# Ultraviolet-visible and fluorescence spectroscopy can be used as a diagnostic tool for gamma irradiation detection *in vivo*

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**Abstract**: The spectroscopic properties can indicate important features about the nature and severity of the disease. However, no earlier studies have been used the spectroscopic properties as a diagnostic tool for radiation detection. This study was aimed to use ultraviolet-visible and fluorescence spectroscopy as a diagnostic tool for gamma irradiation detection in rats *in vivo*. Adult male rats were exposed to 25, 50, 75 and 100 Gray as single dose, using Cobalt-60 (Co-60) source with a dose rate of 0.883 centi Gray/sec (cGy/s). Ultraviolet and fluorescence spectroscopy of rat's blood serum were measured. After gamma irradiation of rats *in vivo*, the blood serum absorbance peaks for 25, 50, 75 and 100 Gray (Gy) decreased and shifted towards the ultra violet wavelength. A maximal change in fluorescence intensity of blood serum at 350 nm was obtained when exciting light at 194 nm after irradiation. The fluorescence intensity also decreased with the dose. The highest radiation gamma dose might be accompanied with the highest oxidative stress. This study suggests that at the above mentioned gamma radiation doses, the blood is highly fragmented; with low aggregation at 25 Gy and with high aggregation at 50-100 Gy.

**Keywords**: Ultraviolet spectroscopy, fluorescence spectroscopy, gamma radiation, blood serum.

## INTRODUCTION

The living organisms, which are continually exposed to ionizing radiation in nature as well as from medical procedures, it will contribute most of the whole-body background radiation. The ionizing radiation can remove the electrons from their atomic or molecular orbital shells in the tissues (Borek *et al*., 1993). The exposure to ionizing radiations may induce adverse health effects, depending on the type of isotope, the absorbed dose, and the dose rate (Keith *et al*., 1999).

Understanding the mechanism of radiation induced damages either X-ray or UV radiation, in biological systems is of prime importance in radiation biology (Durchshlag *et al*., 2003). Proteins are important bio macromolecules that are sensitive to ionizing radiation (Boulton *et al*., 2001, and Davies, 2003). Radiation induced alterations of molecular properties of proteins like breaking of hydrogen/covalent bonds, fragmentation, inactivation has been reported by several investigators (Cho and Song, 2000 and Moon and Song, 2001). The hydroxyl and superoxide anion radicals generated by the radiation, modify the primary structure of the protein, and results in distortion of the secondary and tertiary structure (Davis & Delsignore, 1987). Conformational changes in protein induced by UV, X-ray and γ-radiation have been reported in several studies (Durchshlag *et al*., 2003 and Lee and Song, 2002).

Blood consists of two main parts, firstly is the serum (plasma), and secondly is the cellar part which consists of the red blood cells (RBCs), white blood cells (WBCs) and platelets (PLs) (Kralli, 2006). The blood serum contains the proteins (which are not used in blood clotting), the electrolytes, the antibodies, the hormones, and the lipids.

When gamma radiation interacts with the aqueous solutions, several reactive species like $O^{2-}$, $H^{\cdot}$ and $OH^{\cdot}$ are produced as a result of the radiolysis of water, which, in turn, can change the biological activity of the proteins and peptides through the reaction with certain sites in the molecules (Hati *et al*., 1990, Nascimento *et al*., 1996). In the literature there is no available investigation, which can show the gamma radiation effects on the spectral characterization of the blood serum. Therefore, in the present study, the spectral characterization of the irradiated blood serum *in vivo* was investigated through the absorbance and fluorescence measurements as an important tool for gamma radiation detection in rats.

## MATERIALS AND METHODS

### *UV-Visible spectroscopy*

#### Animals

60 healthy male Wistar-Kyoto rats weighing 200gm were randomly divided into: Group (1) irradiated with 25 Gy gamma radiation (n = 10; n is the number of rats); group (2) with 50 Gy (10); Group (3) with 75 Gy (10); Group

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(4) with 100 Gy (10) and one control group (CG: n = 20) (Abdelhalim *et al*., 2015). The 4 gamma-irradiated rat groups were maintained on standard laboratory rodent diet pellets and housed in humidity and temperature-controlled ventilated cages for a period of 24h day/night cycle (Abdelhalim *et al*., 2015).

