1447.282	-	-	C-C Stretching
1416.680	1415.811	1416.211	C-C Stretching
1381.495	1382.378	1381.753	C-N Stretching
1325.559	1326.889	1328.357	CH_3 Implement bending
1276.184	1277.138	1277.170	CH_2 Implement bending
1085.094	1084.683	1084.770	C-N Stretching
1043.798	1043.692	1043.768	C-O Stretching
878.335	878.101	878.289	C-H Aromatic

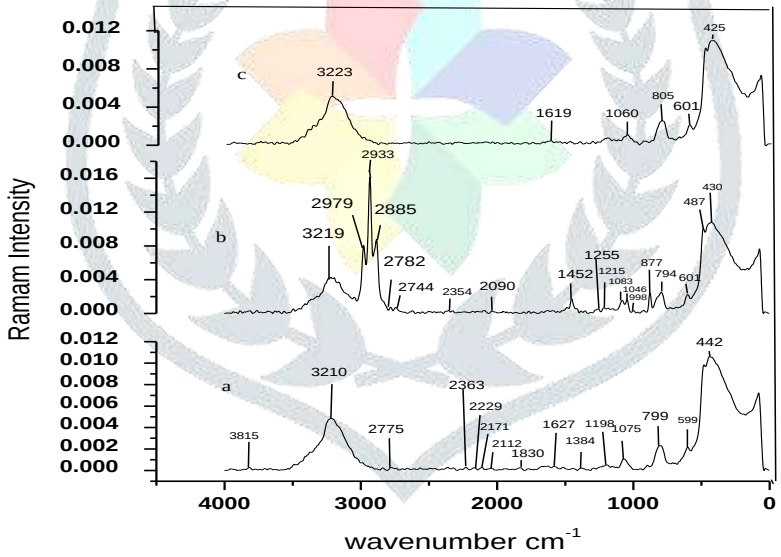

Fig 2: FT-Raman spectra of a) free hemoglobin, b) hemoglobin + curcumin nanoparticles complex and c) hemoglobin + gallic acid nanoparticles complex

Table 2: FT-Raman table for free hemoglobin, hemoglobin and natural dyes complexes

Frequency cm^{-1}			Assignment
Hemoglobin	**Hemoglobin + curcumin nanoparticles**	**Hemoglobin + gallic acid nanoparticles**	
3210	3219.87	3223.41	C-H Vibration
-	2979.10	-	C-H Vibration
-	2933.62	-	C-H Vibration
-	2885.45	-	C-H Vibration
2229.83	-	-	C-H Vibration
1830.16	-	-	C=C Vibration
1627.53	-	1619.19	C=N Vibration
1384.76	1452.31	-	C-N Vibration
-	1215.82	-	C-C Chain Vibration
1075.04	1083.22	1060.17	C-C Chain Vibration
-	1046.44	-	C-C Chain Vibration
-	877.95	-	C-H Vibration of aromatic ring
799.68	794.78	805.27	C-C Chain Vibration
599.89	601.60	601.66	Aliphatic chain vibration
442.14	487.01	425.48	Aliphatic chain vibration

4. Conclusion

The FTIR and FT-Raman results delivered significant proof of human hemoglobin – natural dyes complex development. The cooperation between human hemoglobin and the natural dyes, curcumin nanoparticles and gallic acid nanoparticles has been concentrated effectively by utilizing FTIR and FT-Raman spectroscopic methods.

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