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Optimization and spectrophotometric comparisons of radiochromic dye (2,3,5-triphenyl-2-H-tetrazolium chloride) in gelatin and agar models: gamma and electron beam irradiations

Lakindra P. Frances

Optimization and Spectrophotometric Comparisons of Radiochromic Dye (2,3,5-Triphenyl-2H-tetrazolium chloride) in Gelatin and Agar Models: Gamma and Electron Beam Irradiations

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A THESIS

Presented to

Honors College
Southern University and A&M College
Baton Rouge, Louisiana

In Partial Fulfillment of the Requirements for the

Honors College Degree

By

LaKindra P. Francis

May, 1997

Honors College

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CERTIFICATE OF APPROVAL

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HONORS THESIS

This is to certify that the Honors Thesis of
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ABSTRACT

After irradiation, the colorless radiochromic dye, 2,3,5-triphenyl-2H-tetrazolium chloride (TTC), changes to red triphenyl formazan pigment, and the distribution of absorbed dose in the tissue-equivalent gelatin and agar media can be studied. Using solutions 8% by weight gelatin and 8% by weight agar, both with 3 mM citric acid as buffer and 4 mM concentration of dye, the radiation-induced absorbency of a sample slowly decreased with increasing wavelength. In the gel, TTC is very light sensitive, and 7 days after irradiation, becomes less stable. The samples were irradiated by gamma and electron beam. Nine different doses were used for the ^{60}Co gamma source. Each cylindrical sample of a different dose was sliced one day after irradiation and analyzed by UV-V is spectrophotometry. Absorption maxima were obtained at wavelengths of 520 nm and 570 nm. Using accelerated electrons at an energy of 25 MeV generated by MIRF (Medical-Industrial Radiation Facility), the gelatin samples were irradiated at nine different exposure times. In previous studies of this model, only the gelatin model was used. Now, some comparisons of the radiochromic dyes in different gel matrices of gelatin and agar at different concentrations to optimize dose sensitivity have been completed. Also, some comparisons of the gelatin samples irradiated in borosilicate glass containers at different doses were performed. Along with studies of the concentration of the gels comparing 8% by weight gelatin to 10% by weight gelatin. After analysis, the gelatin samples were observed to be the better gel media, the 10% gelatin samples were firmer and easier to handle, and the borosilicate glass containers were logistically advantageous for the gelatin samples. The dose response will

be observed with both gamma and electron beam irradiation to determine the amount of color change with a spectrophotometer. Studying the dose distribution of the irradiation gelatin and agar tissue models will be significant for the future studies of radiation therapy to estimate dose distribution within patient.

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CHAPTER 1
INTRODUCTION

Radiochromic effects involve the direct coloration of a material by the absorption of energetic radiation, without requiring latent chemical, optical, or thermal development or amplification[1,2]. The production of immediate, reversible or permanent colored images of a radiation exposure pattern in a solid, with or without "fixing," of the sensor medium against further change is generally defined as radiochromic imaging. Radiation dosimetry is the principles and techniques involve with the measurements and recording of dose. Unfortunately, numerous problems have been associated with the measurement of depth-dose distributions in high gradient regions of radiation beams using convention radiation measuring systems. With the introduction of radiochromic dyes some of the dosimetric problems have been resolved[3].

For 30 years, colorless transparent radiochromic thin films have been used as high dose radiation dosimeters to give permanently colored images[3]. Upon irradiation, radiochromic thin films undergo heterolytic bond scission of the nitride group to form highly colored dye salts in solid polymeric solution. They have been used to map radiation dose distributions across material interfaces and high-resolution, high-contrast radiation images. However, this type of radiochromic system is not sensitive enough to be used for clinical or radiological applications. More recently, GafChromic DM-1260 and GafChromic DM-55 have been introduced as another form of radiochromic film. They are colorless before irradiation, and turn blue upon exposure to ionizing radiation. Also, they are used for

radiographic imaging treatment planning, medical dosimetry, and for dose measurements at interfaces.

