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Extraction of some basic optical parameters in normal and cancer DNA

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Abstract

In the present paper, we carried out UV-Visible spectroscopy for normal and cancerous (glioma) deoxyribonucleic acid (DNA) samples. The understudy compound displays absorption bands at around 262nm. Based on this optical data, we calculated various optical parameters for these understudy samples. Some important parameters estimated were optical band gap, disorder energy, refractive index, extinction coefficient, electron polarizability etc. These calculated parameters show change for normal and mutated DNA. These observed properties shown by these compounds could be explored for further confirmation of the diagnostic of the disease.

Keywords: Polarizability, parameter, spectroscopy, energy, material and optical

Full-length article *Corresponding Author, e-mail: famirnit@gmail.com

1. Introduction

Advanced imaging techniques including x-rays, computer tomography (CT), magnetic resonance imaging (MRI) and ultrasound imaging are highly needed as the complemented methods for cancer diagnosis, although the examination of the stained histology slides under the light microscope is still the gold standard for final diagnostic and therapeutic decision. To concurrently increase diagnostic accuracy and detection sensitivity, including fluorescence imaging technology, novel ways based on molecular diagnosis are continuously sought out and investigated (1). The endogenous biomolecules of cells and tissues sometimes create interference during the examination of the final results. Surface-enhanced Raman scattering (SERS) spectroscopy, which combines nanotechnology and molecular vibrational spectroscopy, has inherent benefits in molecular specificity and high sensitivity and is derived from plasmonic nanostructures (2). SERS spectroscopy has been extensively utilised for studying cells, tissues, organs, and even in vivo due to its ability to provide rich molecular information to comprehend biomolecular interaction, cell metabolism, and aberrant biomarker changes during biological processes (3-5). Particularly, early diagnosis, tumour margin identification, and therapy evaluation have attracted a lot of attention [14-17]. One type of multifactorial illness, cancer, multiplies uncontrollably and develops out of control. As a result of the dynamic and abnormal physiological activity of cancer, several biomarkers are frequently produced or expressed more than they would in a healthy condition.

Optical spectroscopy is one of the basic methods which explores the features of materials based on their interaction with incident light, and here the spectroscopic data is usually given by a spectrum. Also, as the optical spectrum of a tissue contains vital information related to the structure and the biochemical constituents of the tissue under investigation which can be explored non-invasively and in real-time manure. Hence the optical spectroscopy is considered an important technique in the diagnosis of cancers [18]. Further depending upon the nature of the interaction between the material and the incident frequency/energy of photon, the spectroscopy is categorized as resonance, absorption, emission, elastic scattering (reflection), and inelastic scattering [19]. The absorption spectroscopy is based on the absorption of the suitable photon from light source by the material and is mostly analyzed by measuring the apart/fraction transmitted light of the photon energy

through the investigating material. Absorption spectroscopy can distinguish the molecular constituents of tissue according to their absorbance in the absorption spectra[20]. Also the DNA is considered to be the fundamental building block of life and is studied for its structure and functions. The fundamental structure of base pair composition and stacking sequence in relation to their optical properties and electronic transitions are of current interest [21].

To investigate the optical absorption is considered to be a vital for explanation of the electronic structure of various type of materials [22]. It could be a helpful tool to determine indirect and direct transition happening in band gap of the materials. The transmittance data could be useful to determine optical constants such as refractive index, extinction coefficient, dielectric constant etc. The evaluated optoelectronic parameters could be further helpful to study the materials in details. The current papers present the UV-Visible absorption spectroscopy of normal and cancer DNA of glioma. As far as literature is concern, this is first kind of paper which represents this type of study. The various optical parameters were evaluated and discussed viz a viz with the nature of samples. The current technique could be explored for the proper and accurate diagnosis of the disease.

2. Experimental Technique

The clinically confirmed or studied DNA samples for the present study obtained at Advanced Centre for Human Genetics in Sheri-I-Kashmir Institute of Medical Sciences (SKIMS), Srinagar, India and this study was already approved by the Regional Ethics Committee SKIMS (IEC-SKIMS). DNA was extracted from the blood, tumor tissues of glioma patients and healthy controls using DNA Extraction kit (Zymo Research Corporation, USA). These samples were also subjected to Polymerase chain reaction (PCR) for amplification. The details related with exaction and other studies are already published elsewhere [23].

