CHAPTER 8

THEORETICAL MODELING FOR PHOTOBLEACHING DURING PHOTODYNAMIC THERAPY: MONTE CARLO SIMULATION

8.1 INTRODUCTION

The detailed study on the pharmacokinetics and the photodynamic effectiveness of ALA induced PpIX and trithia sapphyrin sulfonate (S3S) was discussed in the preceding chapters. However, the ALA induced endogenous PpIX is observed to be highly photolabile, which will certainly play a vital role in the PDT efficacy. Hence the understanding of the fluence distribution in tissues is mandatory to design a PDT dosimetric protocol for a better treatment efficacy.

In general, the effectiveness of photodynamic therapy was assumed to be dependent on the sensitizer concentration and the given light dose. Based on this, earlier researchers have proposed that these two parameters undergo reciprocal relation with each other to produce a desired photodynamic damage (Gibson and Hilf 1985, Grossweiner 1986, Fingar et al 1987). However, this is not validated when the administered sensitizer has the property to degrade by the photobleaching process. As optical dosimetry is clearly an important factor for successful PDT, Moan (1986a) and Potter (1987) first recognized and reported the importance of photobleaching of sensitizer in PDT dosimetry. They have shown that, if the PDT necrosis is achieved only when the amount of the toxic product (singlet oxygen) exceeds a threshold value, then the photobleaching will result in the loss of reciprocity between the treatment variables of the sensitizer concentration and the light fluence. As the sensitizer concentration is reduced due to the photobleaching, the light fluence must be increased disproportionately to achieve the same biological effect. Further it was shown that if the sensitizer concentration is below a certain minimum value, then necrosis will not be achieved regardless of the delivered light
fluence. Based on this many investigators have used a lower sensitizer concentration to exploit the differential retention of sensitizers by normal and malignant tissues to improve the photodynamic efficacy (Boyle and Potter 1987, Khan et al 1993). According to Potter et al (1987), and Boyle and Potter (1987), photobleaching of the sensitizer increases the illumination dose needed to get the same photodynamic effect in comparison with a stable sensitizer. The dependency of the light dose and the concentration for a photostable and photolabile sensitizer was explained by Rotomskis et al (1988, 1997a and b). It was also reported that the photodegradation of the sensitizer may be advantages and disadvantages depending on the initial concentration of the sensitizer and its distribution in tumor and surrounding tissues (Mang et al 1987).

This temporal decrease in the concentration of the sensitizer is normally referred to as photobleaching, due to the following process.

(i) Photodegradation - the conversion of sensitizer into products that do not absorb visible light significantly (Spikes 1992).

(ii) Photorelocalisation - the alteration of photophysical properties due to the light-induced migration of sensitizer from one binding site to another (Moan and Kessel 1988).

(iii) Phototransformation - as in case of some porphyrins, leading to the formation of new red-absorbing photoproducts (Roeder et al 1987, Rotomskis et al 1988).

Further, most of the photosensitizers such as porphyrins, chlorins, phthalocyanines and ALA induced endogeneous PpIX are not photostable both in solution as well as in complex environments. Hence, they are photo-oxidized by the incident light leading to the decrease in the concentration during the treatment procedure. In this context, the photobleaching process observed during the PDT play an important role in the optical dosimetry and the main objective of the PDT is to deliver an adequate light dose to the tumor site to produce the desired tissue necrosis.

The photodegradation of the sensitizer results in a decrease in the initial concentration and, as a consequence, a lower sensitizing effect (Rotomoskis et al 1998).
Further, it is worth to mention that, the desired necrosis could not be achieved when the sensitizer concentration is below a certain threshold concentration. Hence, the sensitizer concentration, the light dose and the time of illumination has to be optimized for an effective PDT. In addition, the exact determination of the light distribution inside the tissue will be very much useful in designing a PDT protocol. In this context, it is mandatory to know the fluence distribution of light inside the tissues under photo-bleaching conditions of sensitizer. Based on this, the present chapter was aimed to develop a theoretical model for the estimation of photon distribution in the tissues using Monte Carlo simulations, by considering the photobleaching of the sensitizer during the PDT process.

