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College of science

Department of Physics



***Study of fatal effects of Neutrons, Beta particles
and UV-Light on Macrophage using
Chemiluminescence Technique***

A Thesis

Submitted to the college of science University of Thi-Qar

As a Partial Fulfillment of the Requirements for

The Degree of Master of Science in Physics

By

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَإِذَا سَأَلَكَ عِبَادِي عَنِّي فَإِنِّي قَرِيبٌ ۖ أُجِيبُ
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وَلْيُؤْمِنُوا بِي لَعَلَّهُمْ يَرْشُدُونَ

صدق الله العظيم

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We, the examining committee certify that this thesis entitled "***Study of fatal effects of Neutrons, Beta particles and UV-Light on Macrophage using Chemiluminescence Technique***" and examining the student **(Doaa Gata Ghali)** in its content and that, in our opinion, it meet the standards of a thesis for degree of master of science in physics.

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Doaa

Dedication

To the Master of martyrs

Imam Hussein

(peace be upon him)

To the Source of my Strength

And my Soul

(My Father)

Abstract :-

This work is concerned with the study of the effects of ionizing radiation involving (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. In addition to these bacteria, 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 chemiluminescence, which produced by free radical, generate from the oxidation of a certain chemiluminescence reagent, named: Lucigenine, to increase leukocyte toxicity from bacterial toxicity, low-dose of ionized radiation were used. The luminescence of individual, mixed microphages and bacteria were recorded, through chemical glare, ie, that the pellets shine larger than the bacteria. The readings were taken individually out-of-body state (invitro). And the readings for whole blood were taken, which is simulate to the readings inside the body (invivo).

A leukocyte is regarded as the main defensive system of animal body against foreign bodies such as (bacteria, parasites viruses). All types of leukocytes have the ability to produce reactive oxygen species such as (singlet oxygen $^1\text{O}_2$, super oxide $\bar{\text{O}}_2$, hydroxyl radical OH^* and hydrogen peroxide H_2O_2) in the process of phagocytosis and respiratory burst. When leukocytes exhibit respiratory burst emits light (chemiluminescence). The emitted light is very weak and requires the introduction of CL materials to increase the efficiency of light detection. The CL solution used is lucigenin dissolved in Dimethyl sulfoxide (DMSO), taking the readings of CL bacteria and white blood cells before irradiation in order to choose the best laboratory conditions for the highest reading CL. These readings were adopted zero-reading., Bacteria

and white blood cells were exposed to different doses of beta, ultraviolet and neutron . Bacteria were added to the white blood cells without exposure to radiation.

Abbreviations	Meaning
CL	Chemiluminescence
Ci	Curie
$_1D^2$	Deuterium
Fw	Molecular weight
H₂O₂	Hydrogen peroxide
KeV	Kilo electron volt
Min	Minute
$^1O_2$	Singlet oxygen
O₂	Superoxide radical
OH[•]	Hydroxyl radical
PMT	Photomultiplier tube
ROS	Reactive Oxygen Species
SV	Sievert
T	Time
V	Volt
WBCs	White blood cells
cpm	Count per Minute
μl	Microliter
CDC	Center of Disease Control and Prevention

DNA	Deoxyribonucleic acid
E.coli	Escherichia coli
EAEC	Enteraggregative E.coli
EHEC	Enterohemorrhagic E.coli
EIEC	Enteroinvasive E. coli
EPEC	Enteropathogenic E. coli
ETEC	Enterotoxigenic E.coli
HUS	Hemorrhagic uremic syndrome
ml	Milliliter
mm	Millimeter
STEC	Shiga toxin-producing E. coli
stx	Shiga like toxins
ADP	Adenosine diphosphate
NADPH	Dihyronicotinamide Adenine Dinucleotide phosphate
NADH	Dihyronicotinamide Adenine Dinucleotide
Gy	Gray
PMNL	Polymorphonuclear Leukocytes

CHAPTER ONE

INTRODUCTION

1.Introduction

1.1 Luminescence

Luminescence is a term used to describe the emission of light, which occurs when molecule in excited state relaxes to its ground state [1]. The various types of luminescence differ from the source of energy to obtain the excited state. This energy can be supplied by electromagnetic radiation (photoluminescence also termed fluorescence or phosphorescence), by heat (pyroluminescence), by frictional forces (triboluminescence), by electron impact (cathodo luminescence), by crystallization (crystalloluminescence), by chemical reaction (chemiluminescence) [2], and Bioluminescence found in biological systems [3]. The properties of any luminescence system which can be measured directly by experimental observation which are, absorption spectra, emission spectra, excitation spectra, lifetimes and rate parameters, quantum efficiency, and yields [4].

1.2 Chemiluminescence

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 [5, 6, 7]. 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) it can shown in figure (1-1). 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[8,6,9]. 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 [9]. CL applications in the solid phase are more limited [10]. 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 [8]. 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 [11]. 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 [12]. 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 [10]. 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 [8].

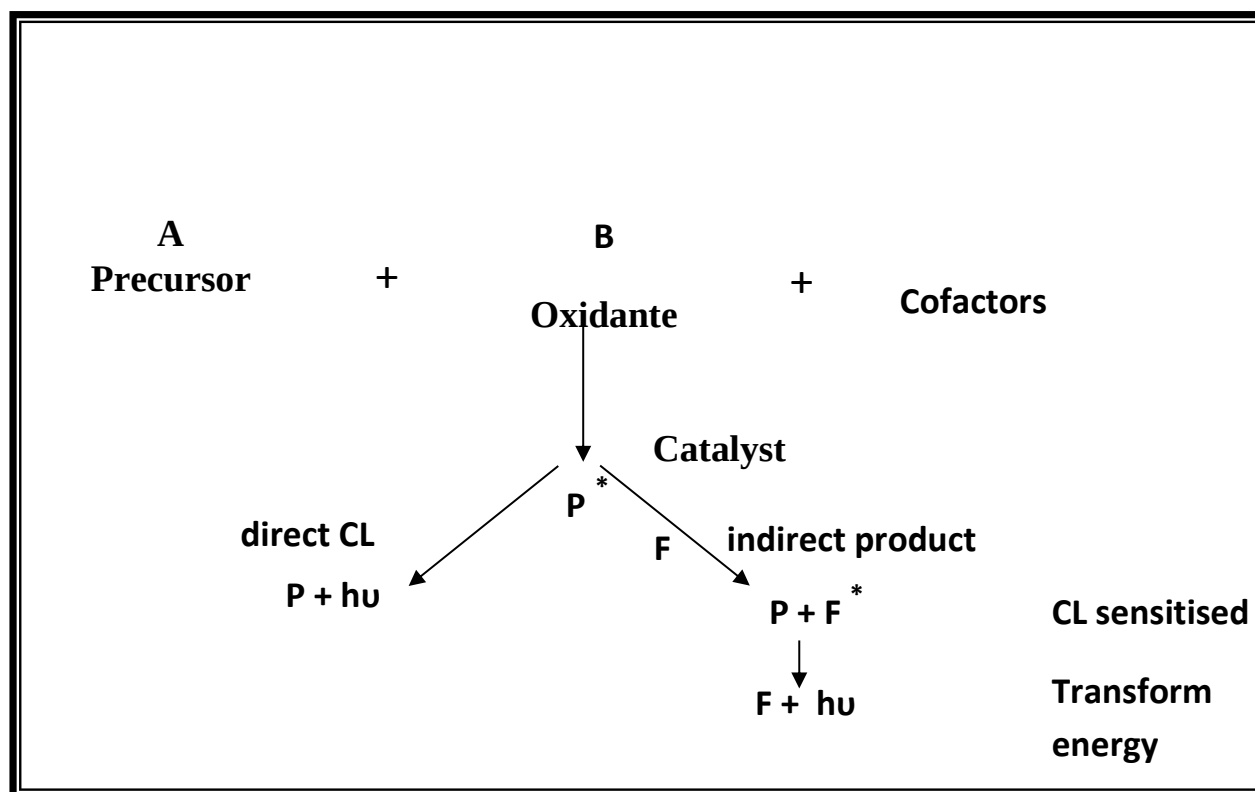


Fig: (1-1) Types of CL reaction (P: product, F: fluorescing substance).

1.3. Chemiluminescence and free radicals produced by

Leukocytes

Chemiluminescence of phagocytes was first described by Allen et al (1972), who noted the emission of light quanta by granulocytes following phagocytosis. CL depends upon the production of oxygen radicals during the respiratory burst (The respiratory burst is defined as an increase oxygen consumption and generation of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) or defined as a complex

series of reactions beginning with assembly of a membrane bound enzyme, the (NADPH) oxidase which is able to catalyze one electron reduction of oxygen to $\dot{O}_2^-$. also the respiratory burst refers to a coordinated series of metabolic events that take place when phagocytes are exposed to appropriate stimuli) [13,14]. Early in the phagocytic process, neutrophils undergo a respiratory burst, generating oxygen-free radicals which, after their production, decay naturally and emit light [15]. Thus CL enhanced by the presence of luminol and lucigenin, may be used as a measure of phagocytic activity. Two types of free radicals are produced by neutrophils, the first type is represented by reactive oxygen intermediates which are formed in neutrophils by the activity of NADPH oxidase, the enzyme of the respiratory burst .The first type includes, superoxide anion ($\dot{O}_2^-$), Hydrogen Peroxide (H_2O_2), Hydroxyl radical (OH^*) and singlet oxygen ($^1O_2$). The second type includes reactive nitrogen intermediates, the first member of them nitric oxide being produced by nitric oxide synthases .

1.4 Reaction Mechanism

It is well known that the production of reactive oxygen species (ROS) by white blood cells (WBCs) can be measured using lucigenin-amplified CL. The lucigenin react with reactive oxygen species, the lucigenin convert to unstable dioxetane intermediate. The lucigenin dioxetane decomposes to produce two molecules of N-methylacridone, one of them is in an electronically excited state, which upon relaxation to the ground state emits a photon [16,17]. As shown in the Figure (1-2). Which illustrated this processes.

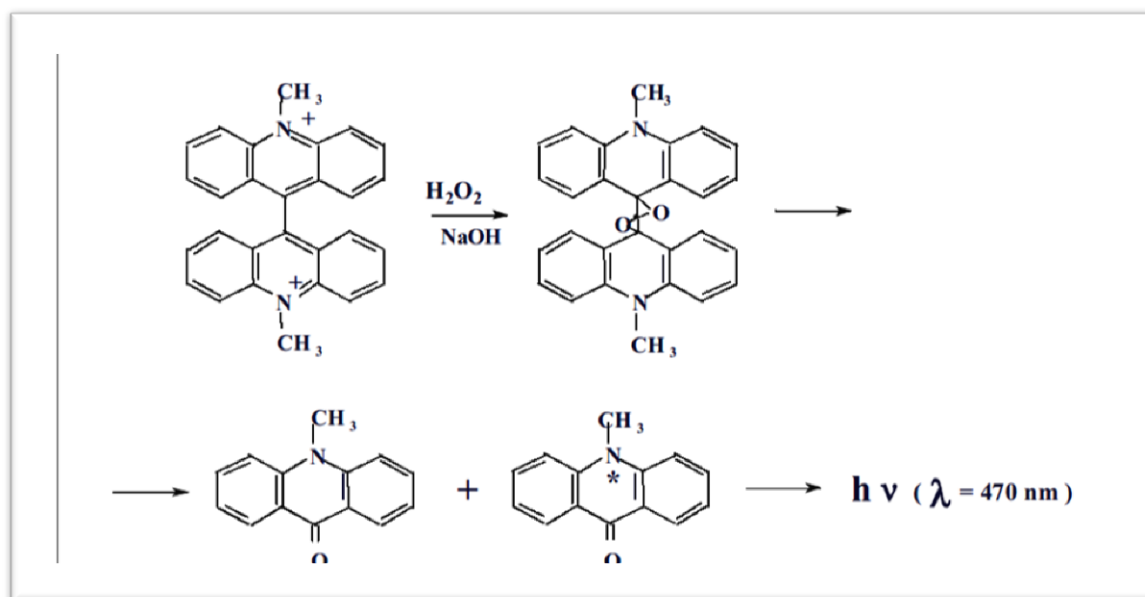


Fig: (1-2) Luminescence phenomena caused by the reaction with reactive oxygen species [16].

1.5 Radiation

Most elements present in nature have no radioactivity but natural elements of 209 Atomic weight and more Their nucleus decay by itself giving energy in a form of radiation such as Alpha particles which are regarded as a large nucleus which is identical to the nucleus of a helium atom which moves in a slow motion in way can be easily stopped, or beta particles which are negative or positively charged electrons which has very small mass compared with alpha particles, its effect extend to several centimeters in the materials, or gamma rays which is electromagnetic rays has a very short wave length. From the naturally radioactive elements are the Uranium, Radium, Thorium, and Actinium, when rays emitted from those elements, they change to a resting non-radioactive elements. Also there are industrial radioactive elements such as Tritium, which can be made by forcing a neutron to the hydrogen ${}^1_1\text{H}$, or a neutron to the heavy hydrogen (Deuterium ${}^2_1\text{D}$).

Our research is using the following radiations

1.5.1 Ultraviolet

The light is just one part of the spectrum of light electromagnetic waves that travel through the vacuum. The electromagnetic spectrum covers a wide range of radio waves with a wavelength of up to one meter or more, reaching x-ray with a wavelength less than one millionth of a meter, UV-A (400-320)nm, and UV-B (280- 320) nm and UV-C (280-200)nm, called bactericidal range because it effectively inhibits bacteria and viruses [18].

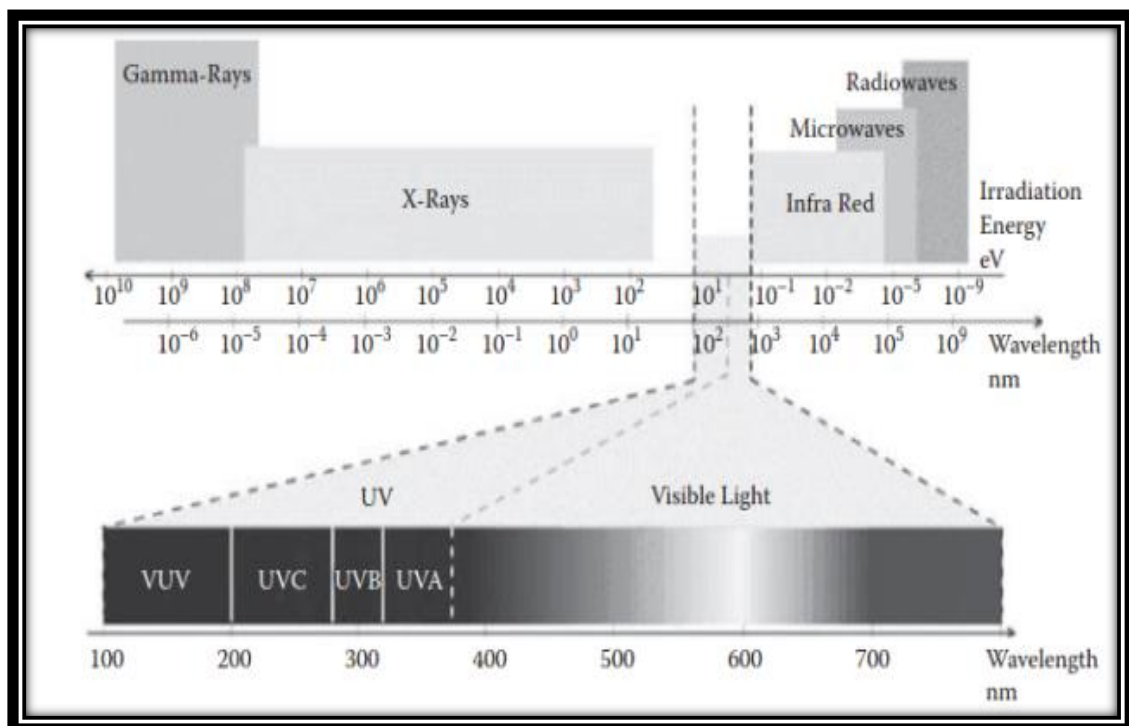


Fig: (1-3)Spectrum radiation electromagnetic with partition cute visible light and ultra violet.[18]

In recent years, ultraviolet radiation has been used for water treatment and disinfection surfaces of a wide range of materials in the food and pharmaceutical industries.

1.5.2 Ionizing Radiation

This type of radiation is characterized by having high energy sufficient for the electronic ejection of the material or the irritation of the substance falling in it. [19] These radiation have very short wavelengths (smaller than nm100), they can focus a large amount of energy on a small space which enough to break down the chemical bonds between the atoms and be able to directly remove the electrons from most atoms. There are several types of ionizing radiation such as gamma rays, X-rays, and particles such as alpha, and beta, [19].

1.5.3 Non-Ionizing Radiation

Is an electromagnetic radiation with wavelengths greater than (100nm), which, it has long wavelengths, with little energy enough to move atoms around the molecules of the material causing vibration, that is not enough to remove electrons from atoms, involving , visible light and ultraviolet light [21,20].

1.5.4 Measurements units

Any radioactive element activity is measured at the number of occurs in a time unit so the activity unit is called Curie, where curie is defined as activity of any radiated element decay in rate of 3.7×10^{10} in the second so,

$$1 \text{ Curie (Ci)} = 3.7 \times 10^{10} \text{ disintegration / sec.}$$

The unit of radiation exposure is called Roentgen and this unit is contained mostly to gamma and X-radiation , defined as the radiation dose which emitting 187.7 erg of the energy in one gram

Chapter One -----

of air and in water or soft tissue is equivalent to 93 erg /gm [22,23]. The absorbed dose unit is called rad and defined as the amount of absorbed radiation equivalent to 100 erg /gm of the exposed material to radiation or (0.01 joule/kgm) this unit is used for all radiations and elements or materials. Lastly the dose equivalent unit is called rem which is the amount of radiation of any kind exerts a biological effect equivalent to one rad of X-rays and it called the sievert (sv)

$$Sv = 100 \text{ rem} = 1 \text{ Joule/kgm.}$$

The relative biological effect can be defined as the amount of radiation energy, which causes equal biological effect. The dose in rem = absorbed dose $\times$ parameter of relative biological effect. The parameter of relative biological effect is different from type of radiation to another so for X-ray and gamma radiation is equal to 1 and for fast neutrons =10, for protons =10 ,but for heavy nuclei like alpha particles =20[22].