Tetrazolium salts are being used as dye agents for radiochromic dosimetric films and for radiochromic gels. 2,3,5-Triphenyl-2H-tetrazolium chloride (TTC) is a tetrazolium salt, which results in the formation of the pink colored triphenyl formazan pigment upon irradiation. Studies have been conducted on the radiation-chemical behavior of TTC both by electron-pulse and steady-state gamma ray radiolysis[4,5]. Kovacs et al has produced and tested radiochromic films containing TTC and found an adequate concentration of components in the 1 to 100 kGy dose ranges.

At present, radiochromic gels are used for routine dosimetry over wide range absorbed doses and absorbed dose rates. Investigators are studying the effects of light, dose, and dose rate, irradiation temperature, and relative humidity to characterize the material in aqueous and alcoholic solutions. In the appropriate mold, such a gel will show a three-dimensional map of dose distribution, which could be extrapolated to estimate what would happen in a patient. In the past, only gelatin media has been used; now we hope to find agar as suitable media to measure the absorbed color within the gel. The scope of this research has been limited to (i) comparisons of the radiochromic dye in different gel media (gelatin and agar), (ii) optimization of dose sensitivity of the radiochromic dye at different concentrations, and (iii) the determination of dose response with both gamma and electron beam irradiation to observe the yield of color change. The purpose of this research is to

develop radiochromic gels with the colorless dye, 2,3,5-triphenyl-2H-tetrazolium chloride (TTC), using gamma and electron beam irradiation to provide a possible model for future studies in human radiotherapy.

Definitions of Useful Terms

Radioactivity is the property of a substance to disintegrate by emission of radiation. **Radiation** is the emission or propagation of energy through space or matter in the form of a wave. The major types of radiation are electromagnetic, particle, acoustic, and acoustic, and in this study, the focus is primarily on electromagnetic radiation. The quantum of electromagnetic radiation is a **photon,** and it is generated when an electrically charged particle changes momentum through collisions. In addition, a photon can be absorbed by any charged particle.

Ionization is the gain or loss of an electron by an atom that results in an ion that will carry a net positive or negative charge. **Ionizing radiation** is energy that has the property of ionizing matter through which it passes either directly or indirectly. It produces random energy releases of great size, and hence generally disruptive effect. To measure the effect of ionizing radiation the radiation must be measured in amount. **Dosimetry** is the principles and techniques involved in the measurement and recording of dose; whereas, the dose is the amount of radiation given. On the other hand, **absorbed dose** is the amount of energy absorbed per unit of mass, and it is measured in gray (Gy = J/kg). Also, it is measured in **rad** which is defined as the amount of radiation which will release 100 ergs in 1 gram of tissue

(units of rad = 10^{-2} Gy). This table list both new and old radiological units used to measure absorbed dose, amount of radiation exposure, and activity.

New and Old Radiological Units

Quality	Name	Symbol	Units
Activity	Becquerel	Bq Ci	1 dps 3.7 x 10^10 Bq
Absorbed Dose	Gray (rad)	Gy rad	J kg^-1 10^-2 Gy
Dose Equivalent	Sievert (rem)	Sv rem	J kg^-1 10^-2 Sv
Exposure	Coulomb per kilogram (roentgen)	R	C kg^-1 2.58 x 10^-4 C kg^-1

Table 1

A huge variety of effects of ionizing radiation exist. In whole-body irradiation, death is due to effects on a variety of organs, and blood-forming organs and tissue are the most sensitive. Individual organs can receive a much higher dose of irradiation before acute systems arise. For example, the gastrointestinal system of the body can be exposed to 10-20 Gy; whereas, a total body dose of 3-5 Gy can be considered lethal. Ionizing radiation produces mutations in any living organism with the possible exception of viruses. In addition, all kind of chromosome breaks are produced by ionizing radiation. It inhibits cell division in which the degree of delay increases with dose. Cells in which division is delayed may grow into giant cells, many times the normal size. So, the aim in radiation therapy is

to deliver the maximum possible radiation to the selected site while minimizing the energy deposited to healthy tissue.

In measuring the effects of ionizing radiation of the gel samples, two important features are taken into account: the incidence peak and the bragg peak. They are the regions within the gels that encompass the irradiated tissue-equivalent gelatin and agar media. The **incidence peak** is the region of initial ionization seen near the beginning of the particle beam; on the other hand, the **bragg peak** is the maximum region of increased ionization seen near the end of the particle beam.