**The irradiation of rats and blood serum**

The rats were irradiated with 25, 50, 75 and 100 Gy single doses using Cobalt-60 (Co-60) source with dose rate 0.883 centi Gray/sec ( cGy/s) at King Saud University (Aabdelhalim *et al*., 2015; Abdelhalim and Sabra, 2015). The rats were anesthetized by inhalation of 5% isoflurane until the muscular tonus was relaxed. 2 ml blood samples were withdrawn from the left ventricle of the heart using needles, and then it was collected into the gel tubes.

For obtaining the serum, the blood sample was allowed to clot, and then the coagulated blood was centrifuged twice at 3000 rpm for 10 min using a centrifugation (Type 7170, Hitachi). All experiments were conducted in accordance with the rules and the guidelines approved by the King Saud University Local Animal Care and Use Committee (Aabdelhalim *et al*., 2015).

**Absorbance spectroscopy**

The absorbance of blood serum of rats exposed to 25, 50, 75 and 100 Gy as a single dose, was measured using a UV-VIS double beam spectrophotometer (UV-1601 PC, Shimadzu, Japan; H14 grating UV) with optical resolution of 0.4 nm. The absorbance measurements were performed over wavelength range 200–800 nm at the room temperature using cleaned quartz cuvettes (1 cm path length). The pH for the different blood serum samples was kept constant during the measurements (Abdelhalim, 2012).

**Fluorescence spectroscopy**

The characterization of fluorescence was made over the wavelength range of 190 -400 nm for the blood serum samples using a FluoroMax-2 JOBAN YVON-SPEX, Instruments S.A., Inc., France. The excitation wavelengths for the blood serum samples were performed at wavelengths 194 nm and 278 nm, and the emission was fixed at wavelength 350 nm. All the measurements were performed on series of samples with a 10-mm light path cuvette (Abdelhalim, 2012). .

## RESULTS

Figs. 1 and 2 show UV absorption spectra of two peaks for the blood serum. The peaks were observed at 195 nm (the aliphatic amino acids) and 280 nm (aromatic amino acids). The data show a decrease in the absorption intensity with a gradual increase in the gamma irradiated doses. The conformational change in the secondary structure of the serum proteins is more distinctive due to increasing the amount of water (figs. 1 and 2).

Fig. 3 shows the fluorescence spectra of blood serum samples measured at an excitation wavelength of 194 nm. A maximal change in the fluorescence intensity was observed at 350 nm when the light was excited by 194 nm. The fluorescence intensity decreased with the gamma irradiation dose (fig. 3).

The blood serum samples irradiated by different gamma radiation doses measured at an excitation wavelength of 278 nm. A maximal change in fluorescence intensity of blood serum observed at 350 nm with exciting the light at 278 nm. The fluorescence intensity also decreased with the gamma irradiation dose (fig. 4).

## DISCUSSION

Our results were supported by the review of Dacie and Lewis, 1991. The decrease in absorbance might be attributed to destruction, fragmentation in the aliphatic and aromatic amino acids, which are concomitant with Gaber, 2005.

Ultraviolet visible spectroscopy measurements indicated fragmentation and aggregation in the serum protein due to the radiation exposure. The irradiation caused disruption of the ordered structure of the protein molecules as well as the degradation, cross-linking and aggregation of polypeptide chains (Gaber, 2005). Martel, 2010 has demonstrated that the irradiation with 5–50 kGy gamma produced extensive dose-dependent serum protein breakdown.

Due to the different gamma radiation doses, a decrease in the protein absorbance can be attributed to the unusual unfolding as well as the random motion of proteins under the different degrees of oxidative stresses. Thus, the absorption spectra of the blood serum are highly dependent on the radiation gamma dose, and the highest radiation gamma dose might be accompanied with the highest oxidative stress (Abdelhalim, 2012; Kaczmarska *et al*., 2011).

The fluorescence spectra obtained by Gaber, 2005, has proved that the irradiation was quenched at the emission intensity when the blood sample was excited at 280 nm.

Gaber (2005) has proved that the fluorescence spectra indicate that the irradiation quenched the emission intensity which excited at 280 nm, which suggests conformational changes within the molecule following irradiation, and with a higher exposure of the tryptophans resulting in higher quenching constants Boni-Mitake (2001). Our study indicates that at the lower and the higher gamma radiation doses, the blood is highly fragmented; with low aggregation at the dose of 25 Gy and with high aggregation at the doses from 50-100 Gy.