1.6 Escherich coli

The German pediatrician and scientist, Dr.Theodor Escherich isolated organism for the first time in (1885) from the intestinal flora of infants and he called them *Bacterium coli commune* [24,25,26]. Later this organism was named as *Escherichia coli* or *E. coli* on his honor [27]. *Escherichia coli* is a member of the family *Enterobacteriaceae*, it is Gram negative, rod shaped, non-spore forming, facultative anaerobe grow in 37°C [28], its length 2 μm while its diameter is 5 μm with cell volume of 0.6-0.7 μm^3 , [29]. *Escherichia coli* colonizes the gastrointestinal tract of

humans and animals in the first hours of life, where a mutual relationship quickly develops between the bacterium and host [27]. Most strains of *E. coli* are non-pathogenic and may serve as protective normal gut flora by preventing the colonization of pathogenic bacteria but in an immunosuppressed host, or when gastrointestinal barriers are broken approximately two million people per year [30].

1.6.1 Serotyping

Strains of pathogenic *E. coli* can be differentiated serologically depending on surface antigens; these antigens are divided into three major groups [27]. These three groups are (O), which is somatic antigen refer to the lipopolysaccharide which is the outer layer of the outer membrane of *E. coli*. There are 174 known O-antigens (numbered 1 to 181, with numbers 31, 47, 67, 72, 93, 94, and 122 were deleted), the second one in classification system is (H) antigen, the flagella antigen group found in the flagella of bacteria, currently, (53) H- antigens are used to serotype *E. coli* strains [31]. The last one is (K) antigen, which represents a class of thermolabile polysaccharides that form sheaths, envelopes, or capsules. This antigen was first identified through bacterial agglutination tests [32]; [33]. However, only extraintestinal *E. coli* are encapsulated [34]. For that, *E. coli* strains are classified using a combination of O and H antigens (ex. *E. coli* O26:H11) however, not all pathogenic *E. coli* contain flagella; in this case, isolates should be serotyped based on the O-antigen only and identified as non-motile (NM) (ex. *E. coli* O26:NM) [35,36]

1.6.2 Biotype

In 1945, John Bray first described Biotype of *E. coli* strains that were responsible for causing a disease syndrome is known as infantile diarrhoea [37].

1.6.2.1 Enteropathogenic *E. coli* (EPEC):

Is commonly associated with infant's diarrhea, the term enteropathogenic *E. coli* was first coined by Neter et al. (1955), to indicate those strains of *E. coli* that had been epidemiologically linked with diarrhea. EPEC is still regarded as an important category of diarrhoeagenic *E. coli*, which after that was linked to diarrhea in industrialised countries and now in the developing world [27].

1.6.2.2 Enterotoxigenic *E. coli* (ETEC):

These pathotypes are an important worldwide cause of diarrheal disease in calves up to 4 days old, although they can occasionally produce diarrhea in older calves [38]. In humans, mainly affecting children in developing countries [27], DuPont et al. (1971), showed that ETEC strains were capable of causing diarrhea in adult volunteers.

1.6.2.3 Enteroinvasive *E. coli* (EIEC):

Is atypical and difficult to be identified antigenically, biochemically, and pathogenically where these strains resemble *Shigella* so more than the illness caused by EIEC has often been mistaken for shigellosis. Clinical features include fever, abdominal cramps, malaise, toxemia, and watery diarrhea or typical dysentery with blood [27].

1.6.2.4 Enteroaggregative E.coli (EAEC):

These types of organism have recently been associated with persistent diarrhea in children and infants in industrialised and developing countries worldwide [27].

1.6.2.4 Sepsis/meningitis causing E. coli (MNEC):

infect Neonates causing meningitis [39].

1.6.2.5 Uropathogenic E. coli (UPEC):

commonly in sexually active women and cause cystitis, and pyelonephritis [39].

1.6.2.6 Enterohemorrhagic E.coli (EHEC):

or Shiga toxin-producing E. coli (STEC). This group is of most concern, they are capable of causing severe disease in humans and animal [27], so we will focus on this pathotype

1.6.3 Bacterial purification:

Five colonies of more common isolated STEC non-O157 were inoculated on (10 ml) of tryptone soy broth at 37 °C for (24) hours then centrifuged in cooling centrifuge (5000 cycle) for (15) minutes then the sediment (pellet) was washed three times with PBS (pH=7.2) then it was suspended by using (9) ml of PBS (pH=7.2) and tenfold dilution (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} and 10^{-10}) were they an done.

1.6.4 Bacterial count:

The viable count of bacteria in each diluent was made according to surface spread [40]. Two Petri dishes per dilution to be tested were set out and labelled with appropriate dishes, using a fresh pipette for each dilution. 100ul of samples were aseptically put at the center of each Petri dishes and surface spread on nutrient agar then all the plates were inverted and incubated at 37°C for 24 hour. Viable counting of bacterial colonies were done. The colony count was calculated by multiplying the average number of colonies per countable plate by the reciprocal of the dilution the reported as colony forming unit per ml of the sample and the diluent which had these concentrations had been selected : (1.5×10^5 cells), (1.5×10^6 cells), (1.5×10^7 cells), (1.5×10^8 cells), (1.5×10^9 cells), (1.5×10^{10} cells) and (1.5×10^{11} cells).

1.7 Human blood and its components :

Although blood is a connective tissue but it is as a homogenous solution composed from red blood cells, white blood cells and platelets. These blood cells moves in a solution of proteins , ions inorganic , organic, and small molecular weights [41]. The different cells which found in the blood will suspend in a liquid its color is yellowish in normal state which is called the plasma [42]. It means that blood is composed from two parts, first one is the cellular Part resembling the different blood cells, and second which the liquid part, which called plasma, which contain blood proteins, enzymes, coagulation factors, and immune bodies.

1.8 Review of literature

1.8.1 Importance of Chemiluminescence (CL)

In recent years CL has become attractive detection method for analytical determination because of the very low detection limits and wide linear working range which can be achieved while using relatively simple instrumentation [43]. CL is a powerful tool for drug analysis since its detection limits are extremely low and its instrumentation very simple and it is low cost .In combination with derivatization techniques in order to increase sensitivity .CL has a wide range applications [44]. CL has been widely used as a sensitive and accurate method for the assessment of capacity of neutrophils to produce ROS by using different chemilumigenic probes such as luminol , and lucigenin [45].

1.8.2 Measurements of Activity of leukocytes by CL

Florfenicol, chloramphenicol , and thiamphenicol in vitro to determine their potential toxic effects on bovine neutrophils by CL and Morphology [46], they found chloramphenicol suppressed phagocytosis and completely blocked respiratory burst activity , of neutrophils , as result is no effects observed in neutrophils exposed to florfenicol and thiamphenicol. studied the phagocytic activity and the respiratory burst of PMNL of whole blood which was activated by Bariumsulphate crystals, demonstrated the peak CL of whole blood was affected by the number of PMNL [47] .It increased with the increase of the number of PMNL in the tested sample, and it was reduced with increase in the number of red blood cells in the tested sample. showed that phagocytic production of (ROS) can be measured using microtitre plate based CL blotting technique. [48].

The production of (ROS) is determined by their ability to catalyze the oxidation of luminol or isoluminol. used (phorbol 12-myristate 13-acetate (PMA) stimulates macrophage to produce respiratory burst [48] .The free radicals play an important role in microbicidal and tumoricidal activity and in protecting the healthy body from diseases, they showed that PMA stimulated macrophage to produce not only superoxide anion but also Nitricoxide free radicals Demonstrated that neutrophil CL during phagocytosis is inhibited by abnormally elevated levels of Acetoacetate. Implication for Diabetic susceptibility to infections. [49]. used CL to investigate the competence of turkey monocytes to mount a respiratory burst response upon interaction with chlamydia psittaci[50]. They demonstrated that this chlamydia psittaci strain induces only a very weak respiratory burst response in turkey monocytes. In contrast, chlamydia psittaci itself elicited an intense non-superoxide mediated lucigenin dependent CL. studied NADPH oxidation and cytochrome C reduction with and without lucigenin as well as NAD(P) H /lucigenin-dependent CL of rat liver microsomes[50] .An increased rate of NADPH oxidation and cytochrom C reduction in the presence of lucigenin was related to one-electron lucigenin reduction by microsomal NADPH reductases. Studied the effect of guanidino compounds known uremic toxins, on neutrophil (ROS) production in vitro [51]. They found diminished reactive oxygen inter mediate production, which is an important consequence of impaired neutrophil function. measured the respiratory burst of leukocytes isolated from sea bass (*Dicentrarchus labrax*) pronephros , poritoneal cavity (P.C.), spleen and blood, was measured by CL assay after stimulation with β -glucan .The CL response by (P.C.) and pronephros leukocytes was significantly

higher than that expressed by a similar number of cells separated from spleen and blood [51] .

1.8.3 Effects of Radiation on Blood

Studied the early effect of sublethal X-irradiation of phagocytic cells in mouse blood and the influence of cystamine as measured by CL. They found that 24 and 48 hours after irradiation (0.24-0.95 Gy) CL of whole blood was slightly decreased[52] .If however CL counts were related to the number of PMNL an enhanced CL per single granulocyte recorded .The administration of cystamine leads to an immune stimulating effect of unirradiated animals. investigated peripheral blood mononuclear cells activation in irradiated and unirradiated whole blood units of human in vitro[53]. They found that it is enhanced in basal conditions and after mitogen activation at 24 hours. found that strongly enhances gamma radiation induced apoptotic cell death of hematopoietic cell in host animals and that opoptosis occurs through associated signaling path way, and the response was not uniform in different host strains[54]. Also found that exposure of lymphoblasts to 20 Gy of X-ray irradiation resulted in 25-30% apoptosis of total cells in normal lymphoblasts [55].

1.9 The aim of the present study

- 1.** Investigate the effect of low doses of Beta radiation , Neutron radiation and Ultraviolet radiation on isolated leukocytes of Human blood in vitro by CL.
- 2.** Investigate the effect of both of Beta radiation , Neutron radiation, Ultraviolet radiation , and E.coli on the activity of the isolated leukocytes in vitro by CL.
- 3.** Investigate the effect of E.coli when added to leukocytes in vitro by CL.
- 4.** Investigate the effect of addition the irradiated isolated leukocytes by Beta radiation , Neutron radiation and Ultraviolet radiation to non ir radiated E.coli strains or addition irradiated E.coli strains to non ir radiated isolated leukocytes.

CHAPTER TWO

MATERIALS & METHODS

2:1 Materials

2:1.1 Instruments

Table (2-1): Instrument Used In This Study And Their Suppliers

No.	Instrument And Supplier
	Autoclave, Hysc, K&K. (Korea).
	Centrifuge, Hettich, Eba20. (Germany).
	Incubator, Bod, K&K. (Korea).
	Microtiter Pipette, Cyan. (Belgium).
	Ph (Potential Of Hydrogen) (Meter, Hanna Ph211. (U.S.A)).
	Refrigerator, Concord. (Lebnon).
	Sensitive Balance, Denver. (Germany).
	Vortex Mixer, Cyan Cl001. (Belgium).
	Light Microscope With Hd Camera, Lica. (Germany)
	Water Bath, K&K. (Korea).
	Hood, Loop, Disposable Petri Dishes, Cotton, Syringes, Beakers, Thermometer (Local Market), Racks, Gloves,

2:1.2 Chemicals And Reagents

The following solutions and chemical compounds were used in this experiment.

Table (2-2): chemical and reagents used in this study and their suppliers.

NO.	Chemical And Reagents Compounds
1.	Ethanol Absolute, Scharlan. (European Union).
2.	Monopotassium Phosphate (K_2HPO_4), Thomas Baker. (India).
3.	Potassium Chloride (KCl), Panreas. (Spain).
4.	Sodium Hydrogen Phosphate (Na_2HPO_4), Qualikems. (India).
5.	Sodium Chloride (NaCl), Avonchem. (U.K.).
6.	H_2O_2 , Thomas Baker. (India).

2:1.3. Culture Media

Table (2-3): Cultural Media Used In This Study And Their Suppliers.

No.	CULTURE MEDIA
	Nutrient Agar,Lab.(U.K.).
	Eosine Methylene Blue Agar (Levine). Oxoid. (England).
	Macconky Agar,Lab. (U.K.).
	Trypticase Soy Broth (Tsb). Oxoid. (England).
	Trypticase Soy Agar (Tsa). Oxoid. (England).

2:2. Methodology

2:2.1. Culture Media Preparation

All media listed in table (2-3) were prepared and sterilized according to the manufacturer's instructions.

2:2.1.1. Levine's Eosin Methylene Blue (EMB)

Eosin methylene blue was prepared by adding 37.5 gm. from powder medium to liter of distilled water then it was heated to boil with repeated stirring to liquefy the medium totally (final ph=7.2) and sterilized by autoclaving at 15 lbs/inch² (121°C) for 15 minutes next cooled to (60°C) and poured into petridishes inside a sterilized hood finally stored in a refrigerator at 4°C. (Emb Used To Distinguish E.Coli From Other Lactose Fermenters).

2:2.1.2. Nutrient Broth

Nutrient broth was prepared by liquefying 1.3 gm. Of the medium in 100 ml of distilled water then heated to boil with continuous stirring to dissolve the medium fully then poured into sterile screw cups and sterilized by autoclaving then stored in a refrigerator at 4°C.

2:2.1.3. Macconkey Agar

Macconkey agar was prepared by mixing 48.5 gm With 1 liter of deionized water allow to soak for 10 minutes then it was heated to boil and sterilized by autoclaving and was lifted to cool to 47°C and mixed well before pouring plates. Then it was stored in a refrigerator

at 4°C. Macconkey agar used for the isolation and differentiation of lactose and non-lactose fermenting enteric bacteria.

2:2.1.4. Tryptone Soy Broth

Tryptone soy broth was prepared by adding 30gm to 1 liter of deionized water, mix well and distributed into a sterile screw cups and sterilized by autoclaving then stored in a refrigerator at 4°C.

2:2.1.5. Tryptone Soy Agar

Tryptone Soy Agar Was Prepared By Liquefying 40gm. In 1 Liter Of Distilled Water And Was Brought To Boil And Dissolved Completely Then Sterilized By Autoclaving, Finally Cooled To (60°C) Poured Into Petridishes Inside The Sterilized Hood And Stored In A Refrigerator At 4°C.

2:2.2. Phosphate Buffer Saline (Pbs) 7.2 Ph

It was prepared according to quinn et al. (2004) by dissolving the following materials in 1000 ml distilled water:

NaCl	8.5 gm
NA ₂ HPO ₄ (ANHYDRASE)	1.15 gm
KH ₂ PO ₄	0.24 gm
KCl	0.2 gm

It was stored in a sterile bottle and adjusted at ph equal to (7.2), autoclaving at 15 lbs/inch² pressure with (121 °C) for 15 minutes finally cooled and stored in a refrigerator in (4 °C).

2:3. Chemiluminescence Reader

The chemiluminescence reader used, as shown in fig. (2-1), consists of two basic systems. These systems are light detection system and the recording system. Below is detailed explanation of each system.

2:3.1. Light Detection System

This system is used to discover the light output and to transform it to measurable electrical sign .it consists of two parts:-the first one is a glass cup (10 ml) that is put in it the solution that is used to get CL that consists of the CL solution (lucigenin in dmsO), and any other material is added to it to measure its luminescence curve which is represented, in this research, by basic blood components and bacteria . The second is photomultiplier tube (PMT) rca31034-20 that consists of cathode, fourteen secondary dynodes and one anode. This tube is kept inside a tight personal cover, which prevents the outside, light from permeating to it. The operating voltage (660v) is distributed between cathode and anode on the secondary dynodes. This voltage had been chosen by examining the response of PMT of thrown potential difference on anode. Because of chemical reaction between the materials of which chemiluminescence solution composes, light emerges and will, fall on cathode. This leads to release light photo electrons that fall on the first dynode which result to emerge secondary electrons. When they fall on the second dynode. This process continues with each dynode in PMT. It leads to increase the current that enters the PMT.

2:3.2 The Recording Systems

The recording system used is (scaler Time ST7), made by the British Nuclear Enterprise. It is designed to measure a sign from scintillation counter or Geiger Muller counter .The system includes a high voltage supplier (0-2KV), high pulsation analyser, and a timer controlled by operations processe. The location of inserting the single sign is used to insert the sign from scintillation counter or Geiger Muller counter.The selection of the type of the counter is done through the revolving keys on the front of the machine .The time of reading is already set at the beginning of the measurement .The machine is operated through two keys on the front, one for the single circuit and the other for the double circuit. The maximum reading of the machine is 5×10^5 cont./s

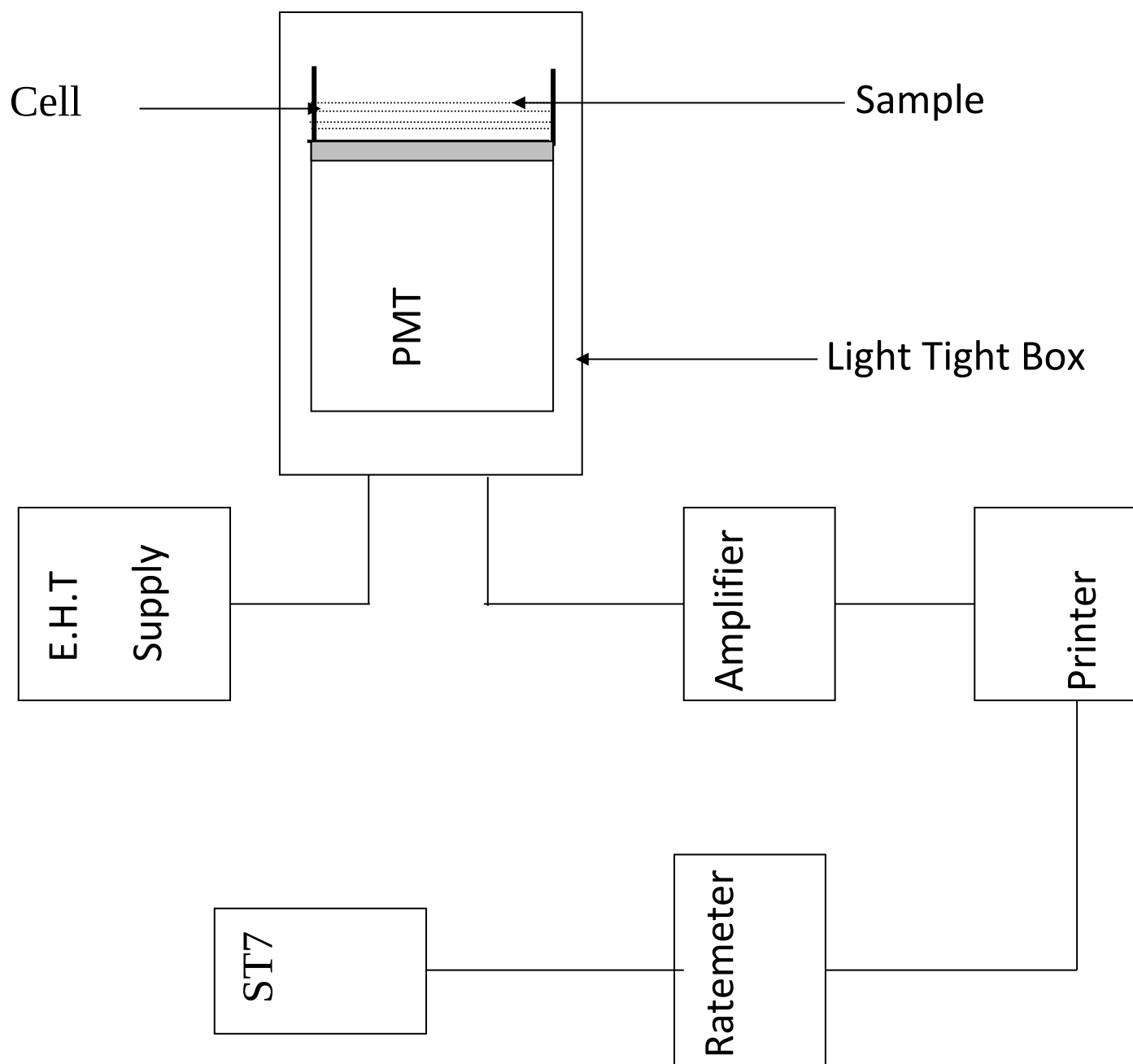


Fig. (2-1) Schematic Diagram of the Chemiluminescence reader.