These terms are directly related to the study and prove useful in the analysis of data. The characteristics of the other tools of analyzing and measuring the effects of ionizing radiation and absorbed dose are discussed in the following sections.

CHAPTER II
BACKGROUND OF STUDY

Description of Radiochromic Dyes

Radiochromic dyes are compounds that change color when irradiated. They color directly and do not require chemical processing because the color change (colorless to red, blue, green, etc.) indicated exposure to radiation. The image formation does not occur as radiation damage but as a dye-forming or polymerization process, in which energy is transferred, from an energetic photon or particle to a receptive part of the leuko-dye or colorless photomonomer molecule[6]. This process usually initiated color formation through radiolytic chemical changes.

One type of radiochromic dye is tetrazolium salt. According to McLaughlin, tetrazolium salts are dye agents for radiochromic dosimetric films[1]. These salts are colorless in aqueous solution but upon irradiation are reduced to the highly colored, insoluble formazan 6. Tetrazolium salts have been used for imaging and mapping of absorbed dose distributions. Also, they have been used for years as one-shot disposable dosimeters for industrial radiation processing. Tetrazolium salts are extremely sensitive to light and unstable after a few days of use.

In this study, the tetrazolium salt, 2,3,5-triphenyl-2H-tetrazolium chloride (TTC), is used. TTC is an off-white powder with molecular formula $C_{19}H_{15}ClN_4$ and formula weight of 316.80. It is very soluble in water and alcohol, soluble in acetone, and insoluble in ether, and becomes unstable after seven days. Upon irradiation, TTC undergoes radiolytic

reduction, which results in the formation of the red dye triphenyl formazan. Figure 1 illustrates the formation of the red dye triphenyl formazan upon irradiation of TTC.

Radiochromic Dyes

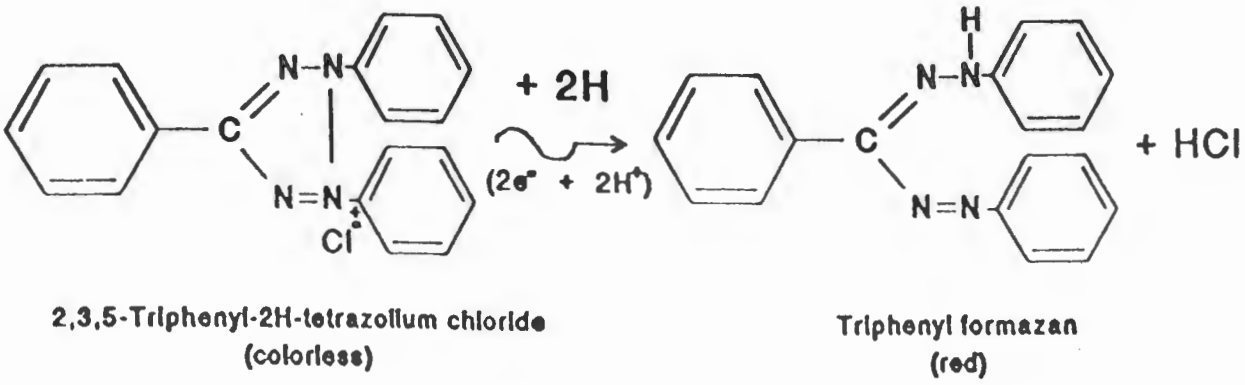

Figure 1

The dye (triphenyl formazan) is extremely fat soluble and sensitive to light. This light sensitivity eliminates TTC as a suitable reagent for definitive histological studies. Also, the dye is moderately toxic so dispose of excess gel appropriately. TTC is a sensitive reagent for reducing sugars and a germination indicator for seeds. The embryonic tissue present in live seeds hydrogenates the colorless reagent converting it to the red dye.

Description of Irradiation Sources

The samples were irradiated by gamma and electron beam. In this study, the gammacell is a ^{60}Co irradiation source made by the reactor irradiation of cobalt metal. Figure 2 illustrates the gammacell, ^{60}Co irradiation source.