8.2 THEORETICAL MODEL FOR PHOTOBLEACHING

Light propagation in tissue has been extensively studied in the past two decades by many researchers. The fluence rate $\Phi(z)$, at a depth $z$, below the surface along the axis of the irradiating beam is described by

$$\Phi(z) = \Phi_0 K e^{-\mu_{\text{eff}} z}$$

where, $\Phi_0$ is the incident fluence, $K$ is the scalar describes the build-up of fluence just below the surface and $\mu_{\text{eff}}$ is the effective attenuation coefficient of the tissue which is equal to the inverse of the effective penetration depth $z_{\text{eff}}$ at which the fluence is reduced to 1/e of its initial value. The fluence distribution inside the tissue, represented in Equation (8.1), is dependent on the three fundamental tissue optical parameters viz., tissue absorption coefficient, $\mu_a$, in cm$^{-1}$, scattering coefficient, $\mu_s$, in cm$^{-1}$ and the anisotropy factor $g$, of the tissue. However during photodynamic therapy, a known concentration of the photosensitizer with an absorption coefficient of $\mu_a$ is injected into the tissues, which leads to the increased absorption coefficient ($\mu_{ae}$) resulting in an altered fluence distribution inside the tissue. Due to the Photobleaching process, the photosensitizer concentration is assumed to follow a single exponential decay when exposed by the light source during the PDT process, which is expressed as

$$C(\Phi) = C_a e^{-\beta \Phi}$$

(8.2)
where, \( C \) (M\(^{-1}\)) is the concentration of the sensitizer at an incident fluence of \( \Phi \) (J/cm\(^2\)), \( C_0 \) is the initial concentration of the sensitizer in the tissue and \( \beta \) (1/cm) is the photobleaching constant of the sensitizer at which the concentration of sensitizer had reduced to 1/e of its initial value.

The photodynamic dose \( D \), at the tissue surface may be defined as the product of drug concentration and total incident light dose. The definition may be generalized as

\[
D = C(\Phi) d\Phi \quad (8.3)
\]

substitution of Equation (8.2) in (8.3) and integration gives

\[
D = \frac{C_0}{\beta} \left( 1 - e^{-\beta \Phi} \right) \quad (8.4)
\]

However, the fluence described in the Equation (8.4) is dependent on the penetration depth and the tissue attenuation coefficient shown in Equation (8.1). Substituting Equation (8.1) in (8.4) gives the photodynamic dose or the absorbed dose as a function of sensitizer photobleaching and the tissue attenuation coefficient.

\[
D = \frac{C_0}{\beta} \left( 1 - e^{-\beta \Phi_{\text{abs}}(e^{-\sigma L} - 1)} \right) \quad (8.5)
\]

In general, the absorption coefficient \( \mu_{\text{abs}} \) (cm\(^{-1}\)) of the sensitizer is defined as the product of the molar extinction coefficient \( \epsilon \) (M\(^{-1}\).cm\(^{-1}\)) and the concentration of the sensitizer \( C \) (M\(^{-1}\)).

\[
\mu_{\text{abs}} = \epsilon C \quad (8.6)
\]

Hence, the decrease in the concentration of the sensitizer can be directly related to the decrease in the absorption coefficient of the photosensitizer and the Equation (8.5) can be rewritten as

\[
D = \frac{\mu_{\text{abs}}}{\beta} \left( 1 - e^{-\beta \Phi_{\text{abs}}(e^{-\sigma L} - 1)} \right) \quad (8.7)
\]
Based on this, a modified Monte Carlo algorithm has been proposed to estimate the light distribution in the tissues under the conditions of irreversible photobleaching of the sensitizer.