2:3.3 Method

The CL solution was prepared by dissolving 0.07 gm of lucigenin in dimethyl sulphoxide to obtain 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 PMT to begin reading the measurements. To measure the cl of each wbc we added the certain amount of studied component to above cl solution in the PMT and operate the system to get the cl reading on the digital reader.

2.4 White blood cells (WBC)

Form Hussein Teaching hospital in Al- muthanna (MAIN BLOOD BANK).

2.5 Source of neutron

From University of Baghdad college of education for pure science (Ibn Al-Haitham) in nuclear physics lab source name is Americium /Beryllium (Am/Be),

Half life 432 years

Density Flow 3×10^5 n/cm².sec

Energy (14 MEV) .

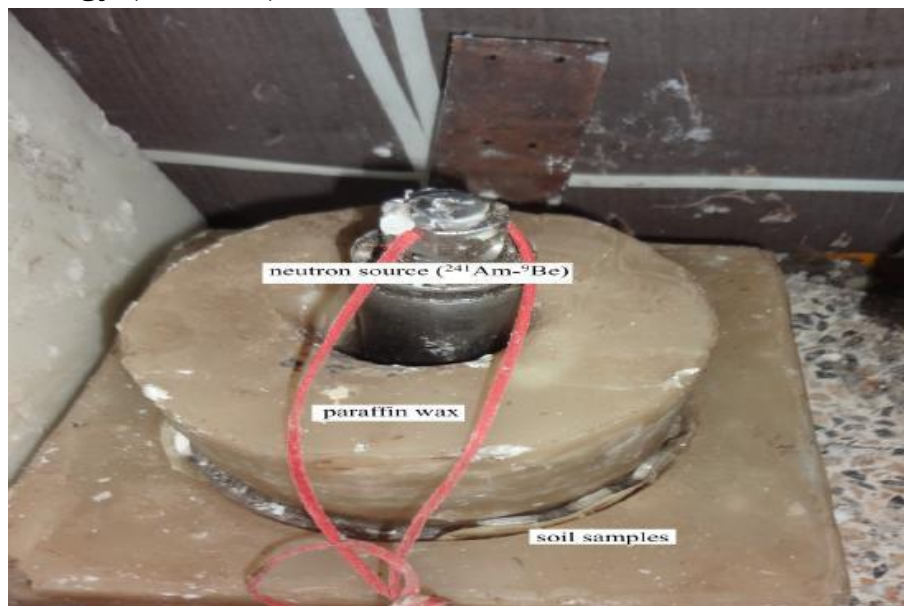


Fig (2-2) Source of neutron.

2.6 Source of beta radiation (β)

From Al muthanna University College of Medicine in laboratory research source name is Strontium-90 (Sr-90)

Half-life: 29.1 years

The activity radiation source 5 μ ci .

2.7 Lucigenin

Lucigenin (5-Nitrobenzo [b]Furan-2-carboxylic acicetnyiester) Net weight: 500mg from Medchemexpress LLC "Research sample Use only" , FW= 510.51 ,chemical formula :C₂₈H₂₂N₂ .

CHAPTER THREE

RESULTS & DISCUSSION

3.1 Identification of STEC identity

3.1.1 Cultural characteristics of O157 STEC

Bacteriological culture revealed different morphological characteristics of O157 STEC on different media, after incubation at 37°C for 24 hours. On MacConkey agar colonies appeared as red/pink color while on Levin's Eosin Methylene Blue (EMB) agar the visible colonies appeared as green metallic sheen. As shown in Figures (3-1),(3-2).

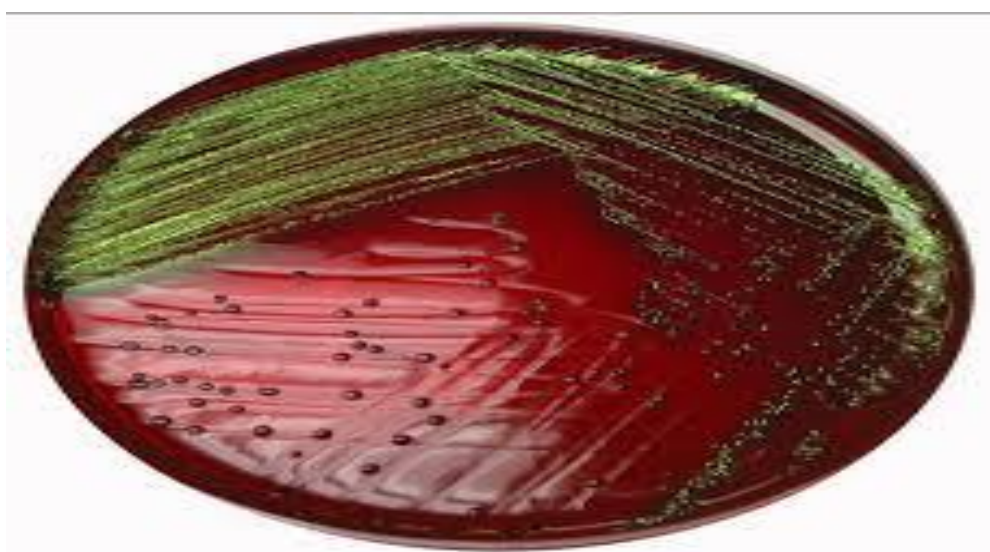


Figure (3-1) E.coli on EMB agar.



Figure (3-2) E.coli on MacConkey agar.

3.1.2 Culturing on CHROMagar STEC

The culturing of sample after enrichment in Tryptic soy broth for 6 hours on CHROMagar STEC showed different colors and the mauve color colonies was an indication for STEC.as shown in Figure (4-3)



Figure (3-3) STEC bacteria.

3.2 Optimum conditions for measurements

The best conditions for suitable reading to get the most proper reading for chemiluminescence. This reading is deal as background .i.e to subtract this values from the total reading for experiment i.e after irradiation .

Table (3-1)

No.	Parameter	values
1.	Operating voltage	660 volt
2.	volume of hydrogen peroxide	2ml
3.	Concentration of lucigenin	7×10^{-4} M
4.	Solution Temperature	37°C
5.	Sample volume	1ml

3.2.1 Determination of hydrogen peroxide volume

Hydrogen peroxide is essential for CL process, because it is the main source of free radical .In our work ,stock solution of 10 ml of H_2O_2 is considered, then use different volumes were taken . to study the relation between CL and $[H_2O_2]$,figure (3-1) explain the most stable reading of CL at volume percent 2ml .

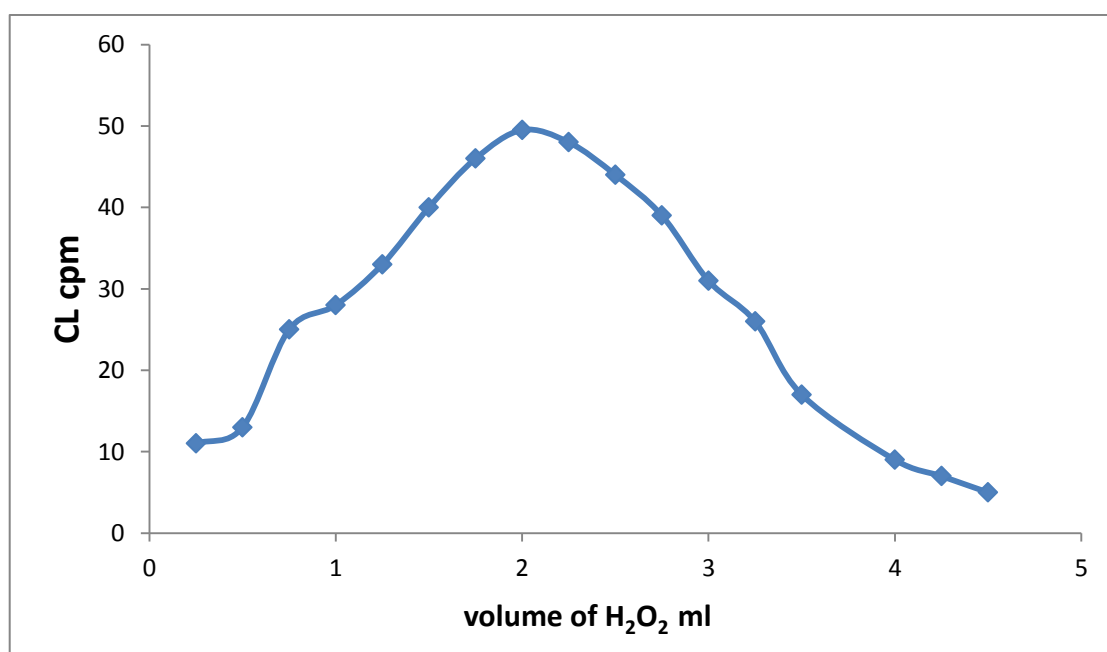


Fig: (3-1) chemiluminescence curves of volume of H_2O_2 .

3.2.2 Determination of lucigenin concentration

Different concentration of lucigenin solution were prepared .Stock solution by dissolving 0.072 gm of lucigenin in 200ml of distilled water, we get 7×10^{-4} M of stock solution. Then this solution diluted to different concentrations , to choose suitable CL concentration .

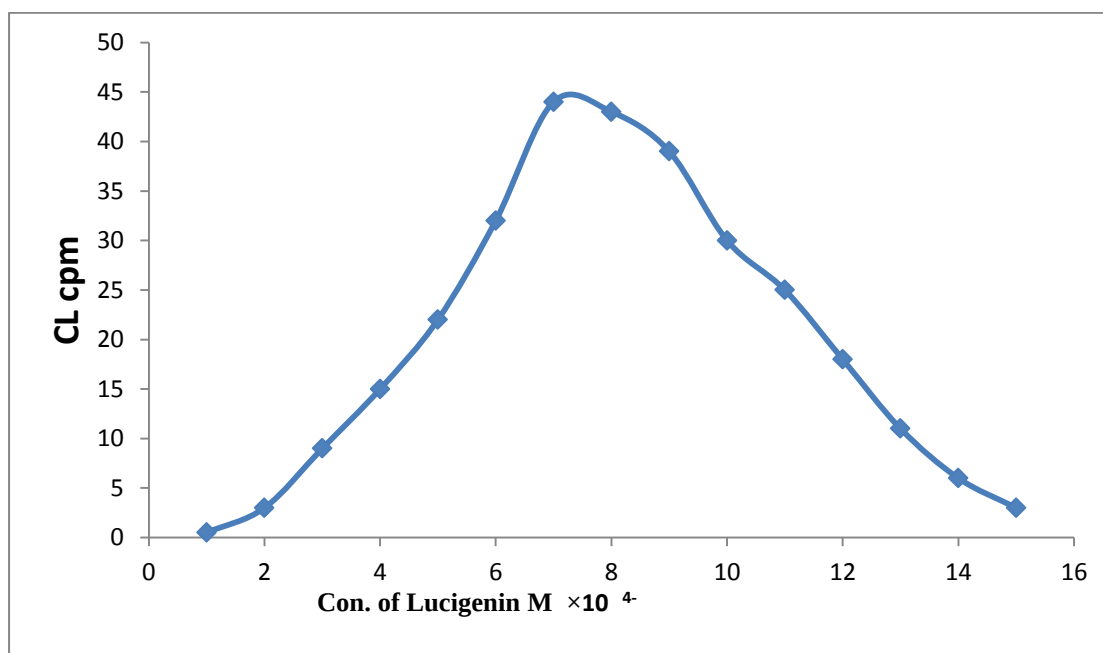


Fig: (3-2) chemiluminescence curves for different lucigenin concentration .

3.2.3 Lab . condition during expirement :

a- Solution Temperature

Solvent temperature effect on the speed of disintegration of free radicals and oxygen concentration . To study the relation between solvent temperature and CL temperature between(30- 50) °C were considered with keeping the volume of the solvent constant because of evaporation process.

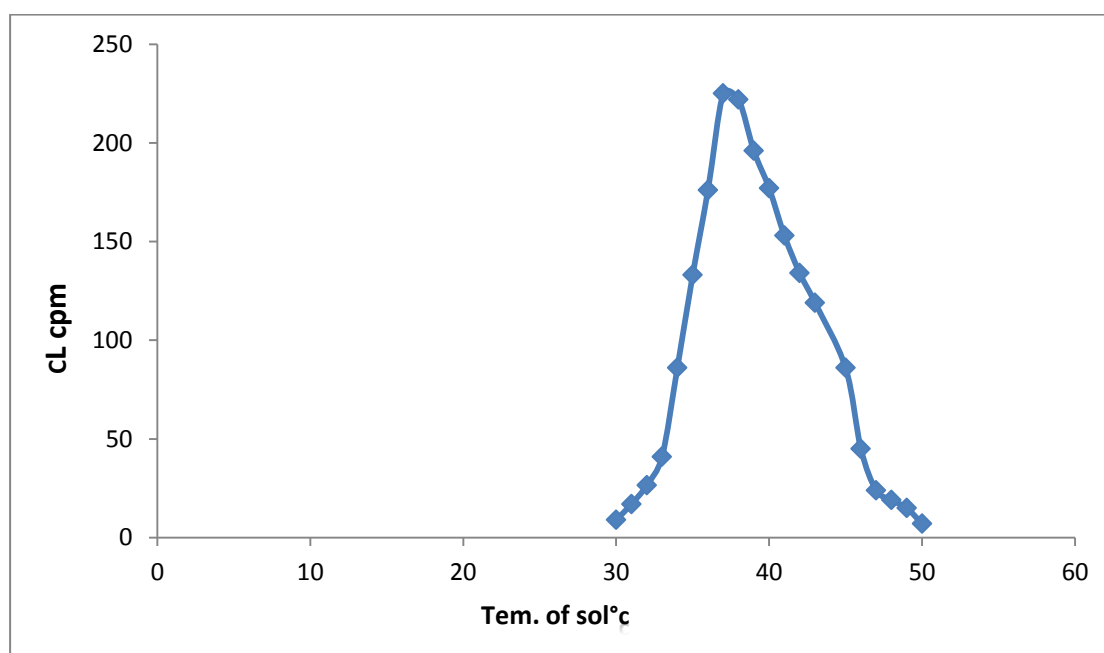


Fig: (3-3) chemiluminescence curves of solution Temperature

b- Lab . Temperature

Temperature of ambient effect on response of CL reader . The dark current at 20°C is 15 electron per 60 sec , but the difference between solvent temp . (37°C) and lab .temp (20°C) will cause quick change in solvent temperature , So 25°C as a lab. Temperature was fixed.

c- Sample Volume

Different volumes of *E.coli* of two strains were added to CL solution .The result were shown in figure(3-4) .As the sample volume increase CL will increase up to 1ml ,then it will decrease at solution point because of the effect of H_2O_2 on bacteria ,then the volume of H_2O_2 will not enough to make reaction with bacteria of CL then will be quenched at different volumes of CL , then the most suitable concentration were concentration of lucigenin 2ml.

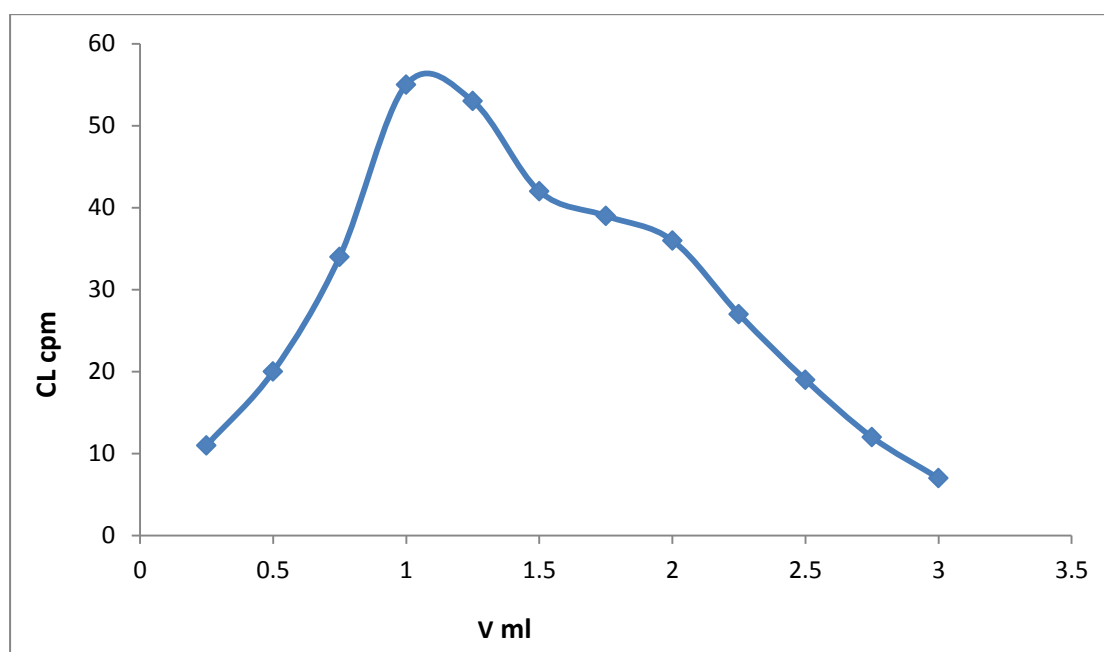


Fig: (3-4) chemiluminescence curves of sample volume .