^{60}Co Irradiation Source

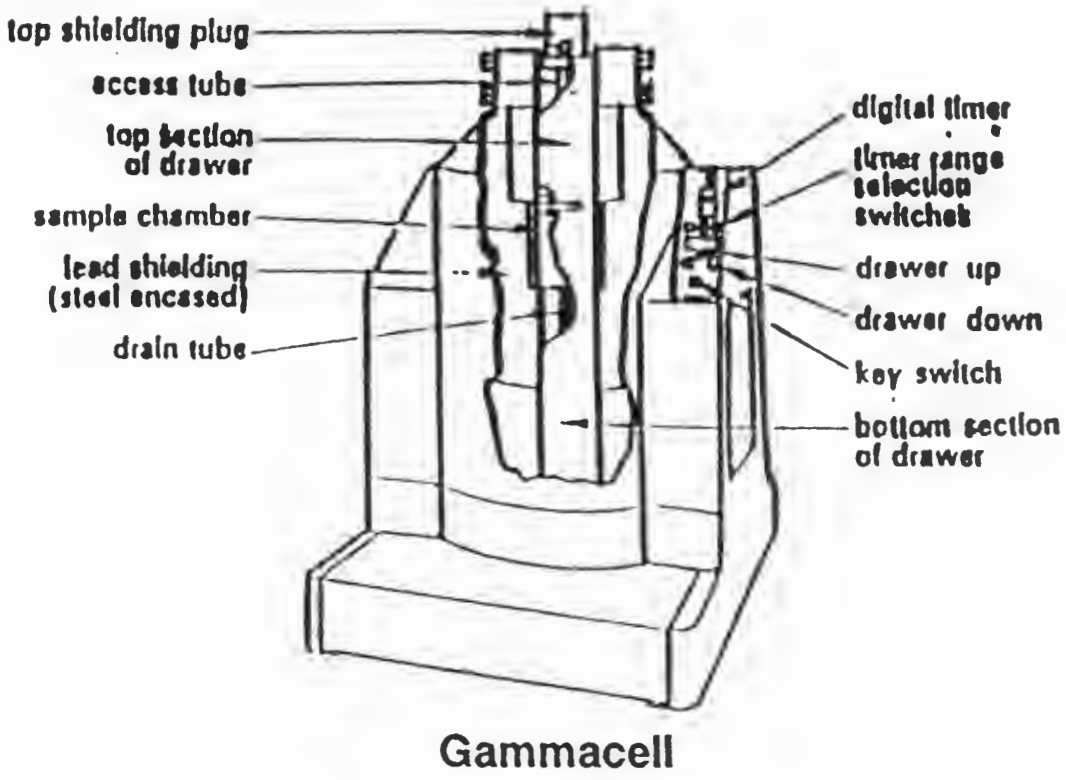

Figure 2

^{60}Co is a form of artificial radioactivity that has a half-life of 5.27 years ($t_{1/2}$ = 5.27). In the gammacell, ^{60}Co is encapsulated within a lead-shielding barrier that surrounds the sample chamber to eliminate the possibility of spreading the radioactivity and the gel sample is centered in the sample chamber on some form of density dense material. The larger samples in the gammacell can receive a uniform dose of radiation throughout their volume. This is possible because the source consists of a cylindrical array of rods. The ^{60}Co artificial radioactivity source has a number of applications including the sterilization of medical

supplies, the promotion of chemical reaction, and some sources are used as calibration standards.

On the other hand, MIRF (Medical-Industrial Radiation Facility) is an electron beam irradiation source that utilizes accelerated electrons that can be introduced into the samples directly from a source of beta particles. A beta particle is a species having a mass of 1/1837 of a proton. When it is negatively charged, it is an electron, and whenever it is positively charged, it is referred to as a positron. The machine parameters of MIRF detail an electron energy of 7-32 MeV and a photon energy of 25 MeV with a repetition rate of 100 pps (parts per seconds).