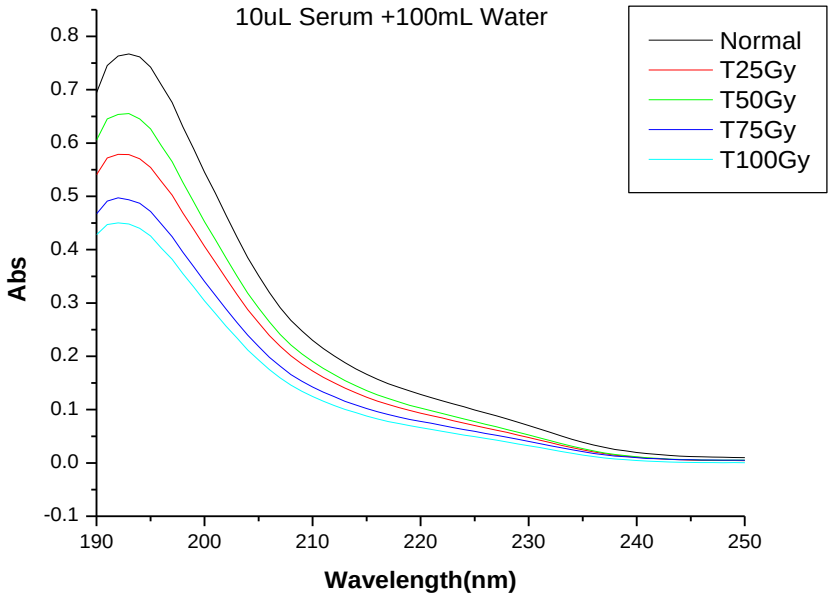


**Fig. 1**: The absorbance for the blood serum (10 µL) of rats irradiated with different gamma radiation doses at concentration of 100 ml of water

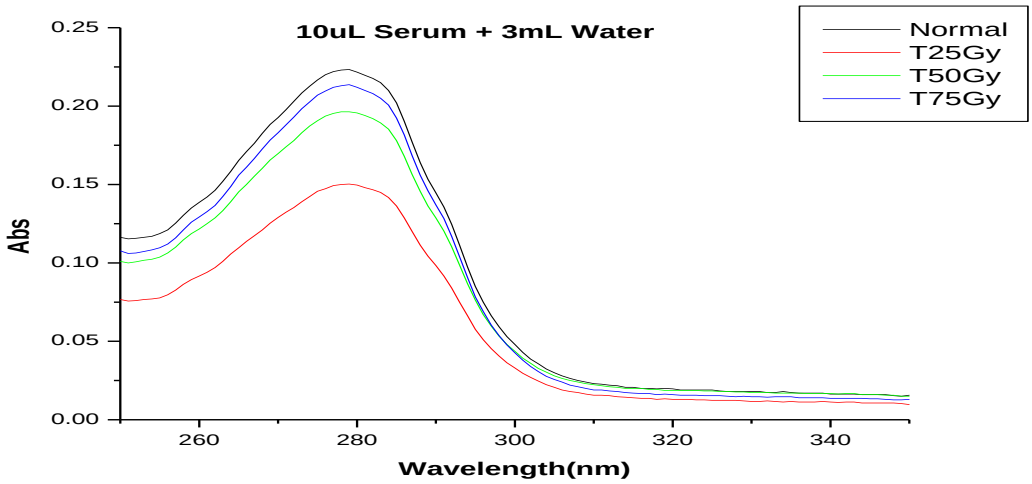


**Fig. 2**: The absorbance for the blood serum (10 µL) of rats irradiated with different gamma radiation doses at concentration of 3 ml of water

The fluorescence intensity variations for the protein were attributed to the conformational changes in the aromatic amino acids, with the absorption values shifted toward the longer wavelengths, due to the protein unfolding (Vivian and Callis, 2001; Eftink, 2000). The aromatic amino acids are buried inside the proteins due to their hydrophobic character, and shift the maximum fluorescence intensity to the longer wavelengths, which are attributed to the protein unfolding (Eftink, 2000). Our observations are agreed with the conformational changes and the protein unfolding induced by the different gamma radiation doses.