8.3 MONTE CARLO SIMULATION FOR IRREVERSIBLE PHOTobleACHING

A modified Monte Carlo algorithm for the irreversible photobleaching process was developed based on the simple Monte Carlo code proposed by Jacques (1998) for the photon distributions, which is based on the following assumptions:

1. The tissue layer is assumed to be an infinite plan-parallel slab with finite thickness with uniform optical properties and index of refraction. Such assumptions leads to a generalization to a layered tissues or extension to an infinitely thick tissue.

2. The air/tissue surface or other mismatched boundaries and the polarization effects are ignored.

3. The photon is launched with an initial weight of 1

In the present study three coordinate systems are used to estimate the fluence rate distribution in the tissues. Cartesian coordinate system is used to trace the photon packets in the tissues. The origin of the coordinate system is the point at which the photon is incident on the surface of the tissue. The z-axis is always the normal of the surface pointing towards the inside of the tissue and the xy-plane is therefore on the tissue surface. A cylindrical coordinate system is used to score internal photon absorption. Both the Cartesian and the cylindrical coordinate system share the same origin and the z-axis and they are represented in Figure. 8.1.

In the case of spherical coordinates, a dynamically moving coordinate system whose z-axis is aligned with the photon propagation direction is being used to trace the propagation fraction of the photon packet. Further a photon inside a turbid media is characterized by position \((x, y, z)\) and the direction cosines \((\mu_x, \mu_y, \mu_z)\). The direction cosines are the cosine of the angle that the position vector makes with the unit vectors corresponding to the coordinate axes.
The spherical coordinate system and the direction cosines of a position vector are represented in Figure 8.2 (a) and (b) respectively. Based on this, and the theory of photobleaching, the fluence rate distribution in the tissues in three different coordinate systems (spherical, cylindrical and planar) and three-irradiation geometry was estimated using the Monte Carlo simulations. The flow chart for the modified Monte Carlo code is shown in Figure 8.3, and the various steps involved in the simulations are as follows:

8.3.1 Launching a Photon

All the photons are launched at a single point and it is eventually the origin of the Cartesian coordinate system. N photons are launched, each with an initial "photon weight" set to 1. If a collimated light is incident on the tissue surface, then the photon is normally incident in to the tissue. The advantage of having the photons entering through a single point is that the results constitutes a spatial impulse response, which can be convoluted over any profile of the incident beam, thus eliminating the need for many length Monte Carlo simulations. For an isotropic source, the photons initial direction was chosen to take random direction based on the random variable generated which is distributed normally between 0 and $2\pi$ for azimuthal angle ($\psi$) and between 0 and $\pi$ for longitudinal angle ($\theta$).
Figure 8.2  (a) Spherical coordinate system for generating the scattering directions and (b) Direction cosines of a position vector

\[ \mu_x = r \cdot x \]
\[ \mu_y = r \cdot y \]
\[ \mu_z = r \cdot z \]

\( r \) is the position vector
\( x, y, z \) are the unit vectors of the axes
Figure 8.3 Flow chart of the Monte Carlo simulation for the irreversible photobleaching during PDT
8.3.2 Photon Initialization

Each "photon" is launched at a position \((x, y, z)\) which is set to the origin \((0, 0, 0)\). The initial trajectory of the photons is described by the trajectory vector \((\mu_x, \mu_y, \mu_z)\) which cites the projection of the trajectory onto the \(x, y,\) and \(z\) coordinates. In spherical coordinates, the trajectory is described by the deflection angle off the \(z\)-axis \((\theta)\) and by the azimuthal angle around the \(z\)-axis \((\psi)\).

For an isotropic point source the initial \(\theta\) value is randomly set by selecting a value for \(\cos \theta\), between -1 and 1 (corresponding to \(180^\circ\) - \(0^\circ\) with respect to the \(z\)-axis). The initial \(\zeta\) value is randomly set between 0 and \(2\pi\). The term \(\sin \theta\) is a temporary variable related to \(\cos \theta\). The values \(\cos \theta, \sin \theta,\) and \(\psi\) are used to project the trajectory vector onto the \(x, y,\) and \(z\) axes to yield the values \(\mu_x, \mu_y,\) and \(\mu_z\).