The optical absorption studies of these samples were carried out on Shimazu (Model-2450) dual beam photo-spectrometer. For these measurements certain but uniform quantity (concentration of DNA) of understudy samples was dissolved in double distilled water. Also, in order to come up from low concentrations of the samples, we used quartz micro cuvette for this measurement. Here in current study, the cancer DNA samples studied are called Ca1, Ca2, Ca3, and Ca4.

3. Results and Discussion

The UV spectrum of isolated cancerous and control DNA molecules are shown in Fig.1. The compound displays absorption bands at around 262nm. This edge is due to π–π* absorption band. The corresponding transitions are mostly described by the singly-excited (highest occupied molecular orbital) HOMO–lowest unoccupied molecular orbital (LUMO) configuration, where the HOMO and LUMO are of π-like symmetry and span over most of the system. More precisely, this band is due to the absorption of light by the nucleic acid bases of DNA and the transition dipole moment is directed along the long axis of DNA strands (262 nm absorption band originates along the long axis transition) [24-29]. Here, a significant difference in the observed intensity of this band could be seen with possible mutations. This gives us a clear idea that something is happening in the system. In case of double helix DNA, the two strands of DNA twist around each other forming the so called double helix structure. When the DNA gets mutated, it starts acting at a particular site (A or G or T or C), causing reduction in length of the individual strand of the DNA helix (due to variation in the staging bonds attached with molecule). As a result, the absorbance intensity of the DNA varies with change in DNA structure. In simple words, when some kind of mutation happens, the hydrogen bonds started breaking and the two DNA get separated away from each other. It can also be observed that due to strong mutations (stages of cancer) existing in current cases, the axial length of the DNA strand change in non-monotonous way, consequently absorbance intensity is also varies irregularly. Also the absorption intensity could vary with molecular concentration, a signature of increase of particular nucleotide after mutation could be noticed. Further as the significant potential of π–π interactions among the aromatic molecules is already established [26], hence the concept has been used in the areas ranging from materials science to molecular biology. Also the π–π interactions are believed to play a dominant character in the thermal stability and folding of proteins, thereby controlling various possible mechanisms/features of the body. Also, the *ð*-stacking between adjacent base pairs is the prime deriving force for stabilizing the double helix. It should also be noted that the direction and wavelengths of optical dipole transitions varies with the type of nature of nucleotide (A, G, G, and T) [26].

Therefore, optical absorption spectroscopy could be used to find the nature and as well as concentration of nucleotides. The optical absorption and energy gap of the understudy DNA molecules was deduced by applying the following relationship [27]:

$$\alpha h\upsilon = B(h\upsilon - E_g)^{\delta} \qquad \text{--(1)}$$

where "α" is absorption coefficient, "$h\upsilon$" is the energy (h is a Planks constant having value 6.625 x 10^{-34} J/s and υ is the frequency of incident photon in Hz) of the incident photon, E_g is the value of the optical energy gap between the valence band and the conduction band and "δ" is the power which characterizes the electron transition process. In particular "δ" is related to the distribution of the density states. It has discrete values 1/2, 3/2, 2 and 3 for direct allowed, direct forbidden, indirect allowed and indirect forbidden transitions respectively. The factor B depends on the transition probability and is supposed to be a constant within the optical frequency range (an energy-independent constant).

In order to determine the precise value of energy gap, graphs of $(\alpha h\upsilon)^{1/\delta}$ against ($h\upsilon$) are plotted with δ = 1/2, 3/2, 2 and 3. The extrapolation of this plot for $(\alpha h\upsilon)^{1/\delta}$ = 0 gives the E_g. The best fit were obtained by plotting $(\alpha h\upsilon)^{1/\delta}$ as a function of photon energy ($h\upsilon$). Since, the DNA is considered as an indirect band gap semiconductor [25-32], therefore, we used δ = 2 and the calculated optical band gaps E_g were tabulated Table-I. Here, we have shown that the DNA is a wide band gap material with wide optical band gap (highest occupied molecular orbital–lowest unoccupied molecular orbital gap) of order 4.20-4.12eV.

