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# BREAKAGE OF DNA MOLECULES INDUCED BY ULTRA VIOLET LIGHT

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**Abstract.** The cancer is a major burden of disease worldwide. The studies and applications of the ultra violet (UV) light is not common for cancer therapy because of its low energies compared with ionizing radiations. However, UV sources also have some advantages such as safe and cheap etc. Therefore, in this study, we have studied a possibility of using UV lights in cancer therapy, focusing on the biological effect of the UV light on DNA molecules. Aqueous solutions of deoxyribonucleic acid (DNA) prepared from E-coli bacteria, were irradiated by two kinds of UV sources. The results have shown that the DNA molecules are broken effectively by irradiation of the UV light which has too enough for the ionization of the atomic orbital electrons. The DNA breakage is also analyzed that fragmentened and/or linear forms of the DNA molecules are produced mainly.

***Keywords:*** *Gel electrophoresis, ultraviolet (UV), DNA breakage.*

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## 1. Introduction

Irradiating a living tissue by UV and ionizing radiation, ionizations and excitations cause damage to chemical structures of constituent molecules, resulting in tissue damage. Most importantly, the damage to genetic material of a cell, deoxyribonucleic acid (DNA) has been recognized as one of the most critical of lethal ones. The ultraviolet (UV) light has very low energies compared with the ionizing radiations and it cannot ionize the atomic orbital electrons directly. Many studies showed that free radicals are induced from the water and other molecules by UV irradiation (Jurkiewicz & Buettner, 1994; Sindt *et al., 2019;* Cadet & Wagner, 2013) and the free radicals can be an important cause of the lethal damages of bio-organizms (Sinha & Häder, 2002; Tornaletti & Pfeifer, 1996; Jamsranjav *et al., 2019*).

The ionizing radiations also applied for the cancer therapy based on their effectiveness of the lethal damage. However, Applications of the UV lights is limited only for the skin cancer treatment (Narayanan *et al., 2010*).

Where through, a unique property of plasmid DNA that is its structure is changed from the supercoiled form to an open circular and linear form depending on single strand breaks (SSB) and double strand breaks (DSB), respectively. The linear form can be generated by two SSBs only if they are located sufficiently close to each other, at a distance of 5–10 bases, to constitute a DSB (Jamsranjav *et al., 2019*, Levin *et al.,* 1973). If DSB is occurred two times in a plasmid, fragmented linear forms (fragmented DNA) will be generated. Agarose gel electrophoresis (AGE) was used to analyze the breakage of the DNA molecules based on the differences of their moving speeds in the electric field

(Davis *et al.,* 1986). By using this method, it is possible to measure the DNA structure forms of linear, open circular and fragmented due to the DNA strand breaks which can be an important cause of the lethal damages.

In this work, we have studied a possibility of UV sources in cancer therapy, namely biological effect on breakage of the DNA molecule irradiated by UV light. The irradiation experiments were performed on DNA aqueous solutions and the DNA structure forms were examined from the irradiated samples. The results showed that lethal damages can be induced to the DNA molecules by the irradiation of UV light.

## 2. Materials and methods

In this study, DNA molecules, which is prepare from E. coli bacteria were obtained from anaspex Co., Ltd., Ulaanbaatar, Mongolia. The DNA was irradiated at a concentration of 10.4 μg/ml, which was obtained by diluting concentrated DNA (500 μg/ml) with TE buffer (10 mM Tris-HCl, 1mM EDTA adjusted to pH 7.5). Each sample was 20 μl for the irradiation experiment.

Two UV sources were applied for the irradiation. First, one is a mercury vapor lamp which generates UV lights with 120 nm-700 nm wavelengths. UV lights with 365 nm wavelength were separated by using a UV filter with 3-UFC-6 model (The model is described with English alphabets instead of the Cyrillic alphabets of Russian). Second one is a LF 104.S generator containing a monochromatic UV lamp system of 254 nm wavelength. It was applied for the confirmation of the results obtained from the first experiment. Those output spectrums of UV lights were measured by a spectrofluorometer with SF-4 model and output spectrum of the sources were illustrated in Fig.1.

