Journal of College of Education for Pure Sciences
Volume 8, Number 2, June 2018
Website: jceps.utq.edu.iq Email: jceps@eps.utq.edu.iq

Study of Killing Effects of Neutron and UV Light on Bacteriophage in a Dry State by Chemiluminescence

DoaaGata Ghali[1] ,Emad A. Salman[2], Qahtan A.Abdulqader[3]

(1) Physics Department,M.Sc Student, University of Thi-Qar, Al-Nasiriya, Iraq.
(2) Physics Department, Faculty of sciences, University of Thi-Qar, Al-Nasiriya, Iraq.
(3) Physics Department, Faculty of sciences, University of Al-muthanna , Al-muthanna ,lraq.

Abstract

This work is concerned to the study of the effects of ionizing radiation including (beta, neutron and ultraviolet rays) on the high virulence strains of Escherichia coli (O157: H7 and O26),which are known for their high toxicity and high susceptibility to free radicals . Besidesthe solution of white blood cells containing the microphage were prepared , microphages have the ability to produce free radicals during the process of killing and infection of bacteria. Increasing or decreasing the effectiveness of bacteria as well as white blood cells are done by reading the chemilumenscence,Which produced by free radical, generate from the oxidation of a certain chemilumenscence reagent, named: Lucigenine, to increase leukocyte toxicity from bacterial toxicity, low-dose of ionized radiation have been used. The luminescence of individual , mixed microphages and bacteria have been recorded, through chemical glare, ie, that the pellets shine larger than the bacteria. The readings were taken individually out-of-body state (invitro).The readings for whole blood were taken, which are simulated to the readings inside the body (invivo).

Keywords: Chemiluminescence's, killing effects , neutron particles, UV light, E.coli.

مجلة كلية التربية للعلوم الصرفة - جامعة ذي قار
المجلد ٨، العدد ٢، حزيران ٢٠١٨
Website: jceps.utq.edu.iq Email: jceps@eps.utq.edu.iq

دراسة التأثيرات القاتلة للنيوترونات والاشعة الفوق البنفسجية على الخلايا الالتهامية بوساطة التألق الكيميائي

DoaaGata Ghali[1] ,Emad A. Salman[2], Qahtan A.Abdulqader[3]

(1) Physics Department,M.Sc Student, University of Thi-Qar, Al-Nasiriya, Iraq.

(2) Physics Department, Faculty of sciences, University of Thi-Qar, Al-Nasiriya, Iraq.

(3) Physics Department, Faculty of sciences, University of Al-muthanna , Al-muthanna ,lraq.

الخلاصة

يدرس هذا العمل بدراسة تأثيرات الأشعة المؤينة ذات قدرة أيقاف معينة مثل(أشعة بيتا والنيوترونات والأشعة الفوق البنفسجية) على السلالات ذات عوامل الضراوة العالية من البكتيريا القولونية Escherichia Coli سلالة (O157:H7 و 26O)المعروفة بتأثيراتها السمية العالية وقابليتها العالية على أنتاج الجذور الحرة . مقابل هذه البكتيريا تم تحضير محاليل من كريات الدم البيضاء الحاوية على المايكروفيج والتي لها القابلية على أنتاج الجذور الحرة اثناء عملية قتل وألتهام البكتيريا . أن زيادة فعالية البكتيريا أو نقصانها وكذلك كريات الدم البيضاء يتم عن طريق قراءة التألق الكيميائي لها . باستخدام صبغة اللوسجنين زيادة سمية كريات الدم البيضاء من سمية البكتيريا . وذلك بتحفيزها بواسطة الإشعاع بالجرعات الواطئة . ومعرفة ذلك من خلال التألق الكيميائي أي ان تألق الكريات أكبر من البكتيريا . واخذت القراءات كل على حده في حالة خارج الجسم (invitro) وكانت القراءات مشابهة لأخذ القراءات في داخل الجسم (invivo).