3.3 chemiluminescence curves of E.coli strains.

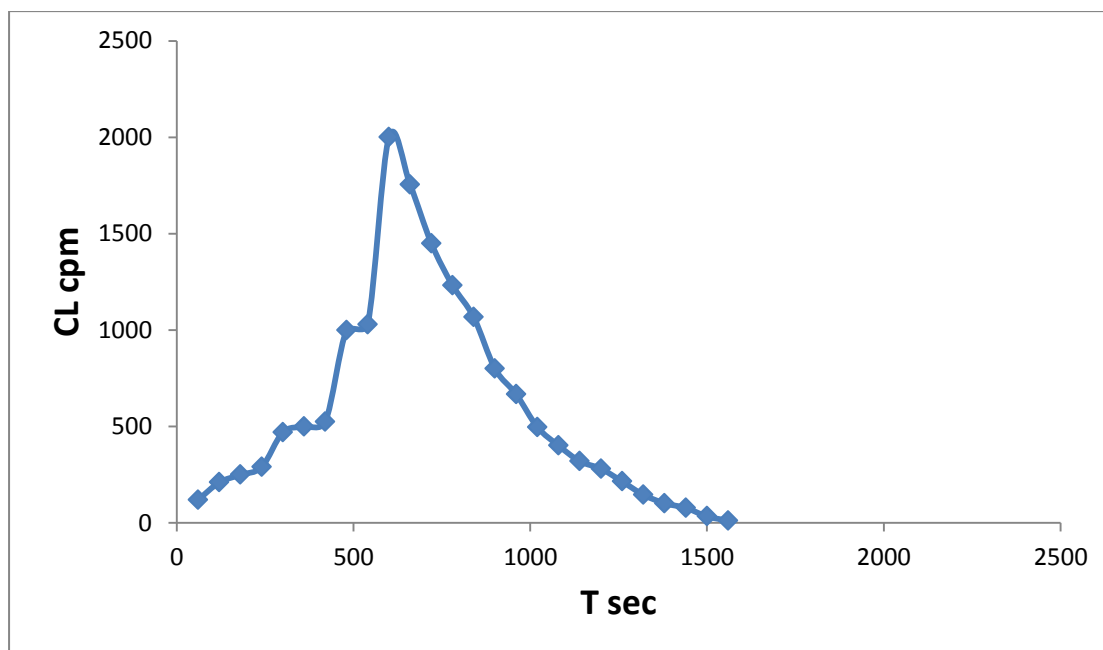


Fig: (3-5) chemiluminescence curve of E.coli strain O157:H7.

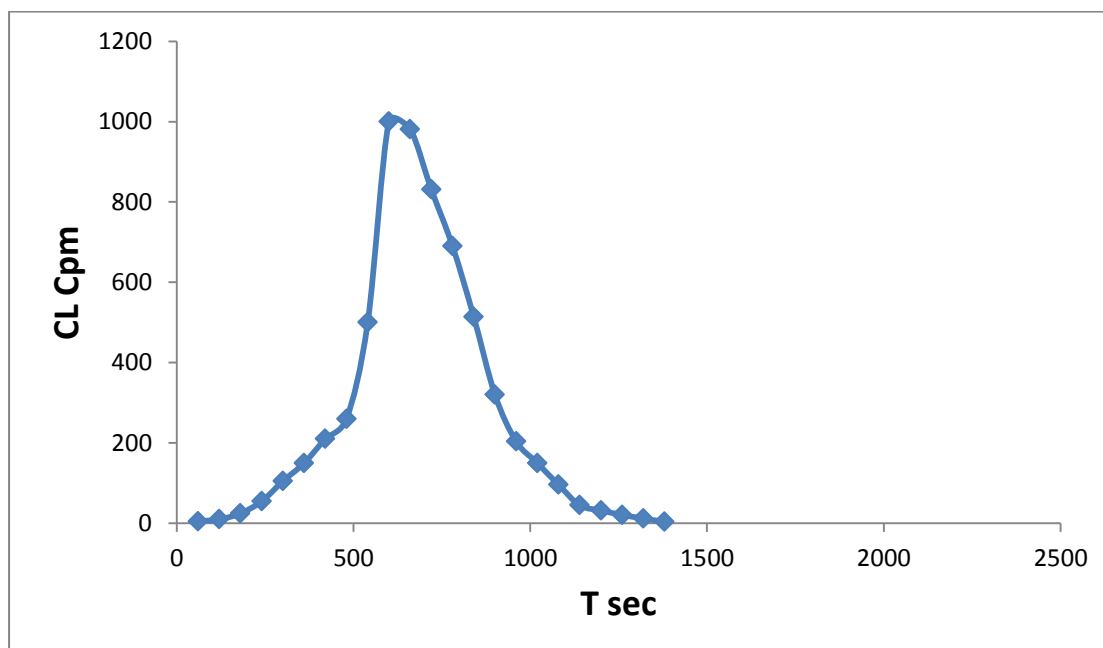


Fig: (3-6) chemiluminescence curve of E.coli strain O26.

3.4 Chemiluminescence curve of leukocytes

A CL curve of leukocytes was obtained by adding 0.25 ml of isolated leukocytes to 5 ml of CL solution. Fig (3-7) indicates the CL of the solution with addition of leukocytes, it is clear that the CL of the solution with leukocytes is greatly higher than CL of the solution only, this is due to that CL depends on the respiratory burst and phagocytosis of leukocytes [56]; especially that all types of leukocytes (neutrophils, eosinophils , basophil, phagocytes , and lymphocytes) have the ability of respiratory burst and produce several types of reactive oxygen radicals (O, OH, H₂O₂, O₂) [57]. There is a relation between those ROS and CL when those ROS act on increasing CL through their reaction with CL material [58].

3.5 Effect of Neutron radiation ,Beta radiation and Ultraviolet radiation on leukocytes .

To study the effect of Neutron radiation ,Beta radiation and Ultraviolet radiation on leukocytes, in different doses . CL curves were resulted from the addition of 0.25 ml of irradiated leukocytic solution for the radiation doses . It is clear from the curves that there is inverse proportion between the peak of CL of leukocytic and radiation doses used for them, whenever the radiation dose was increased the CL was decreased, it means that the height CL curve peak is for the lowest radiation and the lowest CL curve peak is for the highest dose . This result agreed with what found by [60]. It means that the radiation affect leukocytes in two directions the first one is the direct effect on the activity of the cells and the second indirect effect is through the formation of OH and H₂O₂ by the hydrolysis of the medium which can affect the activity of leukocytes. We expect that the formation of these radicals may

be increased when the radiation dose increased leading to an increased toxic effect on the leukocytes and as a result the formation of the ROS will be decreased causing decreased CL; these result agreed with those found by [61] when they suggest the activity of neutrophilic myeloperoxidase decreases with the increasing radiation dosage. It is concluded from these results irradiation by low doses can activate the functions of leukocytes and this is clear from increasing their CL and treatment with high doses of the same rays will inhibit the function of leukocytes by decreasing their CL.

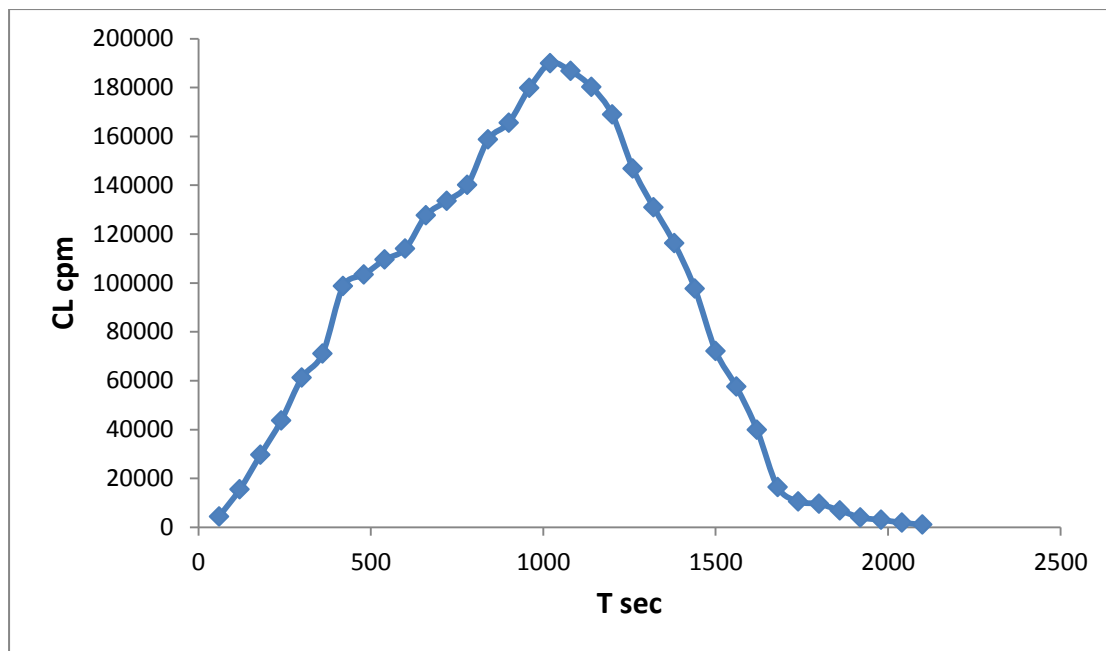


Fig: (3-7) chemiluminescence curve of leukocytes cells only .

3.6.1. Effect of Beta radiation on E.coli strains:-

3.6.1.1 Effect of Beta radiation on E.coli strain O157:H7

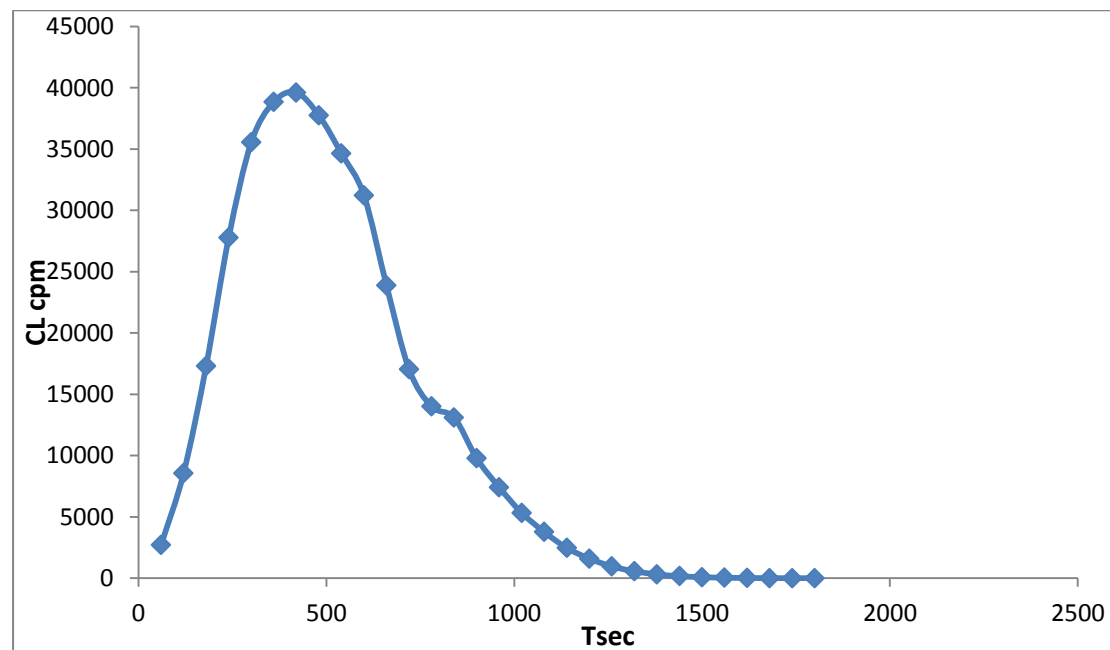


Fig: (3-8) Effect of Beta radiation on E.coli strain O157:H7 after 30 min.

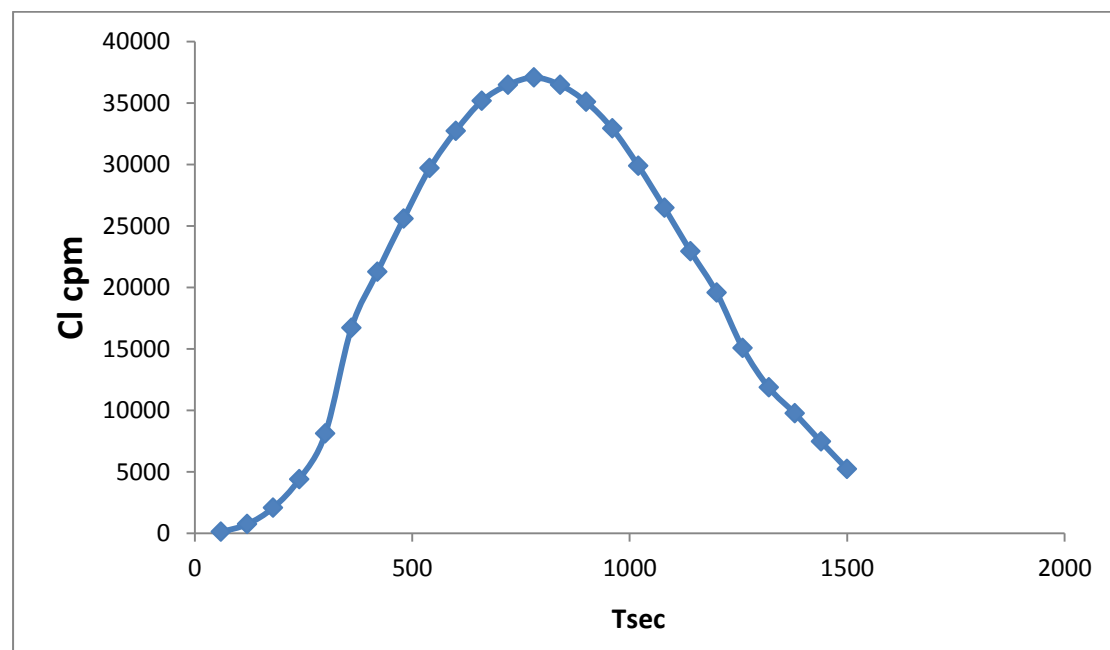


Fig: (3-9) Effect of Beta radiation on E.coli strain O157:H7 after 60 min.

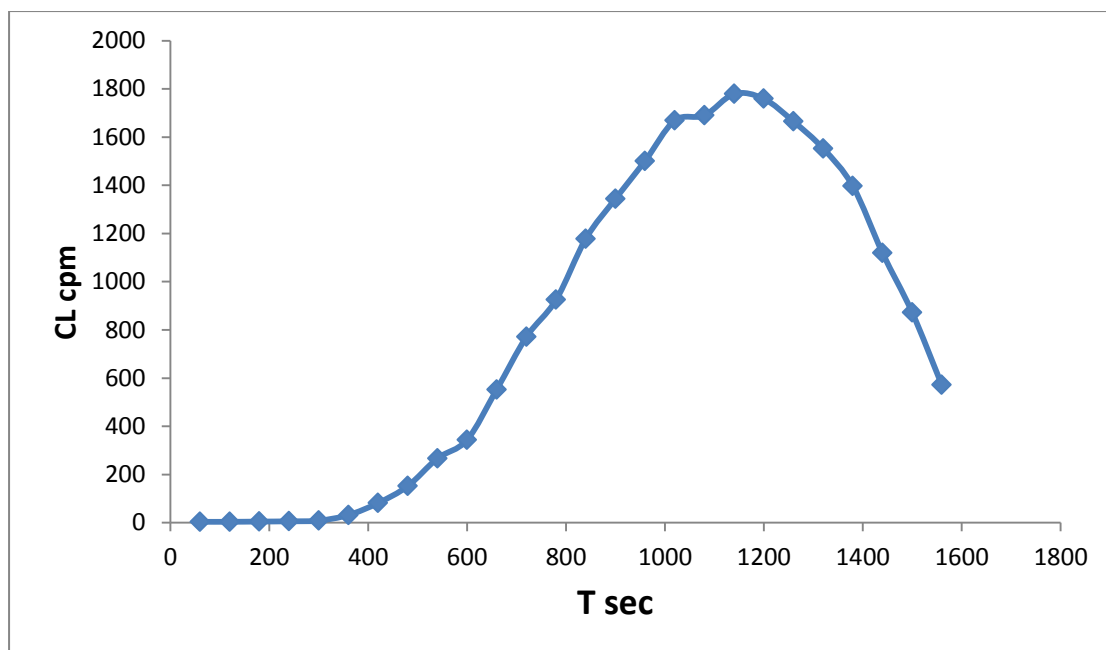


Fig:(3-10) Effect of Beta radiation on E.coli strain O157:H7 after 90 min.