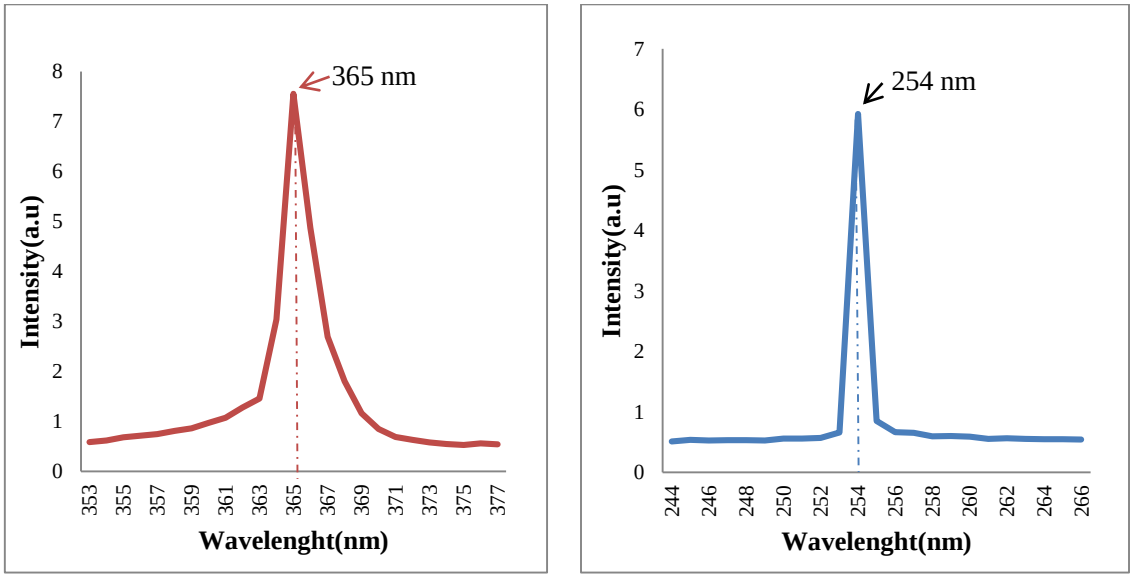


**Fig 1.** Output spectrums of UV sources

Agarose (0.28 g) was mixed in 0.5XTBE (Tris Borate EDTA) for the gel preparation. 5 μl samples was mixed with 2 μl gel loading dye and 5 μl distilled water, and loaded into a hole prepared in agrose gel. A gel electrophoresis system (Mupid-EXu) was used as an experimental apparatus. The gel was run at 100 V voltage for 30-40 min. The run time was enough for the samples migrate to 70–80% of total leight of the gel. After the

electrophoresis running, the agarose gel containing DNA samples was soaked in ethidium bromide solution to visualize the DNA molecules. The binding of ethidium bromide to DNA gives an intense luminescence of visible light, which allows identification of the positions and amounts of separated DNA forms inside the agarose gel. The luminescence were imaged using UV transmissometer GL-3120 and photo digital camera imager. A free software called Scion Image (https://bit.ly/2EyehPT) was used to produce spectrums of the luminescence from the photo images. The spectra describe the dependence between the luminescence intensity (grayscale value) and position (number of pixels) of the DNA molecules.

## 3. Results and discussions

Results for different irradiation times were compared in Fig.2 and Fig.3 for the UV lights with 365 nm and 254 nm wavelengths, respectively.