For a collimated narrow beam, the photon trajectory is assumed to be perpendicular to the tissue surface with the deflection angle \((\theta)\) and the azimuth angle \((\psi)\) at \(0^\circ\), resulting in a trajectory vector \(\mu_x=\mu_y=0\) and \(\mu_z=1\) respectively.

For a collimated beam of diameter \(a\), the position of the launch should be varied in a random manner so that a uniform spatial distribution of photon launching is achieved. Let the beam have a radius, \(a\). The probability of launching at a radial position \(r\) is \(p(r)\) and is given by

\[
p(r) = \frac{2\pi r}{ma^2} = \frac{2\pi}{a^2}
\]

such that

\[
\int_0^a p(r)dr = 1
\]

The Monte Carlo method for selecting \(r\) from \(p(r)\) using a random number, \(\zeta\), uniformly distributed between 0 and 1, inclusively, is:

\[
\zeta = \int_0^r p(r)dr = \frac{r^2}{a^2}
\]
Rearranging the above equation to solve for $r$ as a function of $\zeta$. The resulting expression will allow selection of the radial position $r$ for launching a photon based on a random number $\zeta$

$$r = a\sqrt{\zeta} \quad (8.11)$$

Photons will be launched uniformly in the $x$-$y$ plane within the beam radius, $a$, and the chosen radial magnitude, $r$. Now, the angle $\phi$ is chosen such that the radial coordinates $(r, \phi)$ define the launch point. By choosing $\phi$ relative to the $x$-axis, it is specified by second random number

$$\phi = 2\pi\zeta \quad (8.12)$$

Now the positions $x$ and $y$ are chosen based on $r$ and $\phi$:

$$x = r\cos \phi \quad (8.13)$$

$$y = r\sin \phi \quad (8.14)$$

### 8.3.3 Generating the Step size

The step size, $\Delta s$, is chosen such that the photon takes a step of variable length determined by sampling the exponential probability density function for the stepsize ($s$) before the photon interacts with the tissue by the combination of absorption and scattering,

$$p(s) = \mu_a e^{-\mu_a s} \quad (8.15)$$

where $\mu_t = \mu_a + \mu_s$ and $1/\mu_t$ represents the mean free path between photon-tissue interaction sites. The above Equation (8.15) is based on the Lambert Beer’s law, which states that the probability of a photon moving a large distance between two interaction events is exponentially smaller than it taking a smaller distance.

Hence the stepsize of the photon, $s$, is calculated based on a random sampling of the probability density function for $s$ corresponding to the random variable, $\zeta$, generated by the random number generator. Based on this the sampling of $s$, is given by,
The stepsize, $s$, determined using Equation (8.16) represents the distance that a photon will travel before interacting (through absorption or scattering) with the tissue.

8.3.4 Moving the photon

After setting the photon stepsize, $s$, the photon is ready to be moved in the tissue. The current position of the photon is specified by $(x,y,z)$. The current trajectory of the photon is specified by a unit vector, $r$, which is characterized by the direction cosines $(\mu_x, \mu_y, \mu_z)$ (Witt)

$$\mu_x = r.x \quad (8.17)$$
$$\mu_y = r.y \quad (8.18)$$
$$\mu_z = r.z \quad (8.19)$$

where $x,y,z$ are unit vectors along each axis. For a photon located at $(x,y,z)$ travelling a distance $\Delta S$ in the direction $(\mu_x, \mu_y, \mu_z)$, the new coordinates $(x', y', z')$ are given by

$$x' = x + \mu_x \Delta S \quad (8.20)$$
$$y' = y + \mu_y \Delta S \quad (8.21)$$
$$z' = z + \mu_z \Delta S \quad (8.22)$$

