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FTIR AND FT-RAMAN ANALYSIS OF INTERACTION BETWEEN HUMAN HEMOGLOBIN AND TWO DYES

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Abstract:

Fourier transform infrared spectroscopy (FTIR) and FT-RAMAN as as perceived cycle focused on the depiction of sub-atomic spectroscopic investigation. FTIR spectroscopy is an advantageous process intended for basic description of protein and poly-peptides. It also been conceded as a study of chemical compositions and elements present in the given sample. Raman spectroscopic investigation provides the vibrational spectrum of the exploration which can be considered as its "finger print" region it allows conventional considerate and identification. Association between Human hemoglobin and two colors, to be specific, amaranth and rhodamine 6G have been considered utilizing FTIR and FT-Raman spectroscopy. The atomic design and its utilitarian gathering were portrayed with the assistance of FTIR/FT-Raman spectra in the locales of 400-4000/50-4000 cm^{-1}.

Keywords: Human hemoglobin, Food colourant dyes, FTIR, FT-Raman.

1.Introduction

Human hemoglobin is the heme proteins whose major physiological activity is to tie sub-atomic oxygen. Human hemoglobin (HHb) elites up oxygen from the miniature blood vessels at the support of the lungs and transports it along the conduits to the body tissues, and furthermore wipes up the H_3O^+(aq) particles to switch the hatred of the blood and convey CO_2 from the cells to the lungs to confiscate it. The enzymatic and cancer prevention agent heroes of heme proteins are likewise fine perceived in the literature (R. Mandal et al. 2004, L. Messori et al. 2006). The amino acids in HHb primarily system α-helices which are interrelated by swift nonhelical wedges. The helical fragments favored HHb are habitually reduced by hydrogen holding communications which brings about attractions inside the atom prompting the collapsing of the polypeptide chains into a specific shape (S. Chatterjee et al. 2014).

It is realized that the appropriation of organically dynamic mixes in vivo, including drugs, natural items and harmful materials, depend on their proclivity to the plasma proteins. Despite the fact that HHb isn't carefully plasma proteins. It will be accessible in the plasma on hemolysis. Thusly, examinations on the association of medications, dyes and poisonous little particles with hemoglobin are critical as far as understanding their pharmacological activities. As of late, various examinations on little particles official to Hb have been portrayed (P. Mandal et al. 2010, W. Liu et al. 2011, H. Cheng et al. 2011, S. Chakraborty et al. 2012, Y. Q. Wang et al. 2012, B. Sengupta et al. 2012, Y. Liu et al. 2013, A. H. Hegde et al. 2013, S. Hazra et al. 2013, S. Hazra et al. 2014).

Azo dye which is broadly utilized in food industry to shading numerous food items (W. Peng et al. 2014, G. Zhang et al. 2013, A. Basu et al. 2014, A. Basu and G. Suresh Kumar 2014). Amaranth (AMT) and rhodamine 6G (R6G) to notice the cooperation of this color with the one of the blood proteins Hb. The sub-atomic design of amaranth and rhodamine 6G are appeared in Fig.1a and Fig.1b.

The FTIR spectroscopy was utilized for considering the complex shaped between the azo dyes and hemoglobin; and Raman spectroscopy has discovered expanded application in the drug business lately. It is recommended to be a phenomenal apparatus for considering the unpredictable development (P. Mpountoukas et al. 2010).

IR and Raman spectroscopy react to changes in vibrational methods of covalent bonds because of changes in intermolecular collaborations (R. Spivey et al. 1999). Customarily a large portion of the medication atoms are cationic species and may contain aromatic conjugated systems. Here we explain the similar restricting capacity of the dyes, amaranth and rhodamine 6G, to these heme-proteins as a model system from an assortment of biophysical methods. Thermodynamics of the cooperation has likewise been explained to associate with the underlying information (B.R. Bhogala et al. 2005).

2. Materials and methods

Human hemoglobin and dyes (amaranth and rhodamine 6G) 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.