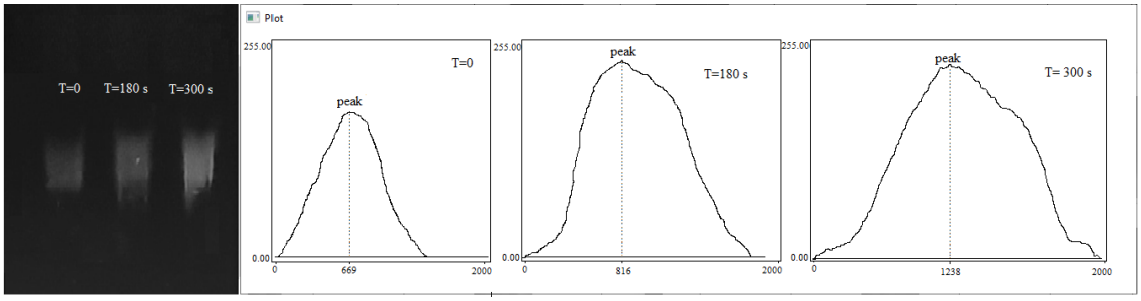


**Fig. 2.** Luminescence images and spectrum of irradiated samples UV light with 365 nm wavelenght. T: Irradiation time (s), X axis: Position (pixels), Y axis: Intensity (gray scales)

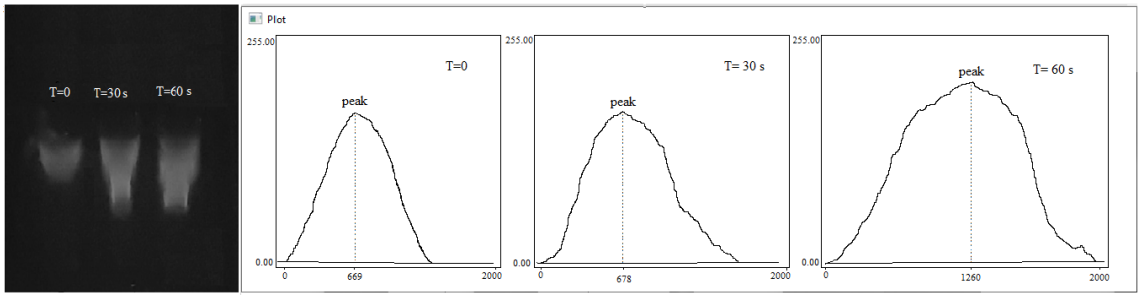


**Fig. 3.** Luminescence images and spectrums from the samples irradiated by UV light with 254 nm wavelenght. T: Irradiation time (s), X axis: Position (pixels), Y axis: Intensity (gray scales)

The white-black images in the left sides of the above figures are images of the luminescence from the ethidium bromide bounded to DNA which were captured with a photo camera installed in AGE system. The white traces placed in lines from left to right on the images show the DNAs irradiated with short to long time intervals respectively. The each trace was analyzed by using Scion image program, and they are transformed to the spectrums shown in the right side of the above figures. As shown in the figures, the spectrum became wider and the peak of the spectrum was also moved to right side due to the irradiation. It is considered that DNA fragments which are possible to move rapidly under the influence of electric field were formed in the sample after irradiation. On the

other hand, it shows that the DNA molecules were broken effective by UV irradiation and changed to smaller fragmented molecules and/or the different forms due to DNA strand breaks. Because, the previous studies have been improved that the DNA molecules with linear and fragmented forms can move faster than other forms in the agarose gel by influence of the electric field (Jamsranjav *et al.,2019,* Peak *et al.*, 1995*;* Maurizot *et al.*, 1991*).*

The spectrum areas were measured and its relation with irradiation time are presented in Fig.4 and Fig.5 for the irradiation experiments.