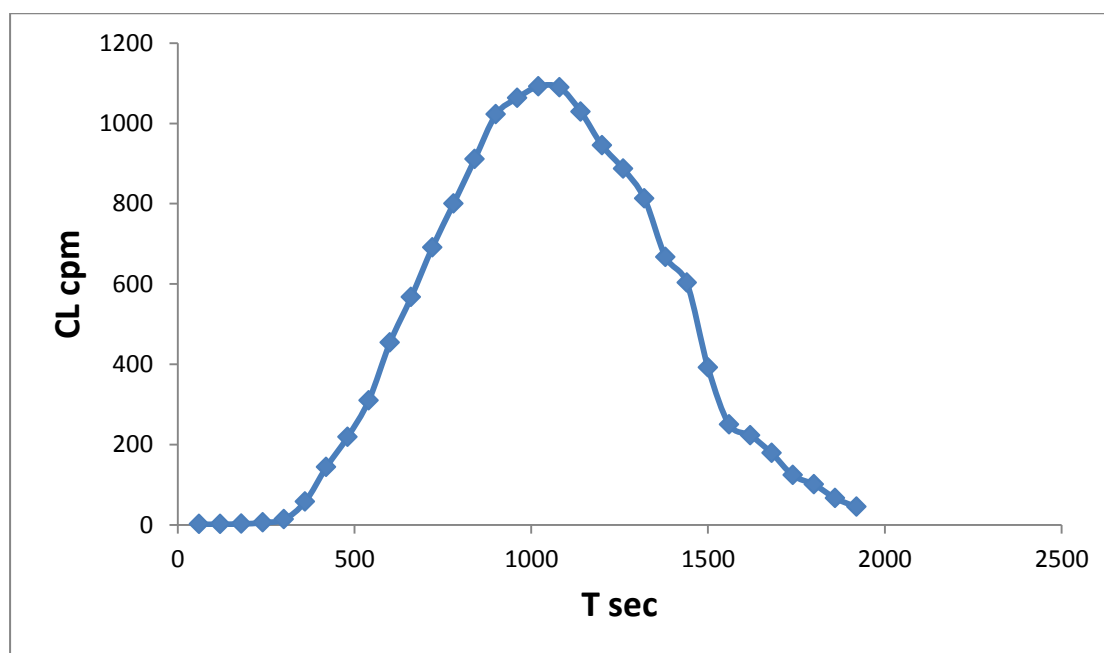


Fig:(3-11) Effect of Beta radiation on E.coli strain O157:H7 after 120 min.

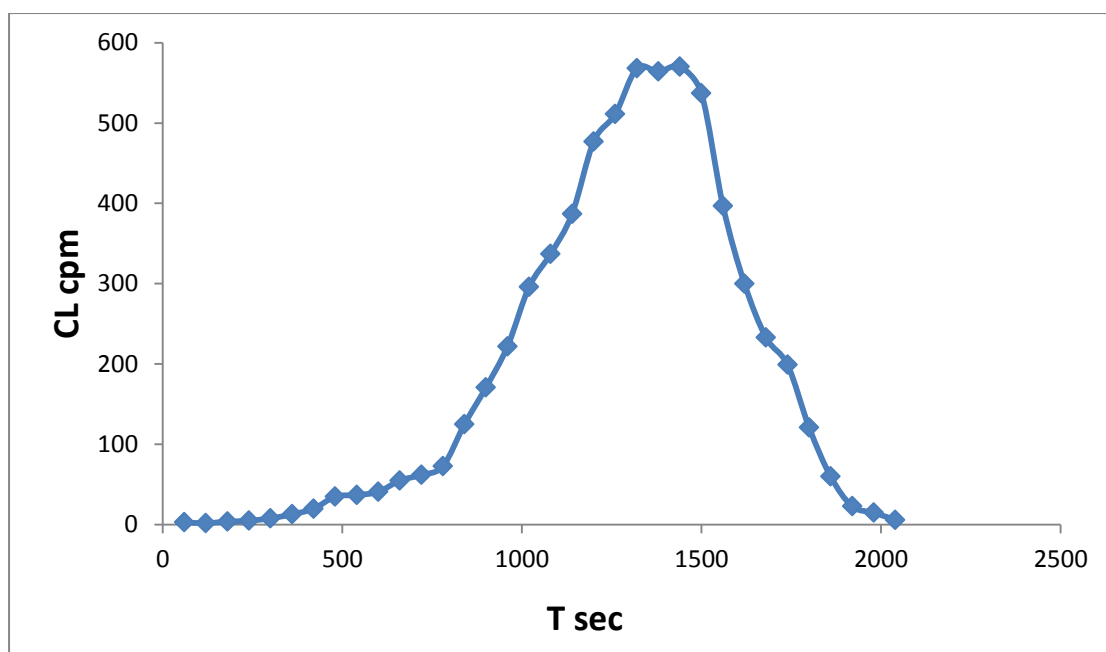


Fig:(3-12) Effect of Beta radiation on E.coli strain O157:H7 after 150 min.

3.6.1.2 Effect of Beta radiation on E.coli strain O26 .

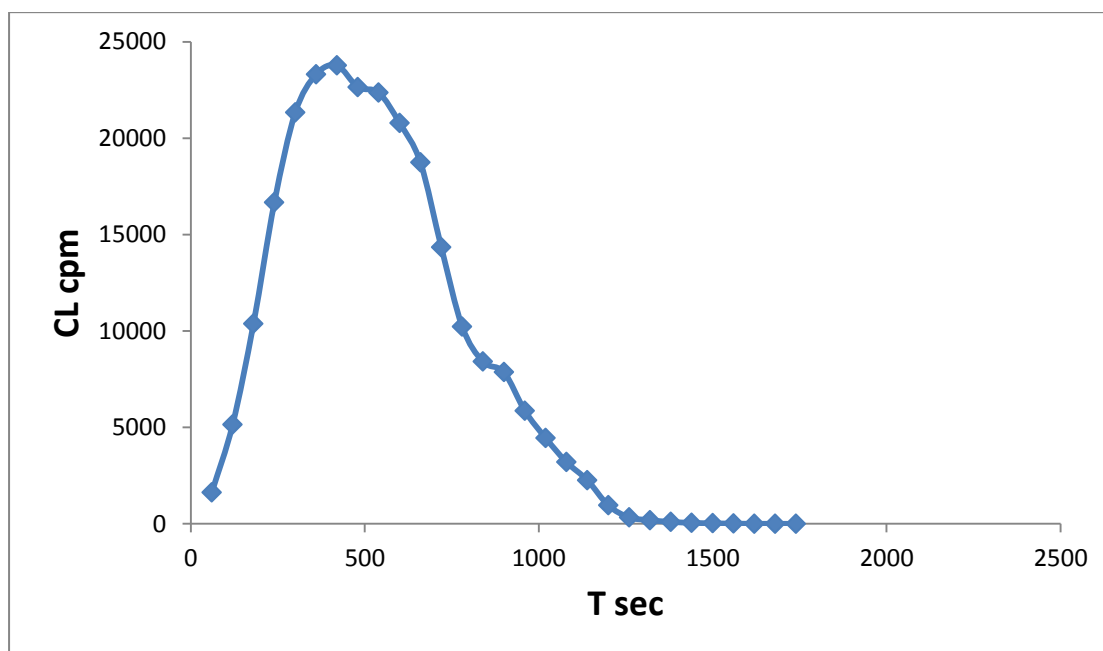


Fig: (3-13) Effect of Beta radiation on E.coli strain O26 after 30 min.

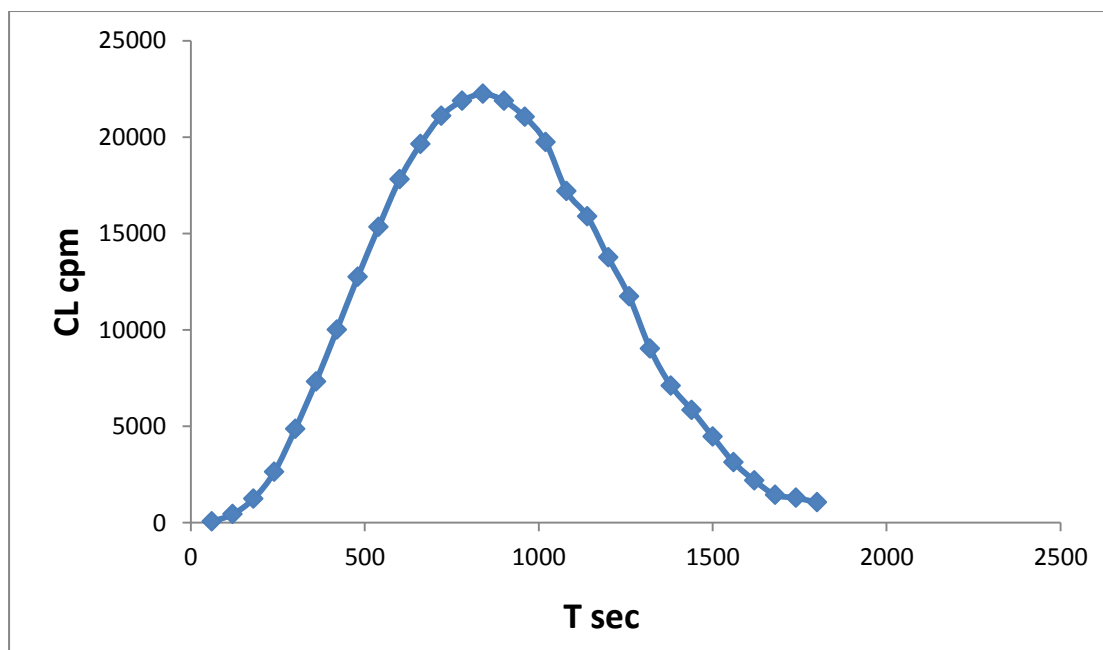


Fig: (3-14) Effect of Beta radiation on E.coli strain O26 after 60 min.

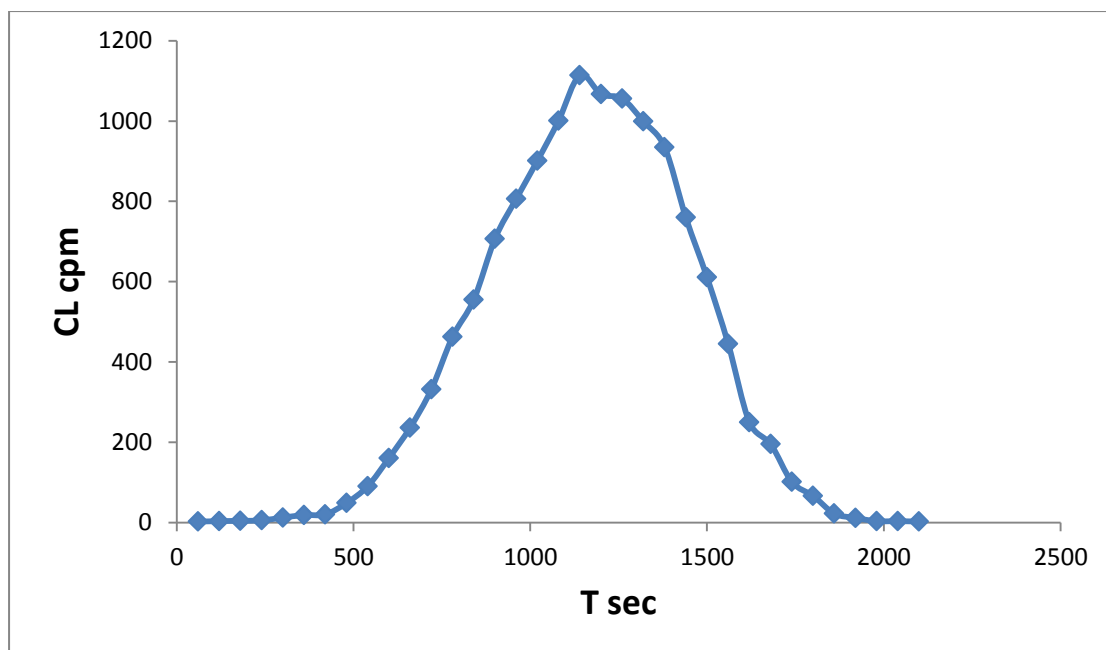


Fig: (3-15) Effect of Beta radiation on E.coli strain O26 after 90 min.

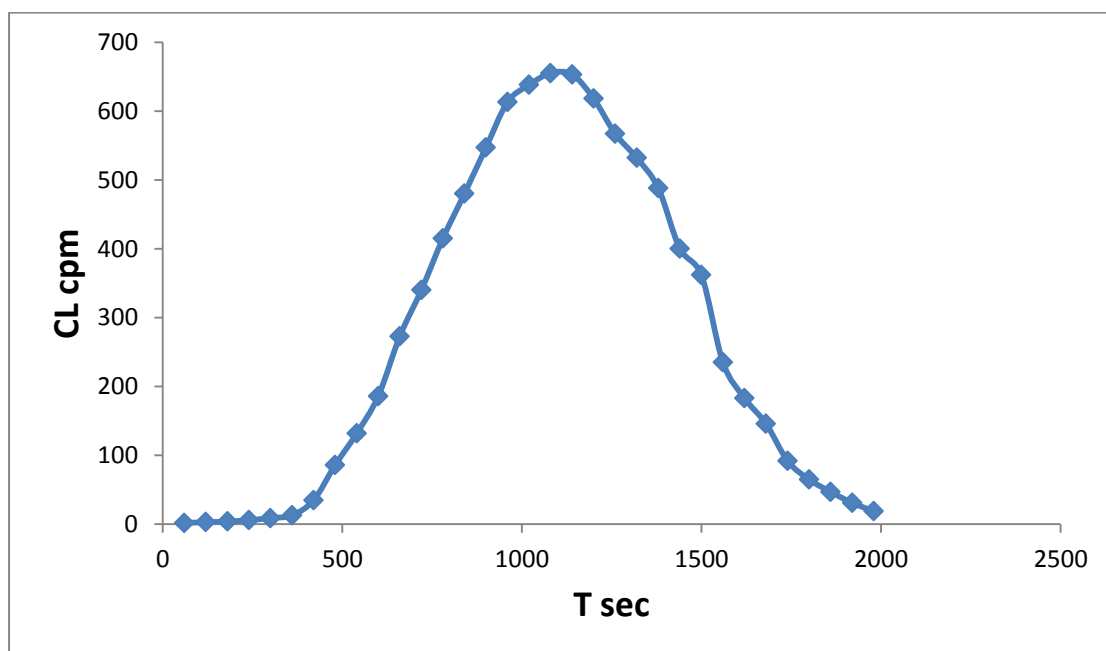


Fig: (3-16) Effect of Beta radiation on E.coli strain O26 after 120 min

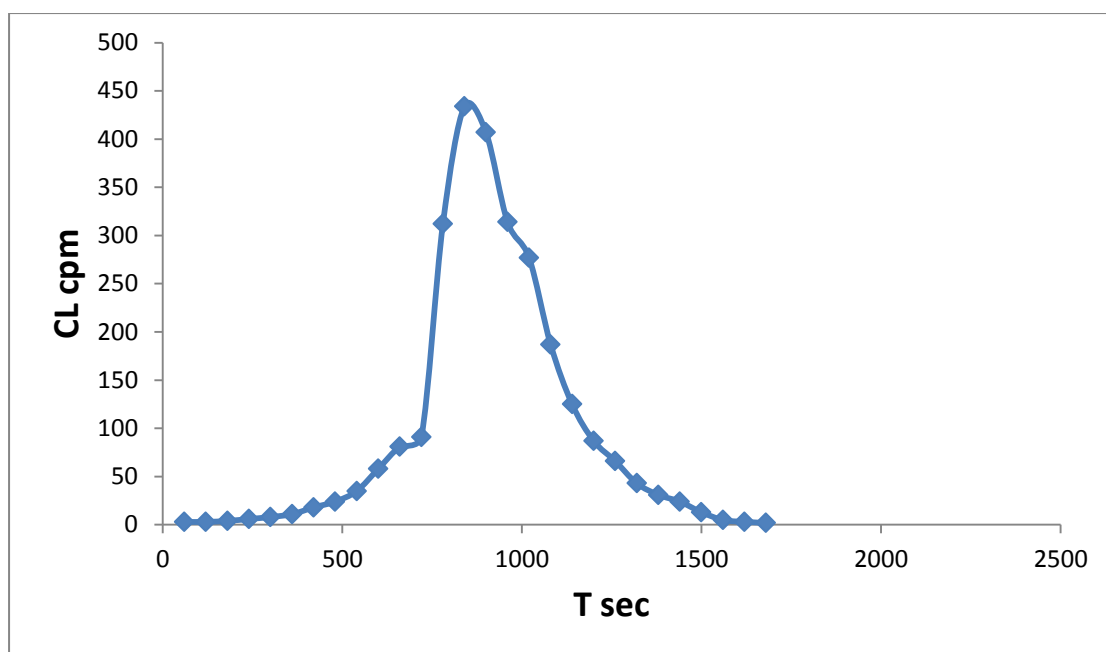


Fig: (3-17) Effect of Beta radiation on E.coli strain O26 after 150 min.

3.6.2 Effect of ultraviolet radiation on E.coli strains.

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

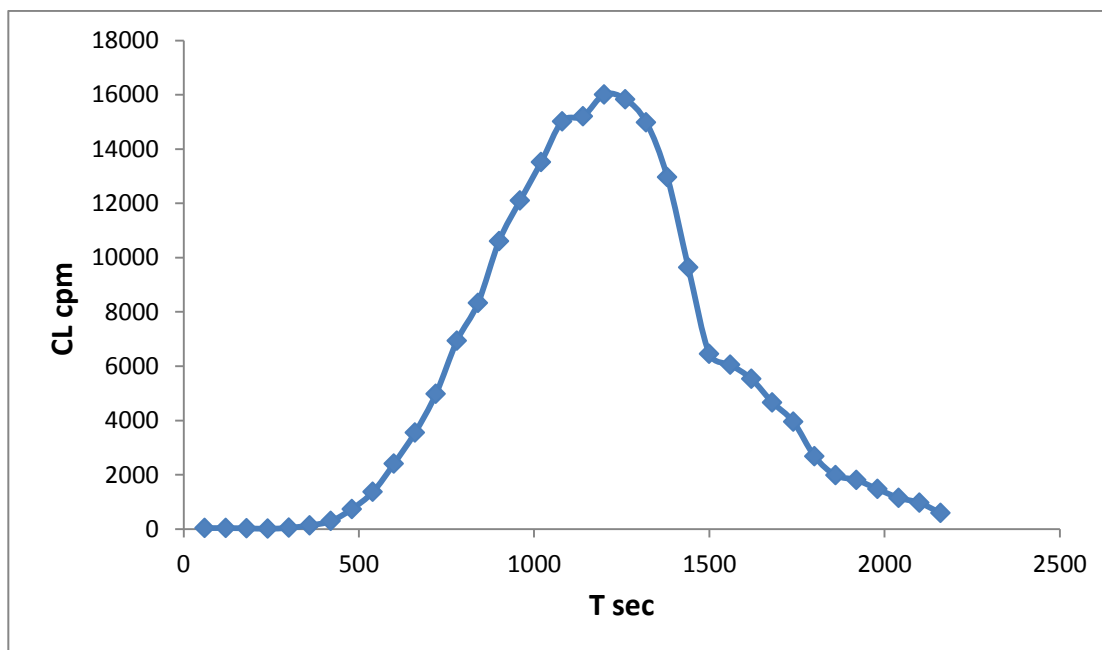


Fig:(3-18) Effect of ultraviolet radiation on E.coli bacteria strain O157:H7 after an hour .