Accelerated electrons dissipate most of their energy in matter by causing excitation and ionization. They can interact with the nucleus by an elastic collision, which deflects electron. The most abundant activation of accelerated electrons is those of valence electrons to the energies of 10-50 MeV. Accelerated electrons have been applied in radiation therapy, and in radiation chemistry for the activation of valence electrons of molecules to produce chemical effects.

MIRF is comprised of a six component system. The first component of MIRF consists of a two-stage traveling radio frequency linac. The gel samples were irradiated at the end of component one using accelerated electrons at energy of 25 MeV generated by MIRF. Figure 3 shows a schematic diagram of the gels irradiated on MIRF. Component two is a collimator head for medical treatment beam, and component three is a motor generator.

The fourth component is an 8-MW Klystron and a wave-guide, while the fifth component is a water cooling system. The last component entails an operator's console and a data acquisition system.

Schematic Diagram of MIRF

Figure 3

MIRF has a number of applications that are utilized in medical, industrial, environmental, and e-beam physics. For example, the medical applications of MIRF have been used for absorbed dose measurements, real-time sensor developments, and benchmark measurements for shielding. The absorbed dose measurements are related to the irradiation of the gel samples and the absorbed color distribution in the gels produced by MIRF. The

industrial applications are electron-beam curing and sterilization, radiation hardness testing, beam diagnostics, and industrial CT scanning. On the other hand, the environmental applications are employed in municipal waste sterilization and hazardous compound degradation. Last, e-beam physics applications are used for channeling radiation and materials modification.

CHAPTER III
EXPERIMENTAL SET-UP AND PROCEDURES

The Knorr gelatin and agar gels were prepared by weight using 3 mM citric acid as buffer. The gel media and citric acid buffer were added to boiling millipore water with stirring until dilution, then the solution was divided in halves within beakers. After the solutions cooled to 70 ^{0}C, 2,3,5-triphenyl-2H-tetrazolium chloride was added with stirring to a final concentration of 4 mM to one half of the beakers, and the other half remained without dye. It is extremely important to keep the lights off because TTC is light sensitive. Aliquot solutions to 50 ml beakers, and allow the gels to set overnight at room temperature covering the top of the beakers with parafilm and wrapping the sides with aluminum foil. On the next morning, the beakers were placed in the refrigerator for one hour to enhance firmness. Once the gels became firmer, they were irradiated in the gammacell or on MIRF (Medical-Industrial Radiation Facility).

The gel samples that were irradiated in the gammacell received uniform dose distribution, and they were centered in the drawer using some type of density dense material such as Styrofoam. Using the dose rate software at NIST (National Institute of Standards and Technology) the clock time for the sample was configured. The transit time is 0.07 minutes. It is the amount of time required for the sample to position itself within the chamber from the top drawer. Subtracting the transit time from the clock time provides the time of exposure or irradiation for the gels, and the dose rate software automatically

calculates this. Throughout the study, different doses were used for comparison, variation, and optimization of the distribution of dose within the gel media.

Table 2 lists the various doses used within the study. All of the exposure times were configured using the same parameters for both Knorr gelatin and agar: dose in water, no attenuation, in red perspex, and 1 dose point.

Various Doses Used

Dose in kGy	Clock Time (min)	Exposure Time (min)
0.25	4.489	4.419
0.3	5.359	5.289
0.5	8.977	8.907
1	17.954	17.884
1.2	21.437	21.367
1.25	22.443	22.373
1.5	26.932	26.862
1.75	31.42	31.35
2	35.909	35.839
2.25	40.397	40.327
2.4	42.874	42.804
2.5	44.886	44.816

Table 2

Time of Exposure and the ChargeDistributed br MIRF

Sample Number	Exposure Time (sec)	Charge, Q (Coulombs)
1	14	2.08×10^{-08}
2	7	1.05×10^{-08}
3	4	4.90×10^{-09}
4	73	1.01×10^{-07}
5	3	3.00×10^{-09}
6	37	5.04×10^{-08}
7	24	3.04×10^{-08}
8	161	2.00×10^{-07}
9	61	7.55×10^{-08}

Table 3

The samples irradiated on MIRF are placed at the end of component one the two-stage traveling wave radio frequency linac. Nine different doses were used for analysis comparison, and variation of dose distribution of dose distribution within the gels. All of the samples were irradiated using 25 MeV, and the dose rate collimator is set at 100 R/min (revolutions per minute) for the gel samples. Table 3 lists the time of exposure and the charge distributed by MIRF.