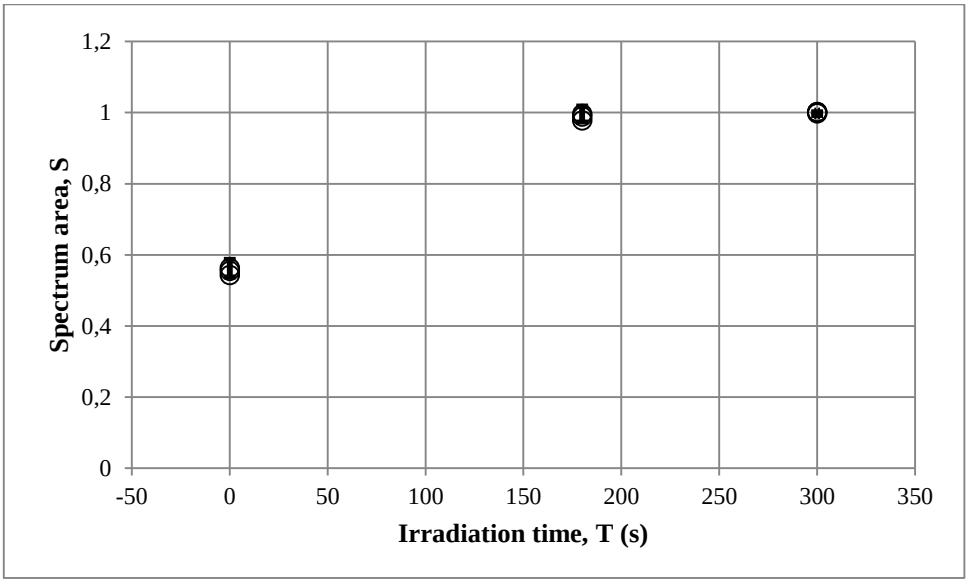


**Fig. 4.** Dependence between the luminescence spectrum area and the irradiation time for the irradiation experiments using UV light with 365 nm wavelenght

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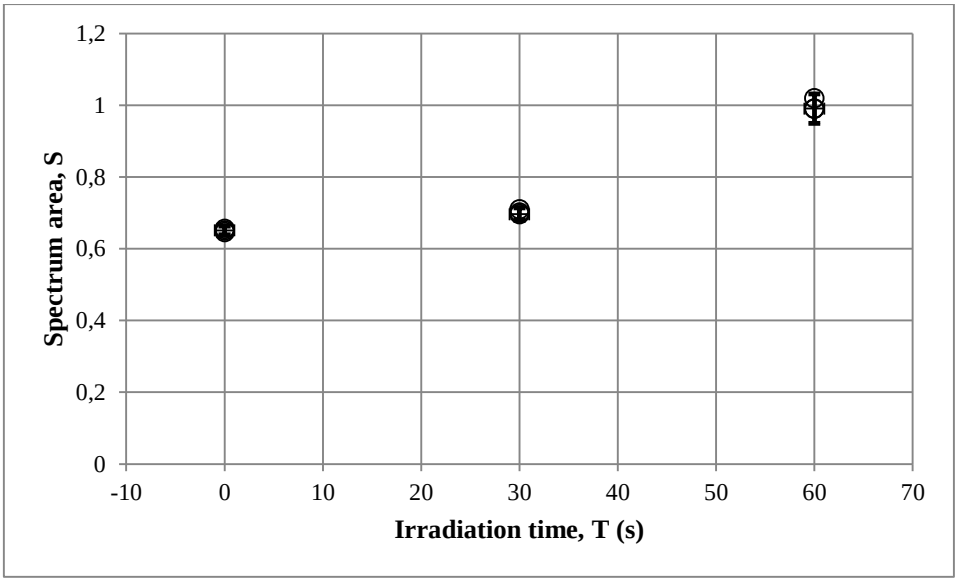


**Fig. 5.** Dependence between the luminescence spectrum area and the irradiation time for the irradiation experiments using UV light with 254 nm wavelenght

The horizontal and vertical axes of the Fig.4 and Fig.5 are the irradiation time and spectrum area which is normalized with the highest value, respectively. Error bars and all data for the 3 repeated experiments also shown in the figures. The both figures show a same result that the areas of the spectrum increased when the irradiation time increases. The reason is considered that the sample was containing numerous of DNAs with super coiled forms before the irradiation experiment, and they are changed to the other forms of linear and open circular etc., due to the breakage of DNA molecules. Because, supercoiled forms bind to the ethidium bromide dye worse than the other forms, and gives lower luminescence (Maurizot *et al.*, 2011).