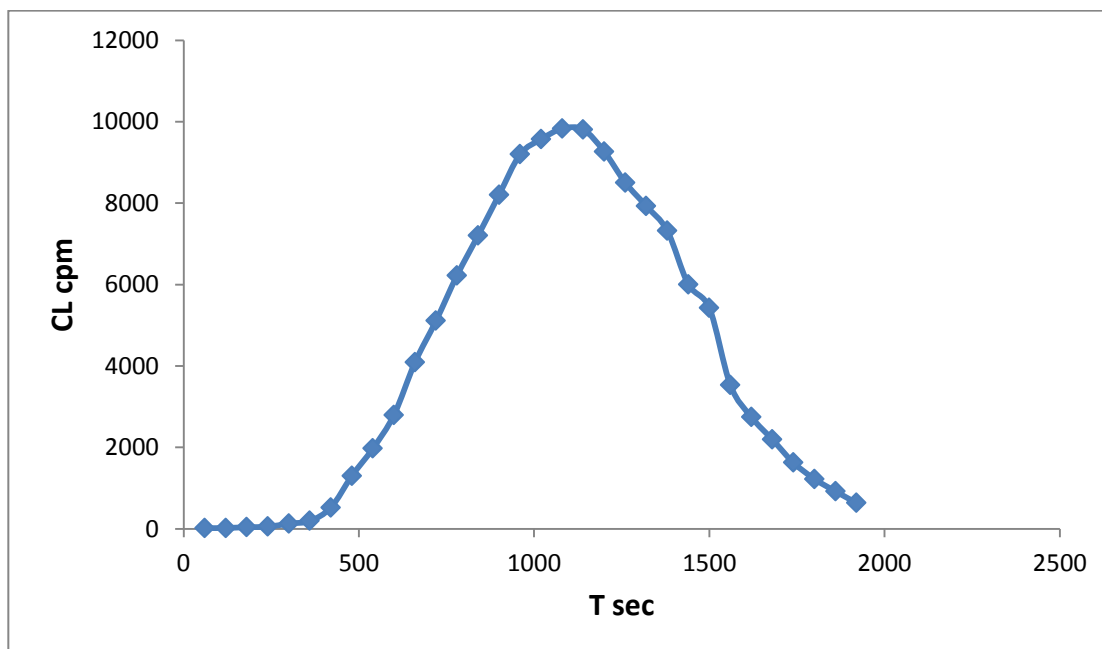


Fig:(3-19) Effect of ultraviolet radiation on E.coli bacteria strain O157:H7 after two hours .

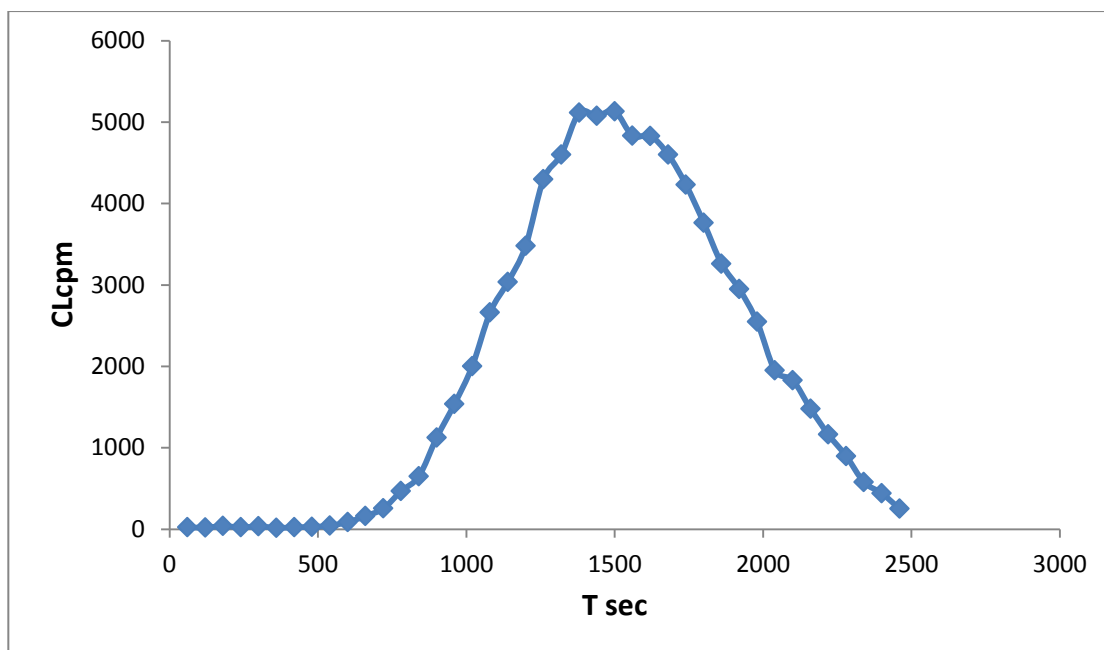


Fig:(3-20) Effect of ultraviolet radiation on E.coli bacteria strain O157:H7 after four hours .

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

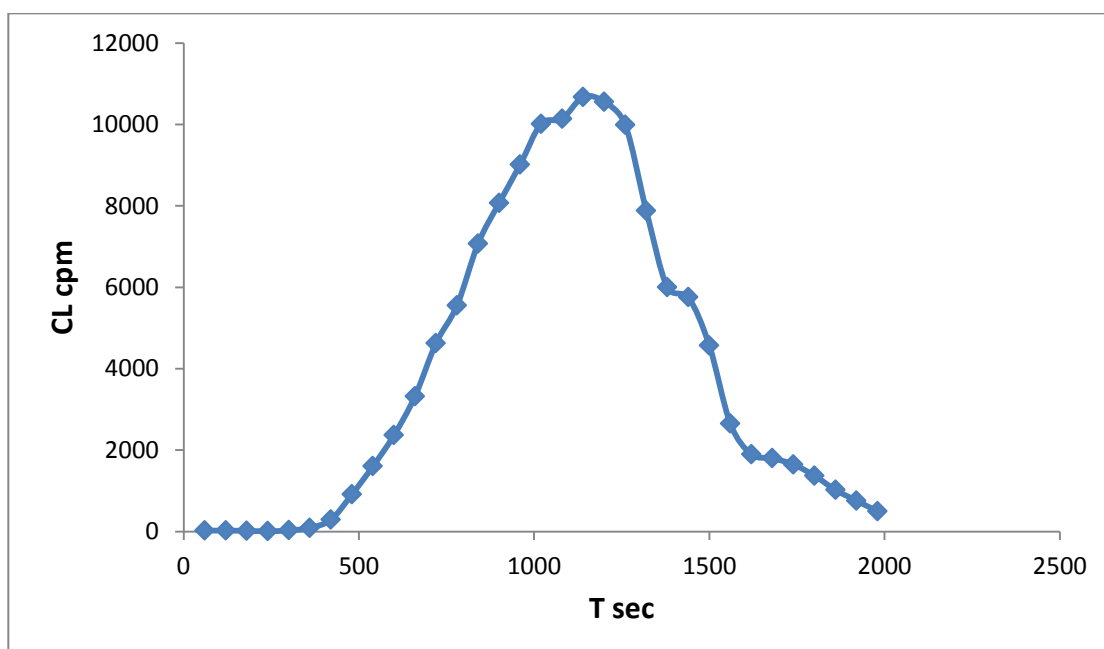


Fig:(3-21) Effect of ultraviolet radiation on E.coli bacteria strain O26 after an hour .

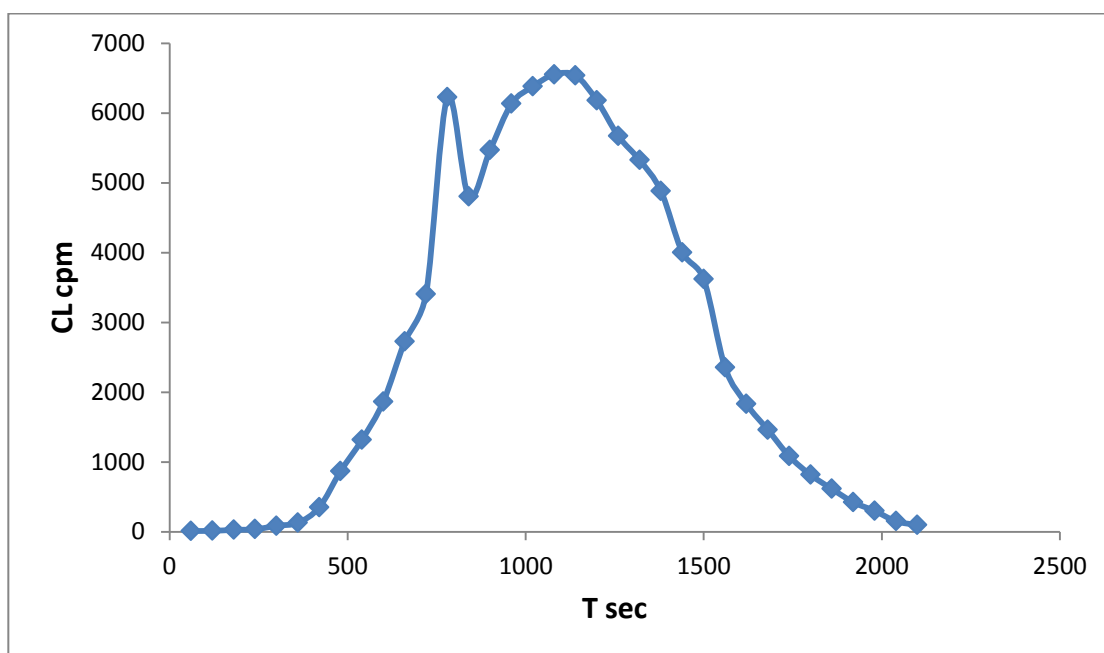


Fig: (3-22) Effect of ultraviolet radiation on E.coli bacteria strain O26 after two hours .

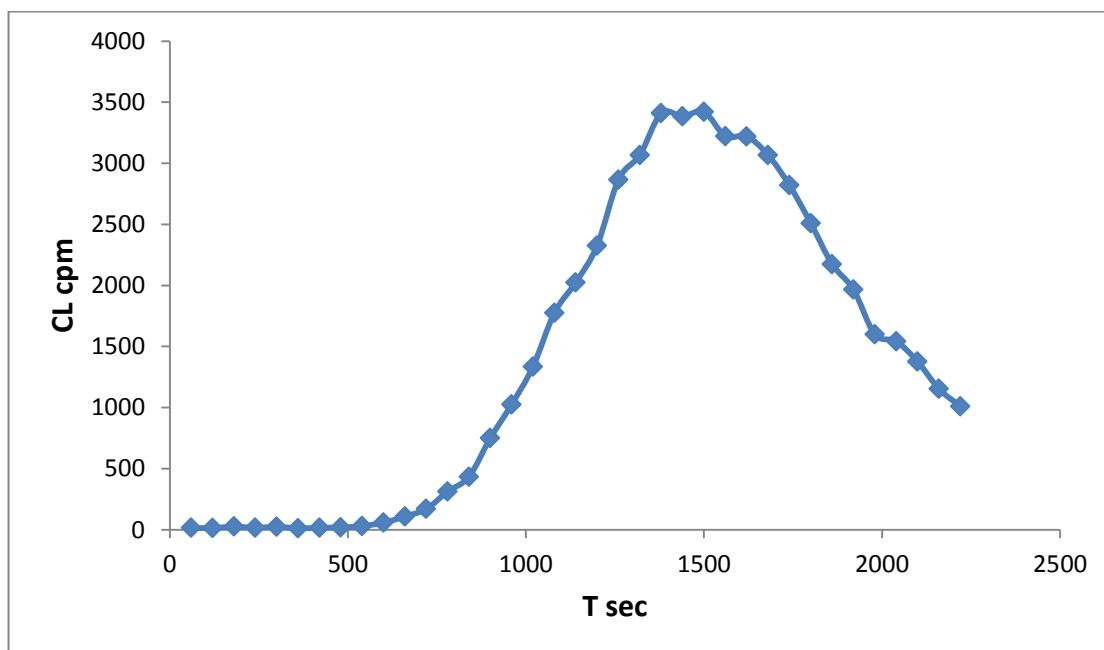


Fig:(3-23) Effect of ultraviolet radiation on E.coli bacteria strain O26 after four hours .

3.6.3 Effect of neutron radiation on *E. coli* strains .

3.6.3.1 Effect of neutron radiation on *E. coli* strain O26 .

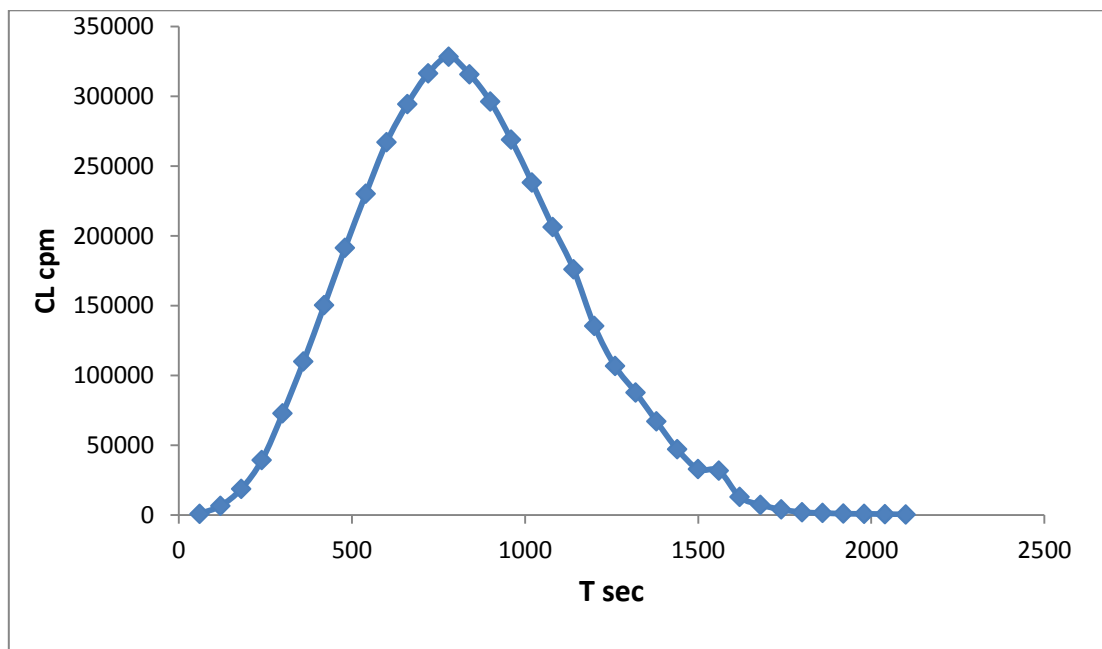


Fig: (3-24) Effect of neutron radiation on *E. coli* strain O26 after one second .

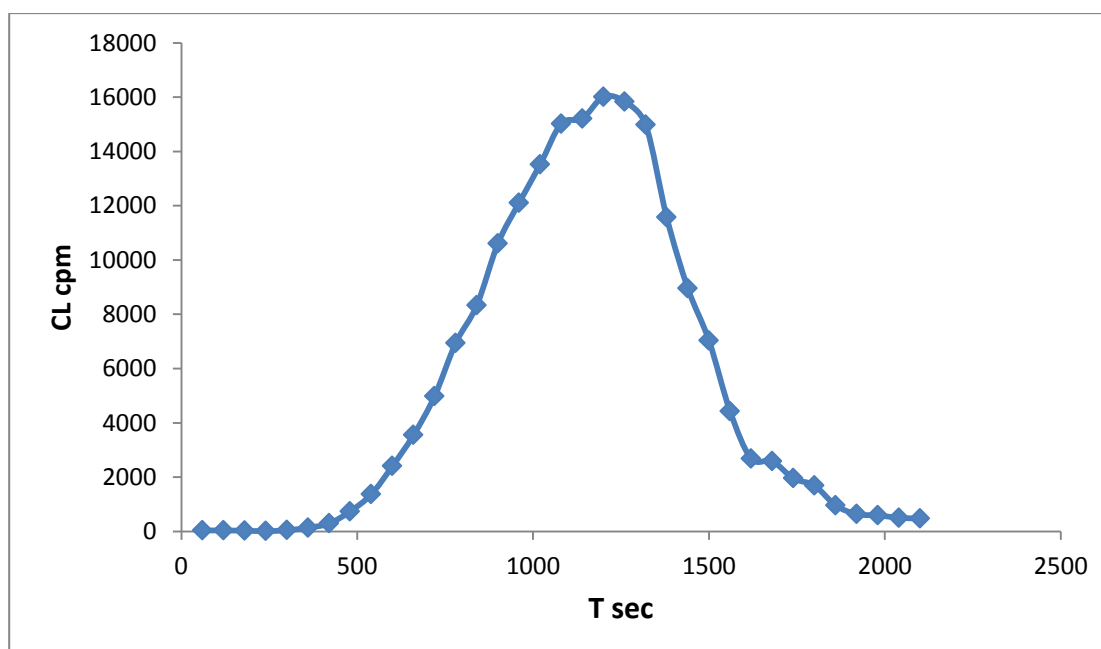


Fig: (3-25) Effect of neutron radiation on *E. coli* strain O26 after Three seconds .