After irradiation, the gels were removed from their containers, sliced and read on the spectrophotometer (Cary 210). To remove gels from the beakers, use a piece of plastic (such as viewgraphs) between the gel and beaker to unmold; or, less preferred, warm beakers

gently in boiling water to begin melting of the gel or line the beaker with viewgraph plastic before pouring the gels to begin with. Do not use a knife or scalpel to slice the gels. A taut stainless steel or piano wire is attached to a cheese slicer or any other conventional instrument to slice the gels thin and consistently.

The samples were analyzed using Ultraviolet-Visible spectrophotometry to measure the extent to which gas, liquid, and solid samples absorb light of different wavelengths. Broad band radiation from tungsten-halogen lamp is collected, focused, and directed through a double pass monochromator which may be adjusted to isolated selected wavelengths. Tungsten-halogen light is directed through "sample" space and then through a "reference" space and the two light beam paths are redirected to converge on the phototube that provides a measure of the amount of radiation absorbed by the sample.

The visible spectra of the gamma-irradiated samples were recorded from 700 nm to 400 nm with maximum absorbency at 520 nm and 570 nm. On the other hand, the visible spectra of the electron beam (MIRF) irradiated samples were recorded at 525 nm horizontally across the gel samples. To record the visible spectra use a sample and a reference (agar for agar samples, gelatin for gelatin samples), and the reference slice should be the same thickness as the sample slice. For instance, the sample of Knorr gelatin plus dye irradiated was referenced to Knorr gelatin irradiated. Also, a sample of agar plus dye no irradiation was referenced to agar no irradiation. The absorbency of distributed color within the gels is recorded on the spectrophotometer. After analysis and data collection the samples are disposed.

CHAPTER IV
RESULTS AND ANALYSIS

In this study, the tissue-equivalent gelatin and agar media are used for the distribution of absorbed dose. Knorr gelatin is an animal protein substance that has gel-forming properties that are finely ground crystals. Whereas, agar is a gelatin-like product made primarily from algae that is a faint yellow powder, and it is a very hygroscopic material. Some comparisons of the radiochromic dye, TTC, in gelatin and agar media at different concentrations to optimize dose sensitivity have been completed. We used both 8% and 10% by weight gelatin and agar; however, the 10% by weight agar samples were very firm and difficult to remove without disfiguring the gel. Halfway into the summer we realized that agar was not an ideal model for the study. It gives a very high background response is due to the very dark coloring of agar. In addition, the separation of its particles causes the light to be reflected revealing a very high background that does not account for the absorbency of distributed color in the gel. So, we proceeded to use only the gelatin media.

The gelatin samples were prepared in borosilicate glass containers (beakers) and red perspex containers. We compared the gelatin samples using both containers. In the gammacell, the dose rate has been characterized out of that in red perspex containers. Even though the dose rate in glass is unknown, the data obtained shows that the borosilicate glass containers receive more dose than the perspex containers. Also, the borosilicate glass containers are plentiful, easier to handle, and less time consuming, so we proceeded to use those containers only.

Along with studies of the gel media containers, we conducted studies of the concentration of the gelatin at 8% by weight and 10% by weight. Initially, we used only 8% by weight gelatin, but we noticed that the gels were spongy and hard to slice consistently for both 1 mm and 3 mm thickness. After experimenting with 10% by weight gelatin, we observed that the gelatin was firmer, easier to handle and slice, and provided comparable data to the 8% by weight gels.

Next, we compared 1 mm and 3 mm gelatin slices 10% by weight irradiated at 0.3 kGy, 1.2 kGy, and 2,4 kGy. As hypothesized, the 3 mm slice gave a higher distribution of absorbed color because it is thicker than the 1 mm slices yield good absorption of distributed color, but it is harder to handle and slice consistently. For the reason that if you are off one tenth of a millimeter with the 1 mm slice then that accounts for 10% error, whereas it accounts for only 3% error with the 3 mm slice.