## CONCLUSIONS

Two peaks for the irradiated blood serum were observed, at 193 nm which corresponds to the aliphatic amino acids, and at 280 nm which corresponds to the aromatic amino acids. The blood serum absorbance peaks for 25, 50, 75 and 100 Gy significantly decreased, and shifted towards the UV wavelength. The decrease in absorbance might be attributed to destruction, fragmentation in the aliphatic and aromatic amino acids. This study demonstrates the following: 1) ultraviolet-visible absorption spectroscopy indicates to the fragmentation and the aggregation that

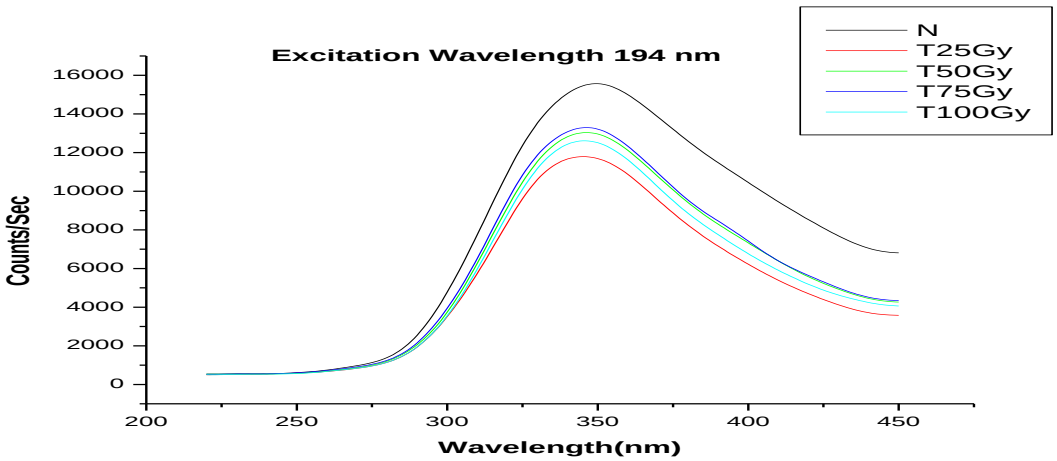


**Fig. 3**: The fluorescence spectra of blood serum measured at an excitation wavelength of 194 nm

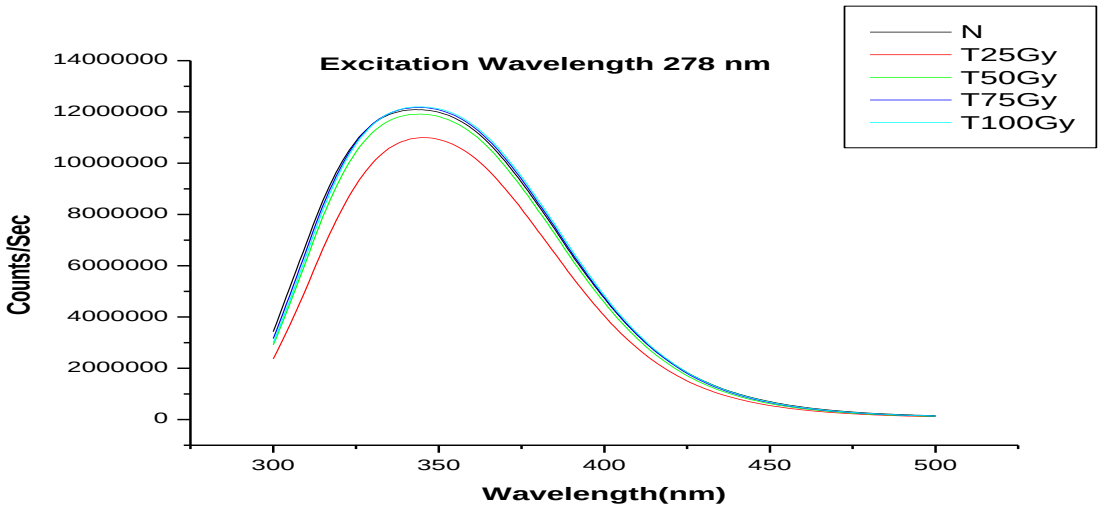


**Fig. 4**: The fluorescence spectra of blood serum measured at an excitation wavelength of 278 nm

might occur in response to the radiation exposure; 2) the decrease in the protein absorbance might be attributed to the degree of unfolding and the random motion under different degrees of oxidative stress; 3) the absorption spectra of blood are highly dependent on the irradiation gamma dose, and the highest irradiation gamma dose might be accompanied with the highest oxidative stress; 4) the fluorescence intensity decreased with the gamma irradiation dose; 5) a maximal change in fluorescence intensity of blood serum observed at 350 nm when exciting the light with 194 and 278 nm.

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