1.Introduction:

Chemiluminescence (CL) is one of the luminescent phenomena and it can be defined as the production of electromagnetic radiation (Ultraviolet, visible or infrared) by chemical reaction [1, 2,3]. The phenomenon can be observed when an electronically excited product or intermediary formed during reaction decays to ground state by emitting a photon. CL reaction can be generated by two basic mechanism direct and indirect (also called sensitised). This results depends on whether the reaction product is responsible for light emission, or on the contrary this excited product is an ineffective emitter but can transmit its energy to an effective fluorophore added to the system[4, 2, 5]. CL reaction can occur in the gas, liquid and solid phase. This liquid shows the greatest potential for analytical application, and proofs of this are used for the numerous publications, which have appeared based mainly on this topic. CL gas phase reactions have become very useful for measuring and monitoring a number of important components related to atmospheric chemistry [5]. CL applications in the solid phase are more limited [6]. The light emitted from CL reactions has differing degrees of intensity, life time and wavelength .The wavelength can extend across the spectrum from near ultraviolet, through the visible and into the near infrared .The intensity of emission of reaction is dependant on the quantum yield, The quantum yield is a measure of the efficiency of the CL reaction [4]. CL from organic molecules is an end result of series of complex chemical and physical transformations. These transformations convert the potential energy contained in the chemical bonds of the reactants to radiant energy in the visible region of the electromagnetic spectrum [7]. The chemical energy conversion into energy of radiation depends on the type of reactions, its thermal effects on structure, properties of the reactions, the products, and the medium in which the reaction takes place [8]. These are known number of reactions of organic compounds, which are emitting visible light in a quantity enough for fine experiments. Among these reactions , the oxidation of lucigenin, luminol.CL is defined as the emission of electromagnetic radiation produced by a chemical reaction. When this emission originates from living organisms or from chemical systems derived from it, it is named Bioluminescence (BL). Both phenomena are luminescence processes that have been traditionally distinguished from related emissions. By a prefix that identifies the energy source responsible for the initiation of emission of electromagnetic radiation [6]. The sufficiently low limits of detection, the excellent sensitivity and the versatility of the CL methods of analysis are the main reasons for the recent surge of interest in CL and BL [4].

2.Experimental Details:

The CL solution was prepared by dissolving 0.07 gm of lucigenin in dimethyl sulphoxide to obtain $0.7*10^{-4}$M. To measure CL curve , we put 5ml from cl solution in a beaker of 10ml and left for 60 second, then the beaker was put in the photomultiplier tube (PMT)to begin reading the measurements. To measure the CL of eachwhite blood cells (WBC)we added the certain amount of studied component to above cl solution in the photomultiplier tube(pmt)and operate the system to get the CL reading on the digital reader.Figure 1, shows the CL reader.

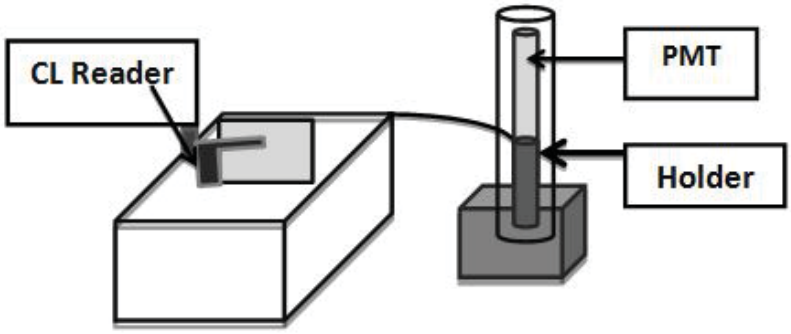

Fig (1): show CL reader detected

3. Results and Discussions:

Firstly we have examined the effect of ultraviolet radiation on E.colistrains.In the following forms, bacterial strains were subjected to ultraviolet radiation at different time intervals. We observed that the bacteria's ability to produce free radicals decreased by increasing the exposure time of radiation, This is evident in the decrease in the curve of chemiluminescence.

a.Effect of ultraviolet radiation on E.coli bacteria strain O157:H7 .

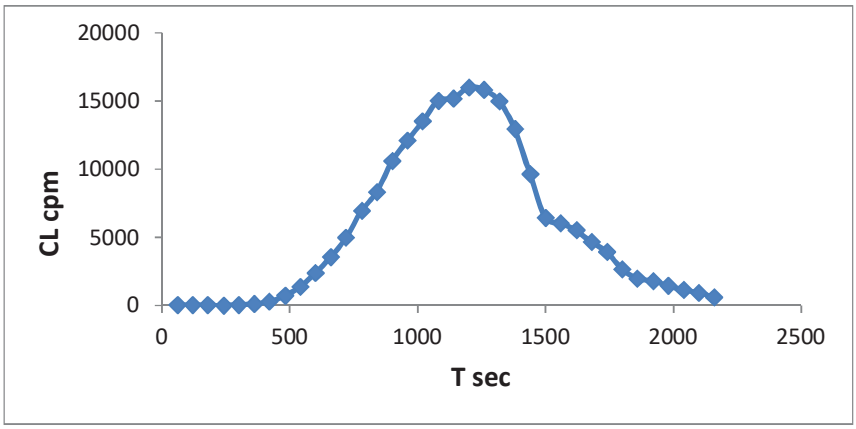

Fig.2.Represent Effect of ultraviolet radiation on E.coli bacteria strain O157:H7 after an hour post irradiation .