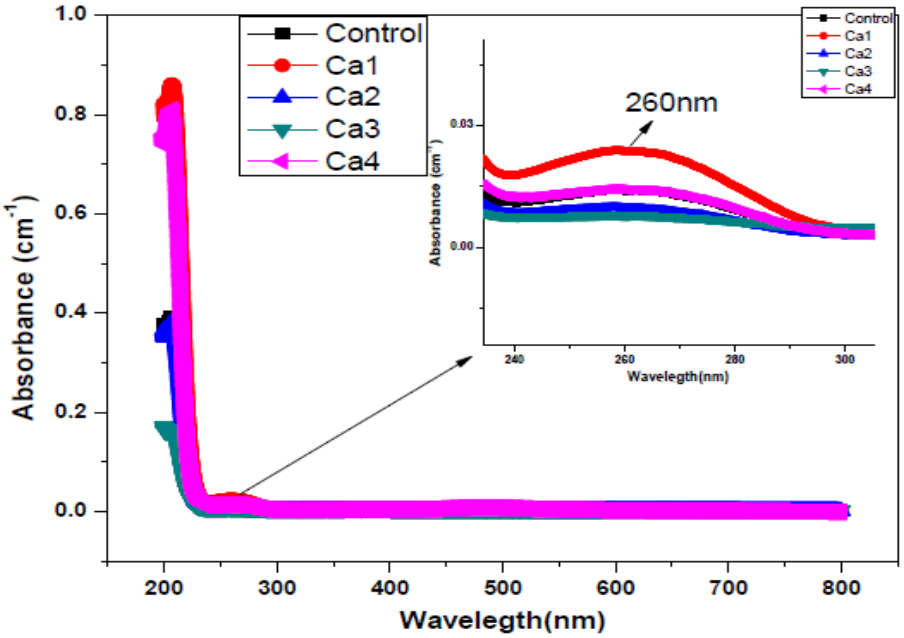

Figure 1: UV-Visible absorptions plots of Control and Cancer DNA samples

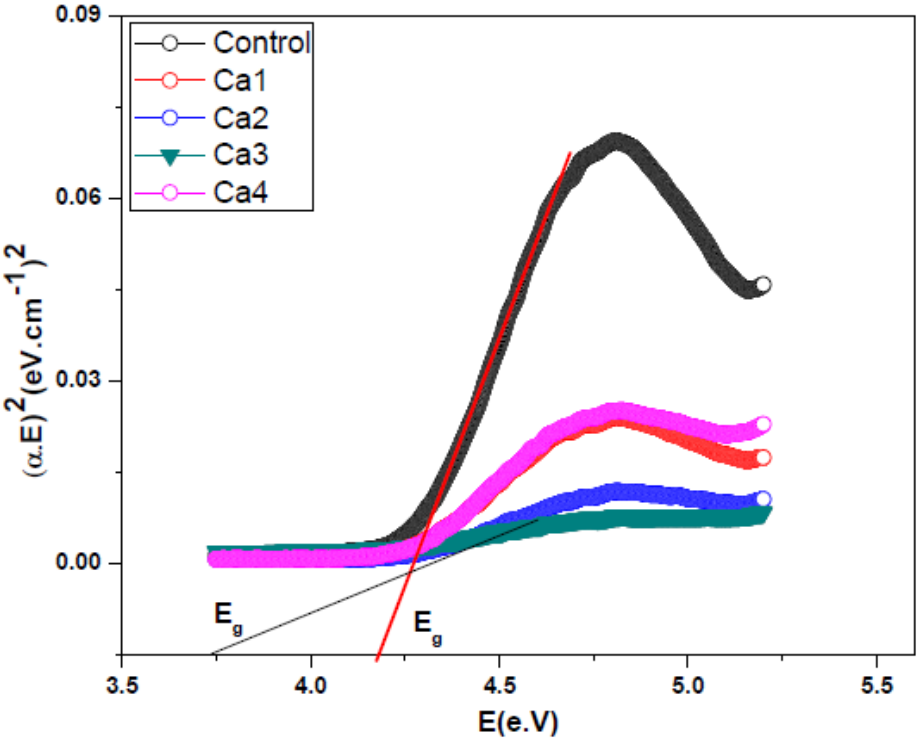

Figure 2: Tau Plots for the control and cancer DNA samples

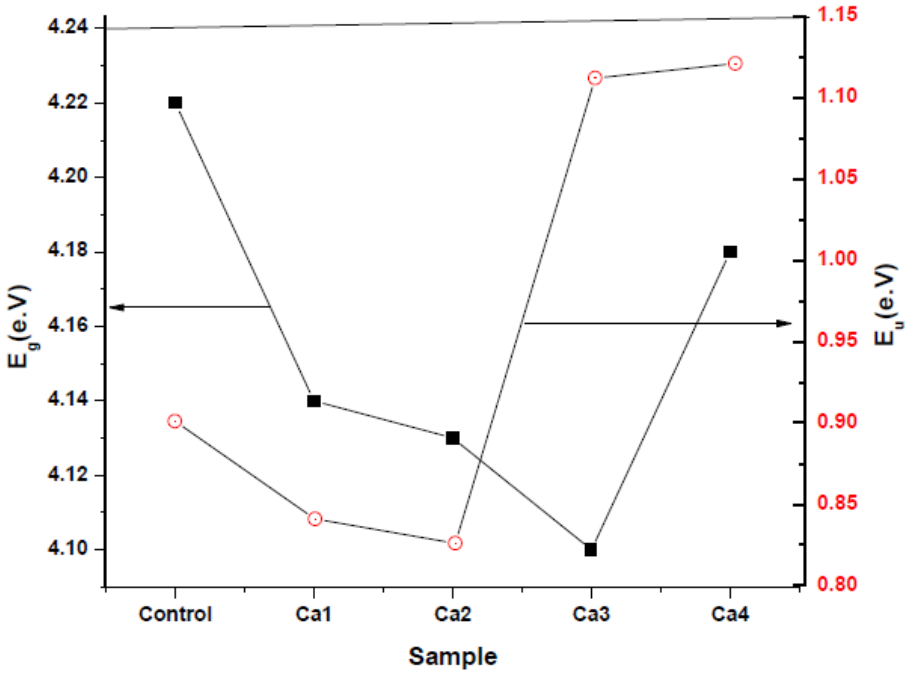

Figure 3: E_g and E_u for the understudy samples

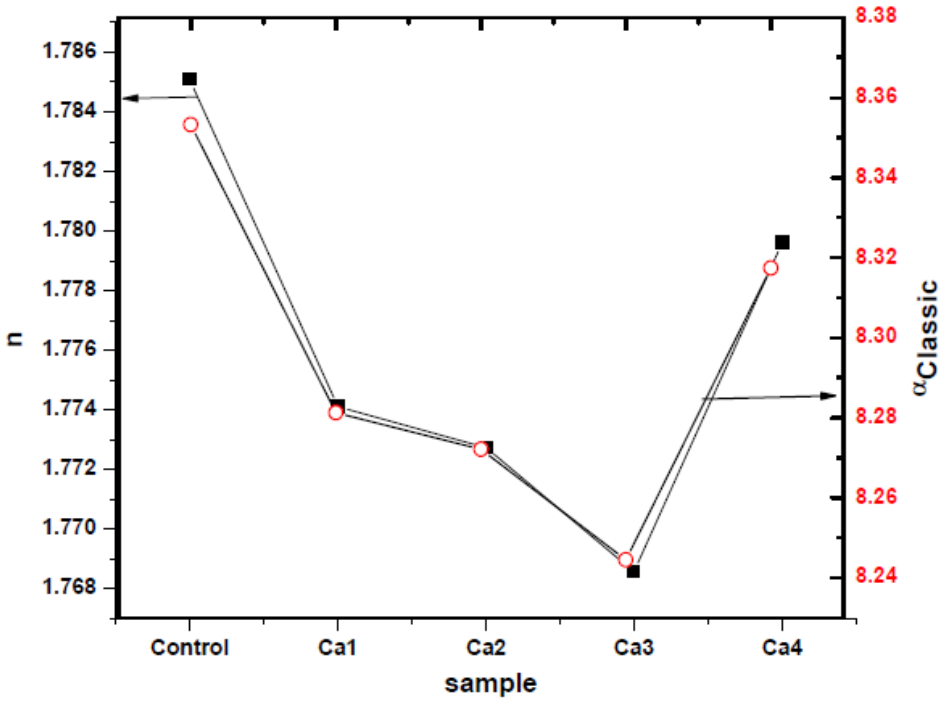

Figure 4: The n and of control and $\alpha'_{Classical}$ cancer DNA sample

Recently, various optical absorbance measurements were carried out on DNA, there the energy gap was found to vary between 4.20 eV to 4.12 eV [27-30]. This observed difference in band gap energy can be attributed to DNA sequence, length of DNA molecule, character of DNA molecule, environment of DNA, influence of water, etc (as DNA is structure with a π-stacked array having four type of different nitrogenous bases like A, G, C and T joined with each other followed by complementary base pairing with further attached with sugar-phosphate backbones resulting a double-helix structure) [31]. A well-established difference between control and cancerious DNA are seen. Here in present case, a possible difference observed in E_g could be due to the nature of the DNA molecule. Also it has been observed that there is effect of stacking sequence on the electronic structure and optical properties of a DNA [33].