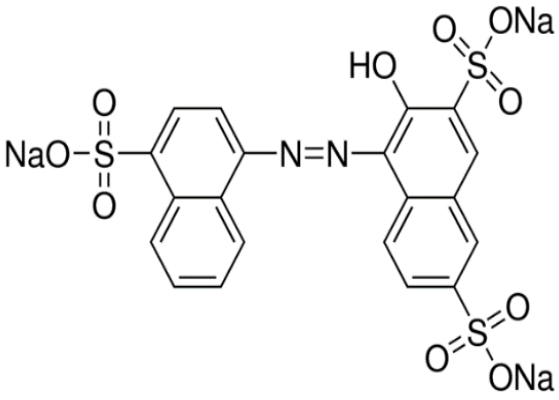

Fig.1a. Molecular structure of amaranth

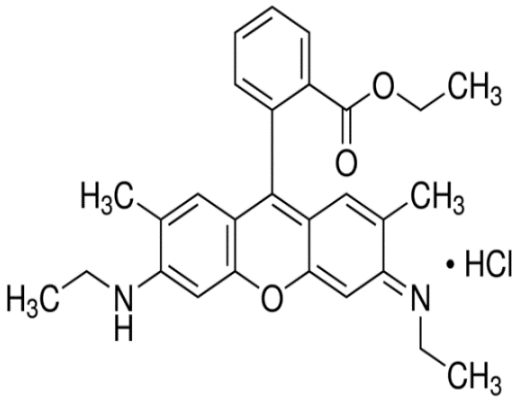

Fig.1b. Molecular structure of rhodamine 6G

3. Results and discussion

Fourier transform infrared (FTIR) spectroscopy was functioned to screen the conformational changes in human hemoglobin (HHb) upon binding of nanoparticle dyes amaranth (AMT) and rhodamine 6G (R6G) (Du et al. 2014). FTIR strategy is an effective apparatus to screen the conformational changes in HHb since it has conformation sensitive spectral signature in the infra-red region (K. Du et al. 2014).

FTIR spectroscopy was utilized for the subjective assurance of the normal optional construction of Hb (P. Mandal et al. 2009 and M. Mahato et al. 2011). For this investigation, we predominantly focused on the peptide spine of HHb because of cooperation with the color atoms. The FTIR range of HHb demonstrated two significant groups in the area of 1500–2000 cm^{-1}, to be specific the amide–I (1600–1700 cm^{-1}) and amide-II (1500–1600 cm^{-1}) groups. The previous band fundamentally starts because of extending vibration of the carbonyl gatherings in the polypeptide chain, while the last is because of a mix of N-H in plane bending and C-N stretching vibrations. FTIR spectra of HHb and HHb - dye complexes are appeared in Figs 2a,2b,2c. Investigation of the spectra uncovered that the peak position of amide-I band moved from 1648 to 1645 cm^{-1} for amaranth and 1644 cm^{-1} for rhodamine 6G and from 1507 cm^{-1} to 1512 cm^{-1} for amaranth and 1514 cm^{-1} for rhodamine 6G for amide-II band are appeared in the Table 1.

Here, it merits referencing that the likenesses in the idea of the two spectra recommended that the fundamental highlights of the hemoglobin auxiliary construction were held within the sight of amaranth. This move in the amide band to bring down wavenumber is likely because of the association among HHb and amaranth and may likewise be credited to a conformational change in HHb resulting from the local unravelling of the α-helix into loops. Subsequently, in the previous case (amide I) the extending recurrence diminished yet in the last case a converse phenomenon was noticed. Along these lines, the dye atoms associated with HHb in a way causing a rearrangement of the polypeptide carbonyl H-bonding network (A. Barth, 2007).

Raman spectroscopy has been utilized in this investigation for the portrayal of human hemoglobin and both human hemoglobin and dyes complexes (C. J. Frank et al. 1999). Raman spectroscopy delineations strong potential for giving nosiness information out of various example critical in science and prescription (H. Schulz, 2007). The medication looks at containing protein - protein intelligence, protein collection then consistence (Z.Q. Wen, 2007). Raman spectroscopy is especially significant while the examination can in like manner be established on the spectra assessed as of isolated materials.

Figs 3a, 3b and 3c are indicated the FT-Raman spectra of human hemoglobin, human hemoglobin with amaranth nanoparticle perplexing and human hemoglobin with rhodamine 6G nanoparticle complex separately. FT-Raman peak assignment values are shown in Table 2.

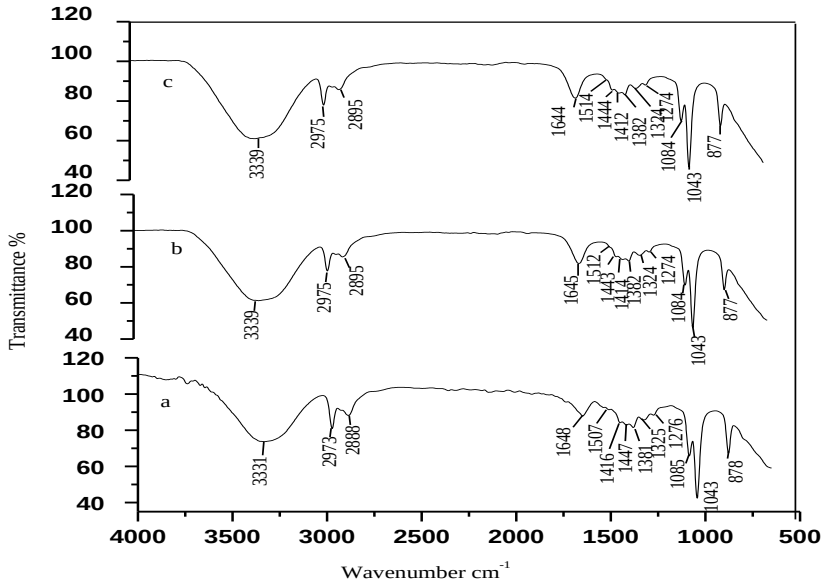

Fig 2: FTIR spectra of a) free hemoglobin, b) hemoglobin and amaranth nanoparticles complex and c) hemoglobin and rhodamine 6G nanoparticles complex