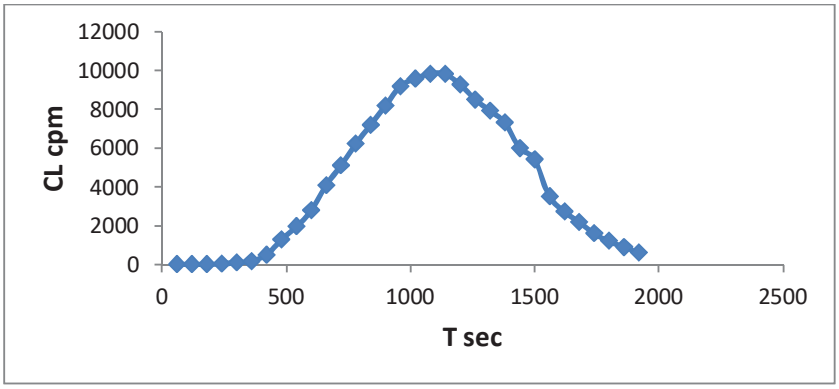

Fig.3.Represent Effect of ultraviolet radiation on E.coli bacteria strain O157:H7 after two hours post irradiation .

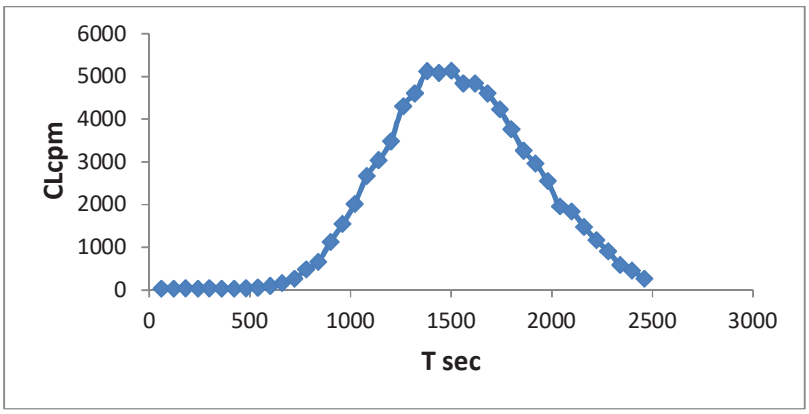

Fig .4. Represent Effect of ultraviolet radiation on E.coli bacteria strain O157:H7 after four hours post irradiation .

b.Effect of ultraviolet radiation on E.coli bacteria strain O26 .

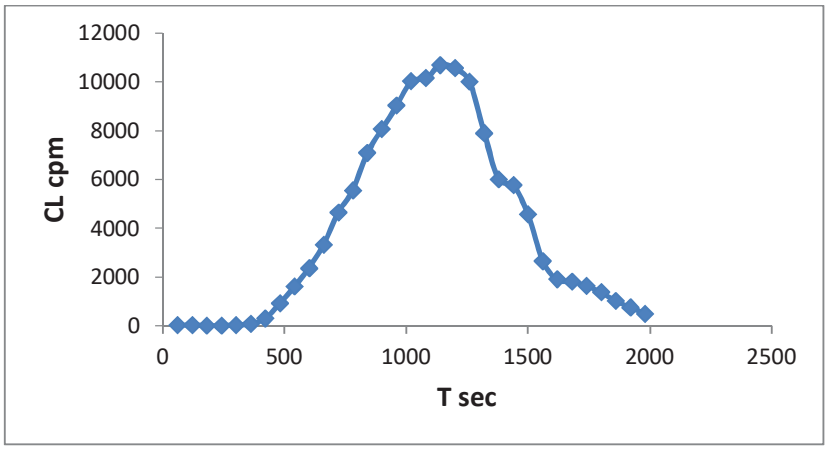

Fig.5.Represent Effect of ultraviolet radiation on E.coli bacteria strain O26 after an hourpost irradiation .