In the following experiment, we used nine different doses for the gamma irradiated gelatin samples: 0.25, 0.5, 1.0, 1.25, 1.5, 1.75, 2.0, 2.25, and 2.5 kGy doses. With increasing dose, we observed a higher absorbency of distributed color within the gelatin medium and a shift of the maximum wavelength 520 nm to 570 nm. Analysis of the mean value absorbency at maximum wavelength 520 nm showed many fluctuations of absorbed dose predicting the 1.25 kGy dose samples to absorb almost as much color as the 2.5 kGy dose samples. These fluctuations at some doses can be attributed to slice irregularity and possibly vibrations of the spectrophotometer, and it can be improved with better sample preparation. In another experiment, we increased the molar concentration of the dye form 4 mM to 12

mM. Although the data was not shown, we observed that increasing the molar concentration of the dye does not change the results even though the gels were darker in color after irradiation.

All of the MIRF samples were irradiated at 25 MeV electrons with increasing exposure times. The exposure time is the time of irradiation measured in seconds. The gelatin samples were irradiated on MIRF at nine random exposure times: 3, 4, 7, 14, 24, 37, 61, 73, and 161 seconds. With increasing exposure time, we observed a darker and broader diameter of the beam of distributed color with the gels, and higher incidence and bragg peaks with the gelatin medium. The MIRF samples were analyzed using single-beam spectrophotometry. The samples were positioned longitudinally on a thin gold o-ring shaped border and the visible spectra was recorded from 16 mm to 48 mm within the gel. This accounts for the gel media not covered by border of the gold o-ring shaped slice holder. For the MIRF samples, the analysis of the mean value absorption maximum at 520 nm at the aperture (the position where majority of the samples yield maximum absorbency of distributed dose) shows some fluctuations between the samples irradiated for 37, 61, and 73 seconds. This can be accounted for by the sample positioning on the border or in the spectrophotometer, slice consistency, and possible vibrations of the spectrophotometer.

Using the data we obtained from the nine doses in the gammacell and the nine exposure times on MIRF, we compared the color off of the gamma cell samples to the color off of the MIRF samples on the same scaling. This is illustrated in figure 4.