It is argued that the decrease in band gap produces defects in the material which increase the disorderness causing the structural deformation in the system [33]. Therefore, it forces us to conclude the observed decrease in band optical band gap could be the disorder and defect creation in the system after any kind of mutations [32]. Also if less ordered the bases then more ultraviolet light is believed to be absorbed.

The Urbach band tail (also called defect tail) was also studied for this system (Urbach rule) [32].

$$\alpha = \alpha_0 \exp(h\upsilon/E_a) \qquad \text{--(2)}$$

where α_0 is the constant and E_a is the Urbach band energy. It should be noted that the various factors like the carrier phonon interaction, carrier-impurity interaction and structure disorder are main reasons for the Urbach band tail in semiconductors like materials [27,32]. Also, we have evaluated the Urbach band tails by fitting above equation, like the absorption coefficient (α) as a function of photon energy ($h\upsilon$) in the near of the fundamental absorption edge. The values of E_a were determined from the inverse slope of the straight line, representing $ln(\alpha)$ versus photon energy ($h\upsilon$) [i,e $\ln\alpha = \ln\alpha_0 + (h\nu/E_a)$]. The value of this activation energy are presented in Fig.3. There variation in E_u is seen with kind of DNA. Therefore, as E_g decreases, the magnitude of defect energy increases. This supports our argument that sub-band states formed in between the HOMO-LUMO bands leading in the narrowing of the E_g. With the intensity of mutations, the number of defect levels below the HOMO band increases to such an extent that the band edge is shifted deep into the forbidden gap, thereby reducing the effective E_g of present system. Hence, it clearly demonstrated that the enhancement in existing defects or disorder after disease may be the prime reason for this observed behavior. Also this observed change in this disordered energy may also be related to the variation in the internal fields due to the chemical group interactions or some of them have ability to transform weak bonds into defects states.

The refractive index (n) is one of the essential parameters of material. Its evaluation can give vital information related with the material. Based on this parameter a number of various optoelectronic parameters can be calculated. The 'n' depends on various material parameters and Eg is one among them. There are different models to calculate n using E_g. Also based on experimental data, the value of n for different type of mixed materials can be calculated using model proposed by Kumar and Singh [34]. As per this model, the n and Eg are related by the following relation:

$$n = K E_g^C \qquad \text{--(3)}$$

where K = 3.3668 and C = 0.32234. Further n also depends on the electron polarizability (α') of ions and well for the material can also be calculated. According to the classical theory, α'classical for a material can be calculated by using Lorentz-Lorentz relation [34]:

$$\alpha'_{Claasical} = [(n^2 - 1)/(n^2 + 1)] \times \left(\frac{M}{\rho}\right) \times 0.395 \times 10^{-24}\ cm^3 \qquad \text{--(4)}$$

where M = molecular weight (g/mol) and ρ = density (g/cm^3) of the material. Further it is also documented that the value of α' can be estimated by using the following relation [34]:

$$\alpha'_{Claasical} = \left[(12.41 - \frac{3(E_g - 0.365)^{0.5}}{12} \times \left(\frac{M}{\rho}\right) \times 0.395 \times 10^{-24}\ cm^3 \qquad \text{--(5)}$$

The values of n, **α' Claasical** based on above relations are given in Fig.4. Here a visual variation in these estimated properties could be observed.

The optical and dielectric properties of nucleic acid can be modulated with an alternation at the base pair [34] but also to apprehend the linear optoelectronic properties of its constituent nucleic acid bases to establish their potential in appropriate fields of photonics. This further indicates that there may be a conformation related to the basic nuclides in the observed structure of the system.

In summary, our study is the first of its kind to show the significance of UV-visible absorption spectroscopy as a probe for estimating the optical parameters of normal and cancer DNA samples. A larger sample size of spectral information would in all likelihood make the prediction model more accurate. We also need an animal model to prove the feasibility of UV-visible absorption spectroscopy for *in-vivo* diagnosis in the future.

4. Conclusions

In the current study, we performed UV-Visible spectroscopy experiments for normal and cancerous (glioma) DNA specimens. After data analysis, we estimated various optical absorption related parameters for these cancer samples. Few significant parameters like optical band gap, disorder energy, refractive index, electron polarizability etc were calculated. These obtained optical parameters show impressive variations for normal and mutated DNA. Based on these parameters, we explore possible mutations (if done in controlled way) and hence could be used for the confirmation of the diagnostic in various possible diseases.

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The authors declare no conflict of interest.

Additional information
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