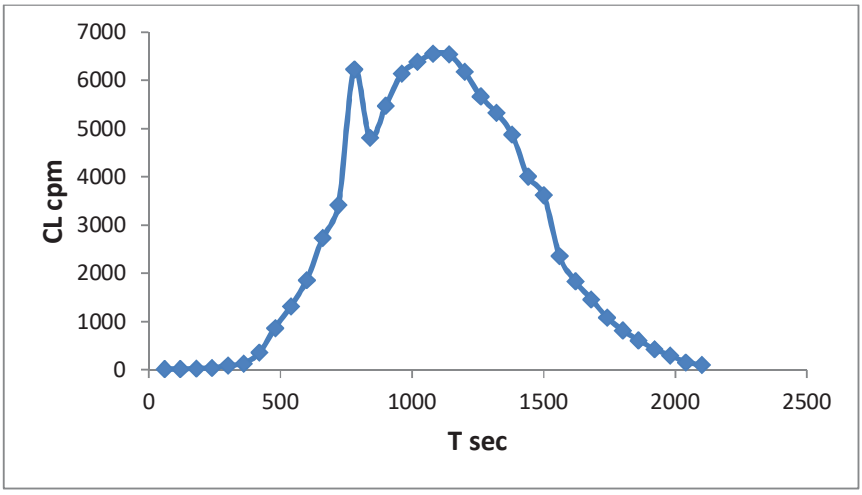

Fig.6.Represent Effect of ultraviolet radiation on E.coli bacteria strain O26 after two hours post irradiation .

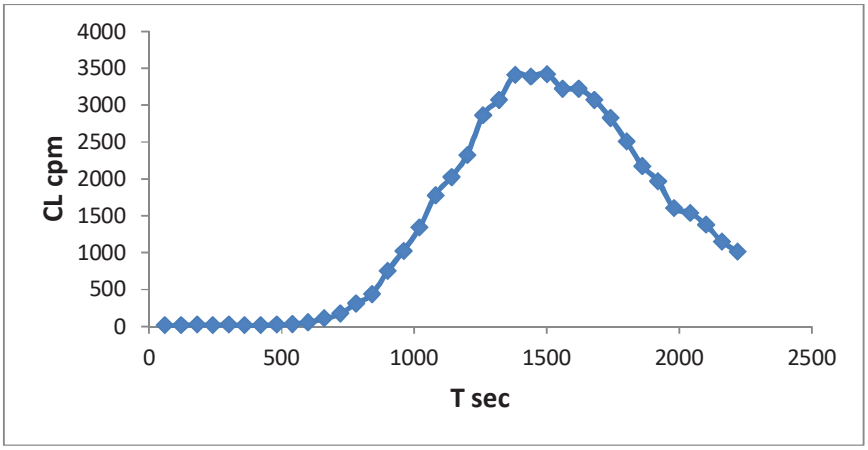

Fig.7.Represent Effect of ultraviolet radiation on E.coli bacteria strain O26 after four hours post irradiation.

In the living organism there are many processes depending on oxygen, and Phagocytosis are the most important process among them, so during the immunological defense, the activity of pentosphosphate cycle is increased through forming NADPH which needed to reduce the oxygen which is bonded to membrane based cytochromes. Therefore the oxygen demand is strongly increased (respiratory burst). During this process, oxygen is converted into superoxide anion, hydrogen peroxide, monomolecular oxygen, and hydroxyl radicals by mean of several kinds of phagocytic cells (neutrophils, eosinophils, basophil leukocytes and macrophages [9]. These extra cellular highly reactive oxygen species (ROS) cause many biological effects such as destruction of bacterial cells, viruses, parasites and tumor cells, lead to promoting inflammation and modulating

the immune reaction. Suggested that radiations are activators of the superoxide anion ($\bar{O}_2$) critical to the microbiocidal action[10,11]. The production of $\bar{O}_2$and subsequent CL is the result of the activation of the membrane bond NADPH, NADH oxidase by radiation.