Table 1: FTIR peak assignment of hemoglobin, amaranth nanoparticle and rhodamine 6G nanoparticle complexes

Frequency cm^{-1}			Peak Assignment
Hemoglobin	Hemoglobin + amaranth	Hemoglobin + rhodamine 6G	
3331.8	3339.0	3339.8	O-H Stretching
2973.5	2975.4	2975.5	C-H Stretching
2888.1	2895.1	2895.2	C-H Stretching
1648.7	1645.7	1644.9	C=N Stretching
1507.8	1512.3	1514.5	C-C Stretching of aromatic ring
1447.2	1443.8	1444.8	C-C Stretching
1416.6	1414.4	1412.8	C-C Stretching
1381.4	1382.4	1382.7	C-N Stretching
1325.5	1324.5	1324.5	CH_3 Implement bending
1276.1	1274.6	1274.5	CH_2 Implement bending
1085.0	1084.8	1084.7	C-N Stretching
1043.7	1043.6	1043.5	C-O Stretching
878.3	877.5	877.5	C-H Aromatic

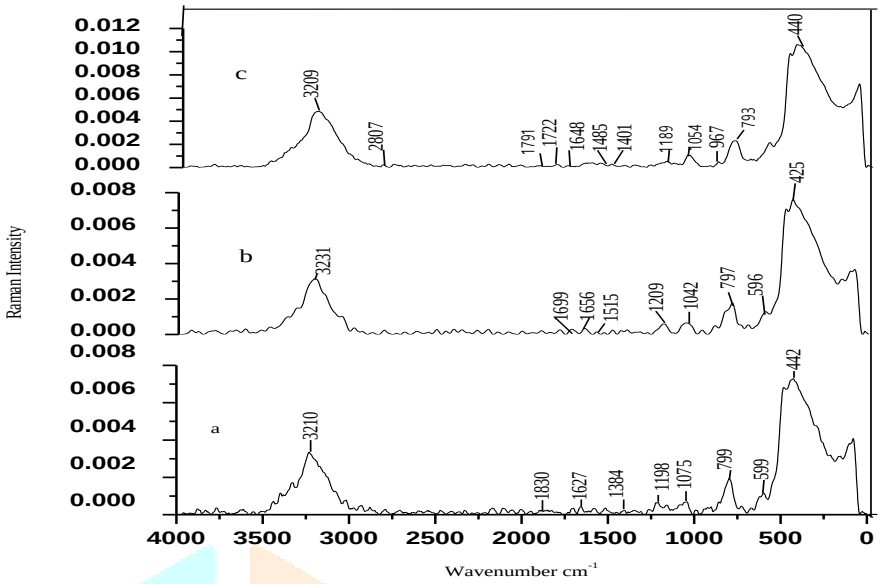

Fig 3: FT-Raman spectra of a) free hemoglobin, b) hemoglobin and amaranth nanoparticle complex and c) hemoglobin and rhodamine 6G nanoparticle complex

Table 2: FT-Raman peak assignment of hemoglobin, amaranth nanoparticle and rhodamine 6G nanoparticle complexes

Frequency cm^{-1}			Peak Assignment
Hemoglobin	Hemoglobin + amaranth	Hemoglobin + rhodamine 6G	
3210	3231.38	3209.69	C-H Vibration
1627.53	1656.75	1648.06	C=N Vibration
1075.04	1042.40	1054.77	C-C Chain Vibration
799.68	797.87	793.03	C-C Chain Vibration
442.14	425.64	440.74	Aliphatic chain vibration

General Raman attributes related through the α-helix arrangement protein human hemoglobin, have been focused on the imitation mixes, for instance poly-L-lysine (J. L. Lippert et al. 1976 and M. C. Chen et al. 1974). Indicative zones are for the most part amide I (1650–1680 cm^{-1}) and amide III (1230–1280 cm^{-1}). The most extreme unsurprising distinction of the helix stood out from both the β-sheet and aimless curl is the nonexistence of ghastly force at 1235–1240 cm^{-1}.

4. Conclusion

The interaction of hemoglobin with the nanoparticle of dyes (amaranth and rhodamine 6G) has been concentrated effectively by utilizing FTIR and FT-RAMAN spectroscopic procedures. It tends to be presumed that the nanoparticles of two dyes cooperated well with the human hemoglobin protein and because of connection it yields the development of complex between them.

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