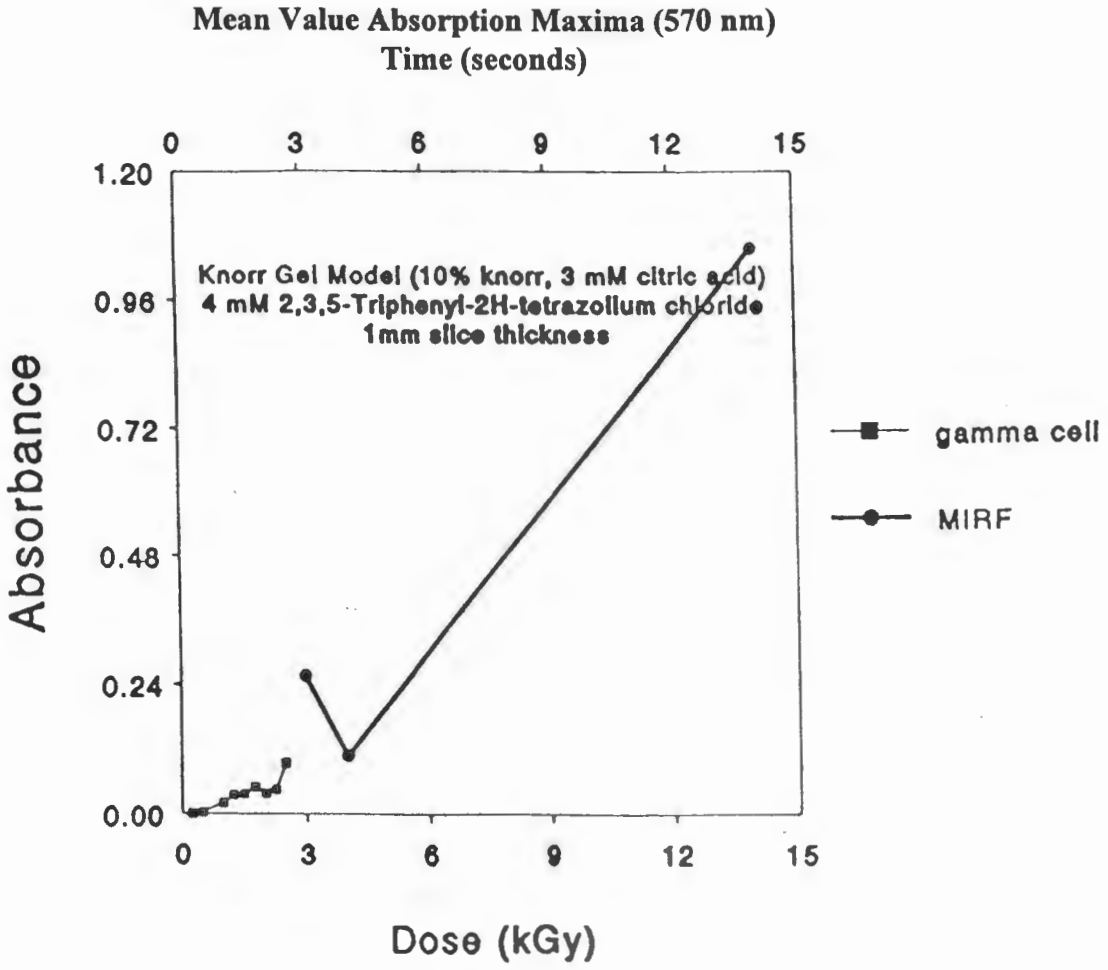

Figure 4

This was an effort to correlate the doses of the gammacell to the time of exposure at 25 MeV on MIRF to estimate the amount of dose absorbed by the MIRF irradiated samples. From the data, we observed that there was not an overlap between the MIRF irradiated samples and the gammacell samples to correlate the times of exposure at 25 MeV. For the reason that the MIRF samples received a much higher dose of irradiation than the gammacell samples.

CHAPTER V
DISCUSSIONS AND CONCLUSIONS

The results of this study are summarized as follows. For the studies in the gammacell, the mean absorbency of the radiochromic dye in acidic gelatin media at maximum absorption wavelengths is proportional to the dose and varies linearly in the dose range used. In previous studies, experiments were conducted to optimize the dye concentration within the gelatin. After optimization, they discovered that 4 mM dye produces optimum results in the acidic gelatin media. In this study, we conducted an experiment increasing the dye concentration from 4 mM to 12 mM, and we noticed that increasing the molar concentration of the dye does not change the results.

The comparisons of the radiochromic dyes in different gel matrices of Knorr gelatin and agar at different concentrations revealed gelatin to be a more suitable media for this study. The separation of the particles of the agar gel caused the light to be reflected revealing a high background that did not account for the distribution of color within the gel. After analysis, the studies of the concentration of the gels comparing 8% by weight gelatin and 10% by weight gelatin bared 10% gelatin to be the better media. Also, the comparisons of the gelatin samples irradiated in borosilicate glass containers to those gelatin samples irradiated in red perspex containers revealed the borosilicate glass containers are logistically advantageous because they receive more dose even though the dosimetry is only calculated for the perspex containers. Nine different doses were used for the gammacell. From the data, the maximum absorption wavelength shifts to the right towards the red colored spectrum of the irradiated gelatin media with increasing dose.

For the studies on MIRF, the change of absorbency in the radiochromic dye in acidic gelatin media at wavelength of maximum absorption is proportional to the energy applied and the time of irradiation (duration). With increasing exposure time to irradiation, the distribution of absorbed color increasing within the gel at the maximum absorption wavelength. At the same energy, the absorbency of the dye at the maximum absorption wavelength is proportional to the distribution of the dose, and it decreases with increasing depth in the gelatin media from the incidence peak to the bragg peak. The absorbency of the dye decreases as the distance between the site of impact of the beam and the border of the model increases with the longitudinal sections of the gels.

Future works include developing the software for the laser scanning densitometer. Slicing of the samples will not be needed; a laser scan of the gel using a computer will help to get the dose distribution.

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