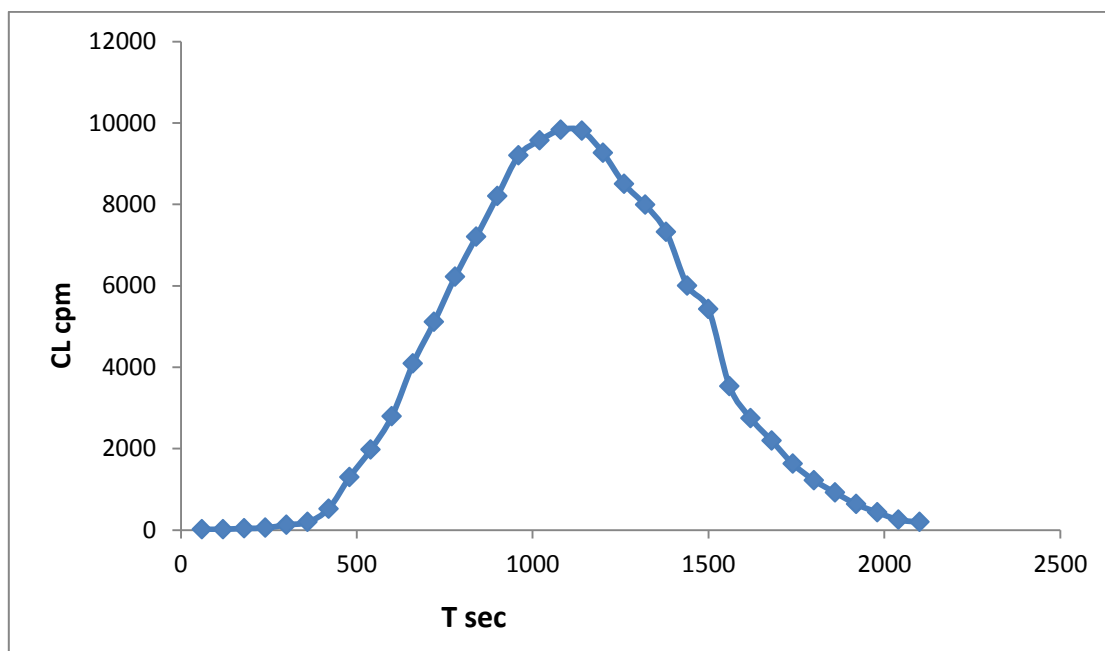


Fig: (3-26) Effect of neutron radiation on E. coli strain O26 after five seconds .

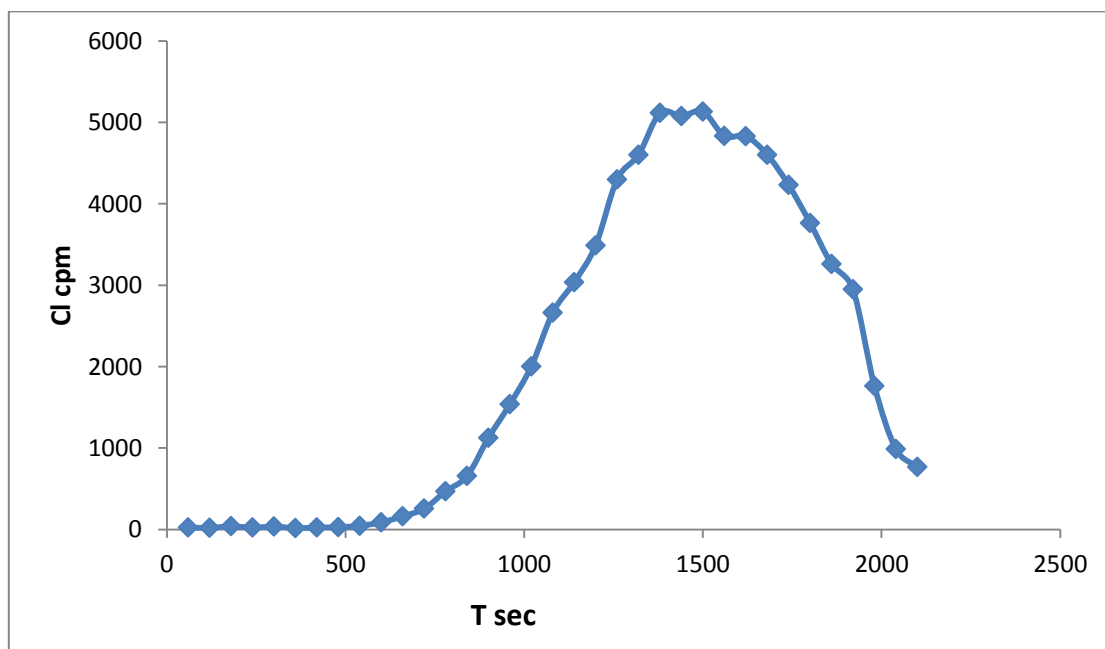


Fig:(3-27) Effect of neutron radiation on E. coli strain O26 after six seconds .

3.6.3.2 Effect of neutron radiation on E. coli strain O157:H7 .

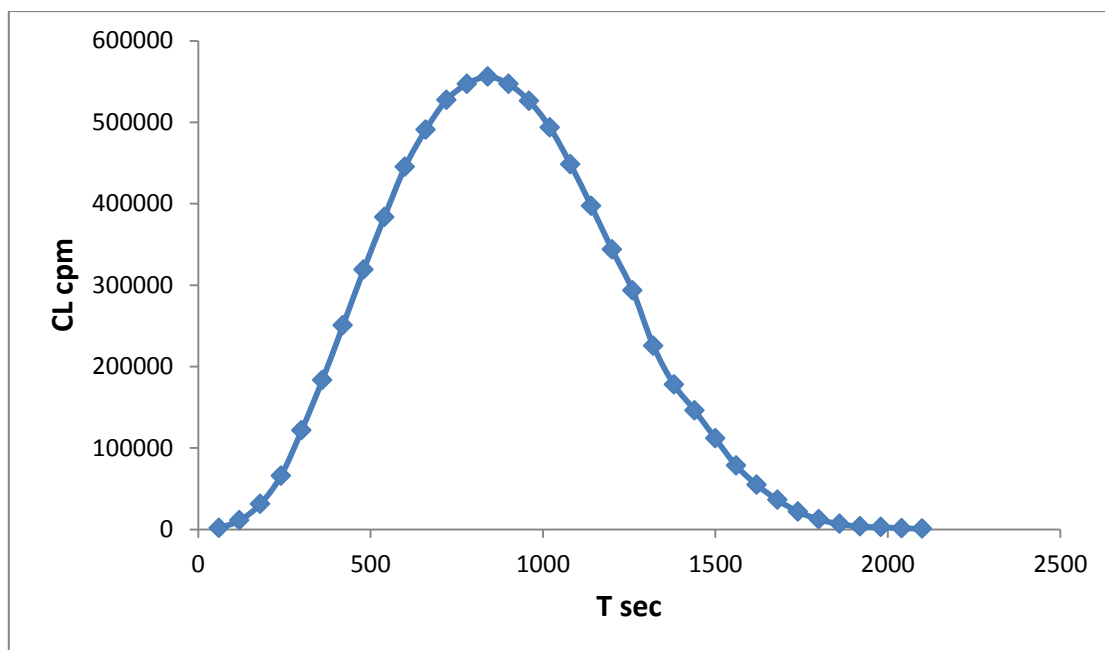


Fig:(3-28) Effect of neutron radiation on E. coli strain O157:H7 after one second .

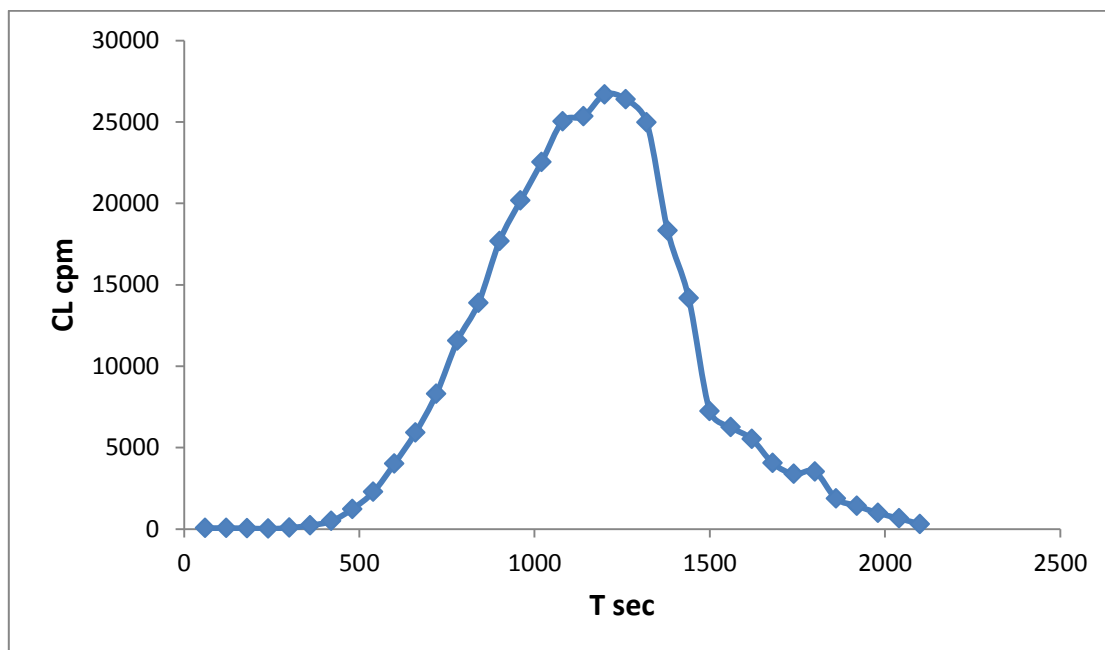


Fig: (3-29) Effect of neutron radiation on E. coli strain O157:H7 after Three seconds .

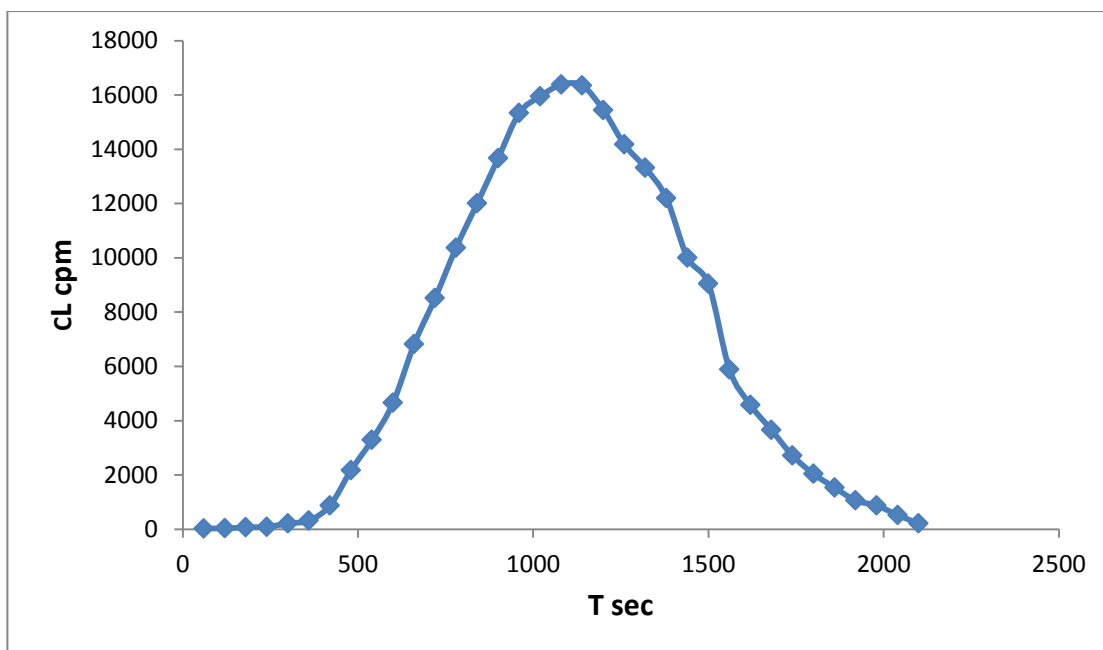


Fig:(3-30) Effect of neutron radiation on E. coli strain O157:H7 after Five seconds .

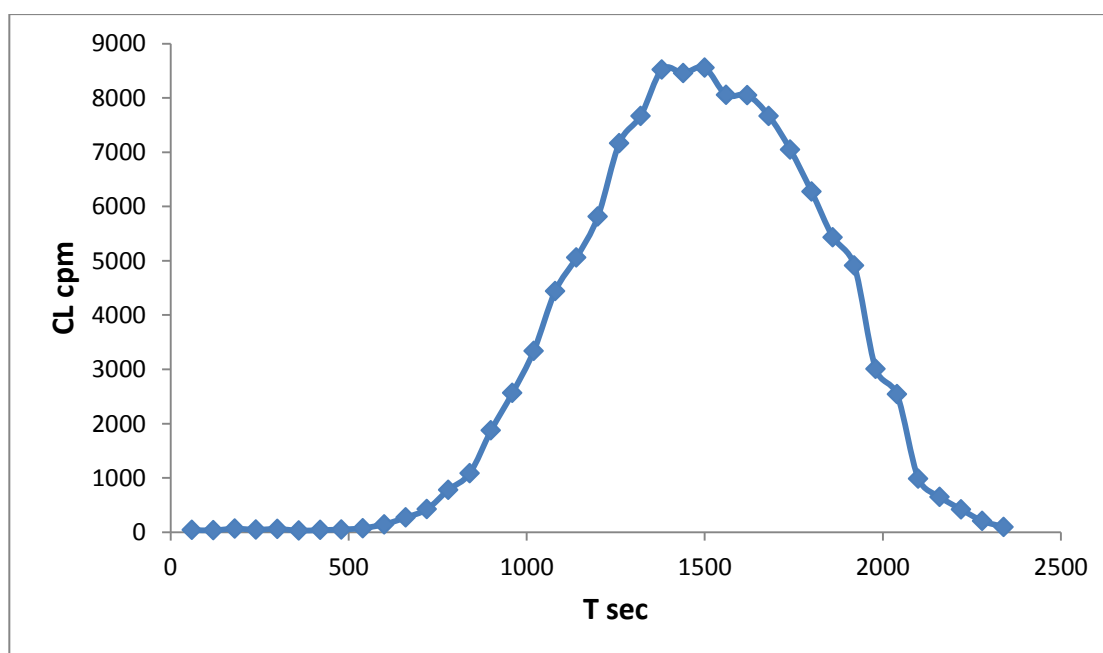


Fig: (3-31) Effect of neutron radiation on E. coli strain O157:H7 after six seconds .

CHAPTER FOUR

GENERAL DISCUSSION

&

CONCLUSION

4.1 Discussion

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 [61]. 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. [62,63] Suggested that radiations are activators of the superoxide anion ($\bar{O}_2$) critical to the microbiocidal action. 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.[64] ADP is known 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 [64,65] 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 [66,67].

4-2 Conclusions

The generation of reactive oxygen species (ROS) can be monitored by means of lucigenin-dependant chemiluminescence. The CL method is sensitive enough to detect very low (ROS) concentration produced by activity leukocytic by low doses . The ROS are aggressive and toxic depending on their concentration. Also it is concluded that CL technique can be combined with other qualitative and quantitative assessments of certain viral infections such as Hepatitis C virus (HCV) in clinical laboratories.

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 and low cost. 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 study 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 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. We conclude that the best treatment case to get best CL

result (best ability of leukocytes to produce ROS) is to radiate leukocytes and bacteria. each one separately before addition at the same time to CL solution.

4.3 Recommendations

We recommend by the followings: -

1. Study the effects of other types of radiations such as X-rays on and isolated leukocytes of human and other mammals such as rabbits using CL technique.
2. Use the laser as well as other bacteria satisfactory , and using CL technique.
3. This technique can be used to in expense of concentration of hydrogen peroxide locted in the atmosphere. As the concentration increases with increasing the air pollution gas resulting from the units productivity .

REFERENCE

Reference -----

1. Narvaiz P. (1995). Chemiluminescence measurements on irradiated garlic powder by the single photon counting technique. Radiat. Phys. Chem., vol. 45, No.2, pp. 203-206.
2. Dodeigne C.; Thunus L. And Lejeune R. (2000). Chemiluminescence as diagnostic tool-Areview. Talanta, vol. 51, pp. 415-439.
3. Larry j.k.;gary h.g.(1983) .chemiluminescence and bioluminescence methods in analytical chemistry . Analyst , vol.108,pp.1206-1274.
4. Michael d.l. (1978) " luminescence spectroscopy" michael d.l.ed,academic press, n,y,ch.2, pp.94-146.
5. 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.
6. Liu y.m.; cheng j.k. (2002) .ultrasensitive chemiluminescence detection in capillary electrophoresis . Journal of chromatography a , vol.595 ,pp.1-13.
7. Kroskocz A. (2003). The effect of hypochlorite on human erythrocytes pretreated with X-radiation, Cellular of Molecular Biologyu letters, vol. 8, pp. 215-219.
8. 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.

Reference -----

9. Navas M.J.; Jimenez A.M. and Galán G. (1999). Air analysis determination of hydrogen by chemiluminescence. Atmospheric environment, vol. 33, pp. 2279-2283.
10. Garcia-campana AM. Baeyens WRG; Zhang X; Ales F and Gamiz L. (2001). Unifamiliar though exciting analytical detection in flowing streams: chemiluminescence. Ars pharmaceutica, vol. 42, No.1, pp. 81-107.
11. Schuster g.b. ; hom k.a. (1982) ." chemical and biological generation of excited states " adam w. And cilento g. Eds academic press , n.y.ch.7 ,pp.229-246
12. Shlyapintokh v.y (1968) " chemiluminescences techniques in chemical reaction " . Academic n.m. emanud n.y. Pp:1-128 .
13. Al-thaher, g.s. (1994). A study of the activity of human granulocytes by mean of chemiluminescence, msc. thesis university of basrah .
14. Allen r.c. And loose l.d. (1976). Phagocytic activity of aluminol - dependent chemiluminescence in rabbit alveolar and peritoneal macrophages. Biochem .biophys .res.commun, vol.69, pp.245-250.
15. Babior ,b.m(1984) :- the respiratory burst of phagocytes. J.clin. Invest. Vol.73, pp.599-601.

Reference -----

- 16 Hipler B. (2003). Chemiluminescence Measurement of
the generation of Reactive oxygen species (ROS).
BMG Labtechnologies gmbh. Application Note 111.
17. Yunbo li; hong zhu ; michael a.t. (1999) . Detection of mitochondria -
derived reactive oxygen species production by the chemilumigenic
probes lucigenin and luminol . Biochimica et biophysica acta ,
vol.1428, pp.1-12 .
- 18.- Tatiana Koutchma, Larry J. Forney and Carmen I. Moraru, 1st
edition, Contemporary Food Engineering, CRC Press, " Ultraviolet Light
in Food Technology: Principles and Applications", (2009).
19. United States Environment Protection Agency, 1st edition, "Ionizing
Radiation Fact Book ", (2007).
20. United States Environment Protection Agency, " Ionizing and Non-
Ionizing Radiation ", (2012).
21. Kwam H., the international conference on Non-Ionizing Radiation at
UNITEN, "Non Ionizing Radiation – Sources ,Biological Effects
,Emission and Exposures ", (2003).
22. Jumá M. And Kammal S.A. (1984). "The atomic radiation", Al-Ratib
universal publishing house.