It is also considered that the DNA molecule breakage is induced by the indirect effects due to the excitation of the water molecules in the sample. Because the photon energy of the UV light is not enough to ionize the DNA molecules directly.

The above considerations were made in the case of that there is no influence from substances other than DNA molecules. In order to examine the influences from the other substances, we measured the purification of our DNA sample by the ratio between UV absorptions at 260 nm and 280 nm wavelengths based on the literature of variations in DNA sample quality (Chang. 2019). The measurement was performed by using a UV spectrophotometer (Beckman, DU-64) and the results are shown in Fig. 6.

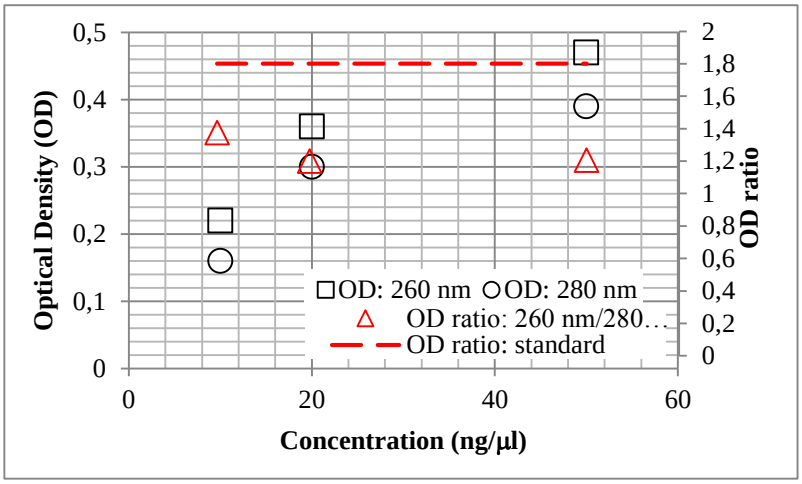


**Fig. 6.** Absorbance value plot for the prepared turbidity standards

Horizontal axis of the Fig. 6 is the concentration of DNA. The vertical axis of left side is optical density showing absorbance of the sample, and the vertical axis of right side is the ratio between the optical densities (OD) at 260 nm and 280 nm wavelengths. Black square and black circle are the measured value of OD at 260 nm and 280 nm wavelengths, respectively.

Red triangle and red line show the OD ratio (260 nm / 280 nm) and the upper limit of the ratio for the turbidity standard. From this result, OD ratio was evaluated 1.26±0.09. It was much lower than the upper limit of turbidity standard (value: 1.8). In other word, our sample is sufficiently met to the turbidity standard.

## 4. Conclusion

In this study, a DNA sample which was prepared from E-coli bacteria was irradiated with UV lights at various time intervals. The irradiation experiment was also performed in two different conditions of UV wavelengths of 254 nm and 365 nm for the improving. The results showed as follows.

- The DNA molecules might be broken by the irradiation of UV lights. It is considered that free radicals due to the excitation of water molecules were produced effectively in the sample, and the DNA molecules were broken by the indirect action via the free radicals.
- The areas of the luminescence spectrum from the DNA increased when the irradiation time increases. After some time it will be saturated.
- DNA samples prepared by our local maker (Zanaspex Co., Ltd.) were sufficiently met to the turbidity standard. It is important for the researchers using DNA in their researches, in Mongolia, especially in present time when imports from other countries are difficult due to Covid-19 pandemic.

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