This study shows that low doses of radiation result in stimulation rather than inhibition of oxidative metabolism, this stimulation might be attributed to a radiation induced alteration of whole blood plasma, here one might consider on increase of Adenosine diphosphate (ADP) concentration which leads to an enhanced CL in irradiated WBC, as it is known from the experiment of the concentration of ADP in serum increases after irradiation.[12] ADP is knwon as a stimulant of platelet aggregation so often in a direct contact of platelet to leukocytes could give rise to enhanced CL after stimulation by low doses of NDV [12,13],showed that the activity of NADPH-oxidase is low in intact cells but can be increased rapidly in response to various stimuli. In other words, the CL response kinetics reflects the process of NADPH-oxidase activation at its early stages as reffered previously in [14,15].

4. Conclusion:

It is concluded from this work that CL is a powerful tool for the analysis since its detection limits are extremely low and its instrumentation is very simple . In combination with derivatization techniques in order to increase sensitivity, it has a wide range of applications. It is also concluded and demonstrated from this work that even very low intensity radiation of blood can effectively influence important physiological processes in isolated leukocytes. This observation is a recent claim that blood may mediate effects upon other body systems. The examination of the influence of various assay conditions on the CL responses of whole blood and isolated neutrophils have optimized CL measurement and demonstrated good reproducibility. Further of more the confirmation of lucigenin CL measures generation. Also the assay provides simultaneous measurements on a large number of samples and the optimized measurement conditions, which can be applied to the accurate assessment of phagocytic function in various biological and clinical studies.

5. References:

1. Calokerions A.C.; Deftereos N.T. and Baeyens W.R.G. (1995). Chemiluminescence in drug assay. Journal of pharmaceutical and Biomedical Analysis, vol. 13, pp. 1063-1071.
2. Liu y.m.; chengj.k. (2002) .ultrasensitive chemiluminescence detection in capillary electrophoresis . Journal of chromatography a , vol.595 ,pp.1-13.

3. Kroskosz A. (2003). The effect of hypochlorite on human erythrocytes pretreated with X-radiation, Cellular of Molecular Biologyu letters, vol. 8, pp. 215-219.
4. Calokerions A.C.; Deftereos N.T. and Baeyens W.R.G. (1995). Chemiluminescence in drug assay. Journal of pharmaceutical and Biomedical Analysis, vol. 13, pp. 1063-1071.
5. Navas M.J.; Jimenez A.M. and Galån G. (1999). Air analysis determination of hydrogen by chemiluminescence. Atmospheric environment, vol. 33, pp. 2279-2283.
6. Garcia-campana AM. Baeyens WRG; Zhang X; Ales F and Gamiz L. (2001). Unifamiliar though exciting analytical detection in flowing streams: chemiluminescence. Arspharmaceutica, vol. 42, No.1, pp. 81-107.
7. Schuster g.b. ; homk.a. (1982) ." chemical and biological generation of excited states " adam w. And cilento g. Eds academic press , n.y.ch.7 ,pp.229-246
8. Shlyapintokhv.y (1968) " chemiluminescencstechniqes in chemical reaction " . Academic n.m.emanudn.y. Pp:1-128 .
9. Babior ,b.m(1984) :- the respiratory burst of phagocytes. J.clin. Invest. Vol.73,pp.599-601.
10. Fujita I.; irita k.; takeshigek.andminikami s.(1984). Diacylglycerol,1- oleoyl-2-acetyl -glycerol stimuli superoxide-generation from human neutrophils .biochemical and biophysical research communications,vol.20,pp.318.324.
11. .Nishizuka y.(1984). The role of protein kinase c in cell surface signal transduction and tumourpromotior .nature,london ,vol.308,pp.693.698.
12. Hovestadt, Ernst M.; Mönig H. And Fischer h. (1983). The early effect of sublethal X-irradiation of phagocytic cells in mouse blood and influence of cystamine as measured by chemiluminescence. INT. J. RADIAT. BIOL., vol. 44, No. 6, pp. 563-573.
13.Karu, t.i;ludmila , v.p. And tatyana, p.r. (1997): nonmonotonic behavior of the dose dependence of radiation effect on cells in vitro exposed to pulsed laser raditaion at h=820 nm . Laser insurgery medicine .vol21,pp.485-492.

14..Baggioloini,m.andwyman ,mp.(1990) turning on the respiratory burst .trends biochem.sci.vol.15, pp-69-72.
15.Segal ,aw.andabo,a.(1993): the biochmical basis of the nadph-oxidase of phagocytosis. Trends biochem.sci.vol.18,pp.43-47..