Reference -----

- 23..Salih A.J. (1988): - "Introduction to nuclear physics", university of Basrah, Basrah.
- 24.Escherich, T. (1885): Die Darmbakterien des Neugeborenen und Sauglings. Fortschritte der Medizin 3: 515-545.
25. Kaper, J. B. (2005): Pathogenic Escherichia coli. Internat. J. of Med. Microbiol., 295 (6-7): 355–356.
26. Shulman, S.T.; Friedman, H.C. and Sims, R.H. (2007): Theodor Escherich: The first pediatric infectious disease physician. Clin. Infect. Dis.,45:1025-1029.
27. Meng, J.; Doyle, P.; Zhao, T. and Zhao, S. (2007): Enterohemorrhagic Escherichia coli. ASM Press, Washington D.C.,:249- - 269.
28. Karmali, M. A.; Petric, M.; Lim, C., Fleming, P.C.; Arbus, G.S. and Lior, H. (2004): The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing Escherichia coli. J. Infect. Dis., 189(3): 556-63.
29. Fotadar, U., Zaveloff, P. and Terracio, L. (2005): Growth of Escherichia coli at elevated temperatures.J. Basic. Microbiol. 45(5): 403-404.
30. Tenaillon, O.; Skurnik, D.; Picard, B. and Denamur, E. (2010): The population genetics of commensal Escherichia coli. Nat. Rev. Microbiol. 8: 207-217.
31. Scheutz, F.; Cheasty, T.; Woodward, D. and Smith, H.R. (2004): Desig-

Reference -----

nation of O174 and O175 to temporary O groups OX3 and OX7, and six new E. coli O groups that include Verocytotoxin-producing E. coli (VTEC): O176, O177, O178, O179, O180 and O181. APMIS 112: 569–584.

32. Gyles, C. L. (1994): *Escherichia coli* in domestic animals and humans. Wallingford, Oxon, U.K. Tucson, Ariz., CAB International.

33. Neill, M.A.; Tarr, P. I.; Taylor, D.N. and Wolf, M. (2001): *Escherichia Coli*. Pages 169 - 196 in *Foodborne Disease Handbook*. Y.H. Hui, M.D. Piersen, and J. R. Gorham (eds.) Marcel Dekker, Inc., New York, NY.

34. Jann, K. and Jann, B. (1992): Capsules of *Escherichia coli*, expression and biological significance. *Can. J. Microbiol.* 38: 705-710.

35. Scheutz, F.; Cheasty, T.; Woodward, D. and Smith, H.R. (2004): Designation of O174 and O175 to temporary O groups OX3 and OX7, and six new E. coli O groups that include Verocytotoxin-producing E. coli (VTEC): O176, O177, O178, O179, O180 and O181. APMIS 112: 569–584.

36. Gyles, C. L. (2006): Shiga toxin-producing *Escherichia coli*: the big picture. *J. of Ani. Sci.*, 84: 99–99.

37. Bray, J. (1945): Isolation of antigenically homogeneous strains of Bact.

Reference -----

- Coli neapolitanum from summer diarrhoea of infants. J.of Pathol. and Bacteriol., 57(2): 239–247.
- 38.Naylor, J. M. (2002): Neonatal ruminal diarrhea. In: Smith, B.P. Large Animal Internal Medicine. 3. Edition., St. Louis, Missouri. :352-366.
39. Croxen, M.A. and Finlay, B.B. (2010): Molecular mechanisms of Escherichia coli pathogenicity. Nat. Rev. Microbiol. 8: 26–38.
40. Ogiehor, I.S. and Ikenebomeh, M.J. (2006): The Effects of Different Packaging Materials on the Shelf Stability of Garri. African J.of Biotech., 523: 2412-2416.
41. AL_Hawary M.F. (1981):- clinical chemistry. Technical institutes establiment, Baghdad, pp. 184.
42. Martin D.W. (1981). Blood plasma and clotting In: Harper's review of biochemistry . (Eds.D.W.Martin P.A. Mayes and V.W. Rodwell). 18th ed..Lange Med. Publ., Losaltos.
- 43.Lizhengping ; lanting d. And zhuiun z. (1995) .study on catalytic effect of copper(II) complex on 1,10 phenanthroline-h₂O-ctmabcl reaction and its analytical applications microchemical journal ,vol.52,pp.208-215.

Reference -----

44. 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.

45. Yunbo li; hong zhu ; michael a.t. (1999) . Deection of mitochondria - derived reactive oxygen species production by the chemilumigenic probes lucigenin and luminol . Biochimica et biophysica acta , vol.1428,pp.1.12

46. Paape m.j ; miller r.h. (1990) .effect of florfenicd , chloramph - enicol , and thiamphaunclear neutrophil leukocytes . J dairy sci, vol. 73, pp.1734-1744.

47. AL_THAHER G.S. (1994). Astudy of the activity of human Granulocytes mean of chemiluminescence, Msc.Thesis university of Basrah.

48. Li h.t. ; ; zhao b.l. ; hou j.w. And xin w.j. (1996) . Two peak kinetic curve of the chemiluminesacence phorbd -induced macrophage . Biochemical and biophysical research communications , vol.223, pp.311-314 .

49. Saeed f.a. And castle g.e. (1998) neutrophil chemiluminescence during phagocytosis is inhibited by abnormally elevated levels of acetoatate : implication for diabetic susceptibility to infections . Clinical and diagnostic laboratory immunolog , vol.5, no.5, pp. 740-743.]

50. Nerom a.v. ; ducatelle r. ; haesebrouck f. (1999).oxidative activity of

Reference -----

turkey monocytes , following the inoculation with chlamydia psittaci . Veterinary microbiology , vol.65, pp.173-184.

51. Hiravama A.; Noronha-Dutra A.A; Gordge M.P.; Neild G.H. and Hothersall J.S. (2001). Uremic concentration of guanidine compounds inhibit neutrophil superoxide production. *Kidney International*, vol. 59, No. 78, pp. S-89-S-92.
52. 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.
53. Bianchi M.; Terenzi CT. And Fagiolo E. (2000). Effects of gamma radiation on peripheral blood mononuclear cells in whole bloods units. Paper presented at the International symposiumon impact of Biotechnology on cancer Diagnositc indicators; Geneva, Switzerland.
- 54.Kitagawo M; Yamaguchi S.; Hasegawa M.; Tanaka K.; Sado T.; Hirokawa K. And Aizawa S. (2002). Friend leukemia virus infection enchances DNA damage-induced apoptosis of hematopoietic cells, causing lethal one in C₃H hosts. *J viral*, vol. 76, No. 15, pp. 7790-7798.
55. Mikami T.; Takashi T. Ishida A. Minamiya Y. Ida H. And Takada G. (2002). Signaling pathway for radiation induced a poptosis in

Reference -----

the lymphoblasts from neurinopathic (type A) and non-neuronopathic (type B) forms of Niemann-Pick disease. J Neurol sci., vol. 15, No. 199, pp. 1-2.

56. Saeed f.a. And castle g.e. (1998) neutrophil chemiluminescence during phagocytosis is inhibited by abnormally elevated levels of acetate : implication for diabetic susceptibility to infections . Clinical and diagnostic laboratory immunology , vol.5, no.5, pp. 740-743.]

57. Hipler B. (2003). Chemiluminescence Measurement of the generation of Reactive oxygen species (ROS).

BMG Labtechnologies gmbh. Application Note 111.

58. Allen r.c. And loose l.d. (1976). Phagocytic activity of aluminol - dependent chemiluminescence in rabbit alveolar and peritoneal macrophages. Biochem .biophys .res.commun,vol.69,pp.245-250.

60. Bianchi M.; Terenzi CT. And Fagiolo E. (2000). Effects of gamma radiation on peripheral blood mononuclear cells in whole blood units. Paper presented at the International symposium on impact of Biotechnology on cancer Diagnostic indicators; Geneva, Switzerland.

61. Babior ,b.m(1984) :- the respiratory burst of phagocytes. J.clin. Invest. Vol.73,pp.599-601.

Reference -----

62. Fujita I.; Irita K.; Takeshige K. and Minikami S. (1984). Diacylglycerol, 1-oleoyl-2-acetyl -glycerol stimuli superoxide-generation from human neutrophils .biochemical and biophysical research communications, vol.20, pp.318-324.

63. Nishizuka Y. (1984). The role of protein kinase C in cell surface signal transduction and tumour promotion .nature, london ,vol.308, pp.693-698.

64. 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.

65. 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 radiation at $\lambda=820$ nm . Laser insurgery medicine .vol21, pp.485-492.

66. Baggiolini, M. and Wyman , M.P. (1990) turning on the respiratory burst .trends biochem.sci.vol.15, pp-69-72.

67. Segal , A.W. and Abo, A. (1993): the biochemical basis of the NADPH-oxidase of phagocytosis. Trends biochem.sci.vol.18, pp.43-47..

Reference -----

الخلاصة :

توجد العديد من الدراسات التي تهتم بالتأثيرات الإشعاعية على المايكرو بات (البكتيريا والفايروسات) ومعظم هذه الدراسات تهتم بالتأثيرات التي تسببها أشعة كاما على المايكرو بات .
أهتم هذا العمل بدراسة تأثيرات الأشعة المؤينة ذات قدرة أيقاف معينة مثل (أشعة بيتا والنيوترونات والأشعة فوق البنفسجية) على السلالات ذات عوامل الضراوة العالية من البكتيريا القولونية **Escherichia Coli** سلالة (O157:H7 و O26) المعروفة بتأثيراتها السمية العالية وقابليتها العالية على إنتاج الجذور الحرة . مقابل هذه البكتيريا تم تحضير محاليل من كريات الدم البيضاء الحاوية على المايكروفيج والتي لها القابلية على إنتاج الجذور الحرة اثناء عملية قتل وألتهم البكتيريا . أن زيادة فعالية البكتيريا أو نقصانها وكذلك كريات الدم البيضاء يتم عن طريق قراءة التآلق الكيميائي لها . باستخدام صبغة اللوسجين زيادة سمية كريات الدم البيضاء من سمية البكتيريا . وذلك بتحفيزها بواسطة الإشعاع بالجرعات الواطئة . ومعرفة ذلك من خلال التآلق الكيميائي أي ان تآلق الكريات أكبر من البكتيريا . واخذت القراءات كل على حده في حالة خارج الجسم (invitro) وكانت القراءات مشابهة لأخذ القراءات في داخل الجسم (invivo).

أن كريات الدم البيضاء تعتبر خط الدفاع الأول في الجسم ضد الأجسام الغريبة (البكتيريا ، الطفيليات، الفايروسات،...) ، حيث أن جميع أنواع كريات الدم البيضاء لها القدرة على إنتاج أشكال متعددة من جذور الأوكسجين الفعالة مثل الأوكسجين الأحادي $^1O_2$ ، سوبر اوكسيد O_2^- ، الهيدروكسيل OH^* ، بيروكسيد الهيدروجين H_2O_2 بعملية البلعمة والتنفس الحياتي ، وبما أن كريات الدم البيضاء التي تعاني تنفس حياتي تبعث ضوء (تآلق كيميائي) ألا أن هذا الضوء يكون ضعيفا لذلك تحتاج إلى مقدمة لبدء التآلق الكيميائي (مواد التآلق) لزيادة كشف الضوء الناتج. لذلك استخدمت تقنية التآلق الكيميائي في هذه الدراسة لمعرفة تأثير الإشعاع والفايروسات على الدم الكامل وكريات الدم البيضاء والحمراء المعزولة ، أن محلول التآلق المستخدم هو اللوسجين Lucigenin المذاب في (DMSO) Dimethyl sulfoxide . أخذ قراءات التآلق للبكتيريا وكريات الدم البيضاء قبل التشعيع وذلك لاختيار احسن ظروف مختبرية لاعلى قراءة تآلق ، واعتمدت هذه القراءات القراءه الصفريه ، تم تعريض البكتيريا وكريات الدم البيضاء الى جرعات مختلفة من اشعة بيتا والفوق البنفسجية و النيوترونات، تم اضافة البكتيريا الى كريات الدم البيضاء دون التعرض الى الاشعاع .



جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة ذي قار

كلية العلوم

قسم الفيزياء

**دراسة التأثيرات المميتة للنيوترونات وجسيمات بيتا والاشعة فوق
البنفسجية على البكتيريا باستخدام تقنية التألق الكيميائي**

رسالة مقدمة الى مجلس كلية العلوم جامعة ذي قار وهي جزء من متطلبات نيل درجة
الماجستير في علوم الفيزياء

من قبل

دعاء كاطع غالي

بإشراف

الاستاذ الدكتور عماد عبد الرزاق سلمان

الاستاذ الدكتور قحطان عدنان عبد القادر

المقومان

المقوم اللغوي

أ.م.د. سامر مهدي عبدالمحسن

جامعة ذي قار/كلية العلوم

طبقاً إلى الأمر الإداري ذي الرقم ٣٠/س ٢٥٠١ بتاريخ ٢٠١٧/١٠/٨ والصادر من
كلية العلوم/جامعة ذي قار.

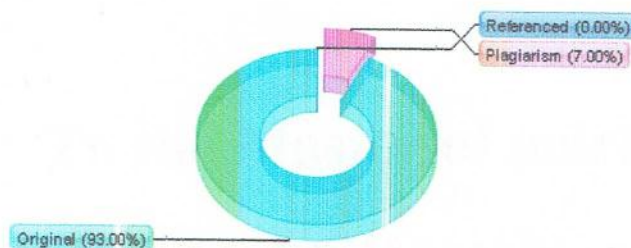
المقوم العلمي

أ.م.د. علي عبدالوهاب رضا

الجامعة المستنصرية/كلية العلوم

طبقاً إلى الأمر الإداري ذي الرقم ٣٠/س ٥٩١ بتاريخ ٢٠١٨/٢/١٣ والصادر من
كلية العلوم/جامعة ذي قار.

Thursday, January 18,
2018



تقرير

فحص أستيلا رسالة ماجستير:

*Study of killing Effects of Neutron and UV Light
on Bacteriophage in A Dry state By means of
Chemiluminescence*

دعاء كاظم غالي
كلية العلوم
قسم الفيزياء





الى / قسم الفيزياء

م/ استلال الكتروني

تحية طيبة

اشارة الى طلبكم الصادر في تاريخ (2018/1/15) نحيل اليكم تقرير الاستلال قبل التقويم
العلمي لرسالة طالبة الماجستير (دعاء كاطع غالي) الموسومة ب : (Study of killing
Effects of Neutron and UV Light on Bacteriophage in A Dry state By means
of Chemiluminescence) , قد بلغت نسبته (9%) فقط.

8-2-2018
أ.م.د. هيثم عبدالامير مينا
م. العميد للشؤون العلمية
والدراسات العليا

المرفقات//
تقرير ورقي
قرص CD

نسخه منه الى/

- ❖ مكتب السيد العميد المحترم/للتفضل بالاطلاع.....مع التقدير.
- ❖ مكتب السيد معاون العميد العلمي المحترم / للتفضل بالاطلاع مع التقدير
- ❖ ملفه الاستلال .
- ❖ الصادرة.

تعهد بالتقيد التام بما جاء في مضمون كتاب وزارة التعليم العالي والبحث العلمي/جهاز الاشراف والتقويم المرقم ج ت/425 الصادر بتاريخ 2014/3/24، والمبلغ اليها بكتاب رئاسة جامعة ذي قار/قسم البحث والتطوير المرقم 4704/54/7 والصادر بتاريخ 2014/3/31، عند أعداد الرسالة أو الاطروحة الموسومة:

study of killing effects of neutron and
uv light on ~~D~~acrophage in a dry state
by means of chemiluminescence

وبخلافه نتحمل نحن الموقعون أدناه المسؤولية القانونية حال وجود ما يخالف مضمون الكتاب أعلاه أو ما ينافي أخلاقيات البحث العلمي.

الاسم	القسم والكلية أو المعهد	التوقيع	التاريخ
1- المشرف الاول:			/...../.....
2- المشرف الثاني: د. محمد طه عبد السلام عبد الجبار	جامعة ذي قار / كلية العلوم / الفيزياء		/...../.....
3- طالب الدراسات: د. عمار كاظم عاني	جامعة ذي قار / كلية العلوم / الفيزياء		/...../.....

ملاحظات هامة:

- 1- تعتبر استمارة التعهد هذه جزء من متطلبات اعتماد مشاريع طلبة الدراسات العليا وتوزيع المشرفين على الطلبة.
- 2- تطبق استمارة التعهد هذه على السادة المشرفين على الرسالة أو الاطروحة وعلى طلبة الماجستير والدكتوراه.
- 3- السطو العلمي هو سرقة نتاج فكري، وعند الاقتباس ضرورة الإشارة للمصدر، وفي حالات الاقتباس ضرورة إعادة صياغة الفقرة المقتبسة دون الخروج من حدود فكرة الفقرة الاصلية المقتبسة والمشار لمصدرها في متن الرسالة أو الاطروحة.
- 4- تشمل استمارة التعهد هذه الرسائل والاطاريح التي لم تنجز حتى تاريخ كتاب جهاز الاشراف والتقويم أدناه.
- 5- أعدت استمارة التعهد هذه استناداً للفقرة 4 من كتاب وزارة التعليم العالي والبحث العلمي/جهاز الاشراف والتقويم المرقم ج ت/425 الصادر بتاريخ 2014/3/24 والمبلغ اليها عن طريق كتاب رئاسة جامعة ذي قار/قسم البحث والتطوير المرقم 4704/54/7 بتاريخ 2014/3/31.



51- يرضع على عاتق الاستاذة الفتي يقومون بدور الاسراف مساندة الزامهم بالتعهد بمنع الطالب من التعامل مع المكتبة التي تقوم بمهمة طبع وبيع البحوث والرسائل والاضريح لطلبة الدراسات العليا، كما وضع صفحته من قبل الكليات بعد ان جيبها من خلال الجامعة.