8.3.5 Photon absorption

After each propagation step, the photon packet is split into two parts – a fraction is absorbed and the rest is scattered. The fraction of the packet that is absorbed is

$$\text{Fraction absorbed} = \frac{\mu_s}{\mu_s + \mu_t} = 1 - \frac{\mu_t}{\mu_s + \mu_t} = (1 - \alpha) \quad (8.23)$$

where $\alpha$ is the single particle albedo which equals the fractional probability of being scattered and $1-\alpha$ equals the fraction probability of being absorbed. To determine whether the photon is absorbed or scattered a random number uniformly distributed between 0 and 1 is generated. If the generated random number is less than the probability of absorption $p(\alpha)$ then the photon is treated to be absorbed. So a fraction of the current photon weight absorbed is
\[ N^* = W \cdot (1 - \alpha) \]  

where \( N^* \) is the \( n^{th} \) increment in the stepsize. Hence the current photon weight is reduced to \( W = W - N^* \), and the updated photon weight is expressed as

\[ W' = W - W \left( 1 - \frac{\mu_s}{\mu_t} \right) \]  

\[ W' = W \left( \frac{\mu_s}{\mu_t} \right) \]  

where, \( W' \) is the change in the photon weight after the absorption process, \( \mu = \mu_a + \mu_s \) is the total attenuation coefficient of the tissue. In the present study the total absorption coefficient, \( \mu_a \), was assumed as the sum of the absorption coefficient of the tissue and the injected sensitizer. Hence,

\[ \mu_a = \mu_{at} + \mu_{as} \]  

where, \( \mu_{at} \) and \( \mu_{as} \) are the absorption coefficient of the tissue and the sensitizer respectively.

As the tissue absorption coefficient (\( \mu_{at} \)) and the scattering coefficient (\( \mu_s \)) will remain constant during the PDT process, the change in the weight of the incident photon during its propagation is very much influenced by the change in the sensitizer concentration due to the photobleaching process. Further, it is worth to mention that the sensitizer absorption coefficient is proportional to the concentration of senistizer, which is given by

\[ \mu_{as} = \varepsilon C \]  

where \( \varepsilon \) is the molar extinction coefficient of the sensitizer, which is a constant for a particular wavelength of interest. Hence, the change in the concentration of the sensitizer by the photobleaching process is assumed to influence sensitizer absorption coefficient in a proportionate manner.

Based on this, the fraction of photons absorbed is calculated at each depth by considering the photobleaching of the sensitizer, using the following relation
where $\mu_m(z)$ is the photosensitizer absorption coefficient at depth $z$ prior to the delivery of the $n$th fluence increment and $\Delta W'(z)$ is the fluence increment at depth $z$. This equation accounts for the photobleaching during the fluence or photon increment and it reduces to the simple product $\mu_m(z) \Delta W'(z)$ for very small increments.

After all $N$ photons have been propagated, each bin array, $A[ir]$, contains an accumulated weight of absorbed photons, where $ir$ denotes the index number indicating the distance from the source to the bin. Dividing each bin array by the total number of photons ($N$) and by the volume of that particular bin ($V[ir]$) yields the concentration $C[ir]$ [cm$^{-3}$] of absorbed photon:

$$C[ir] = A[ir]/(N \cdot V[ir])$$  \hfill (8.30)

Dividing $C[ir]$ by the absorption coefficient $\mu_a$ [cm$^{-1}$] yields the relative fluence rate $F[ir] = C[ir]/\mu_a$.

From the estimated values of the fluence rate, the photosensitizer concentration (proportional to the sensitizer absorption coefficient) at each depth is updated according to the following expression,

$$\mu_m^{*+}(z) = \mu_m(z) e^{-\Delta W'(z) \beta}$$  \hfill (8.31)

### 8.3.6 Changing the Photon direction - Scattering

Once the photon has been moved and its weight decremented, the photon is ready to be scattered. If the anisotropy, $g$, equals 0, then the scattering is isotropic. If $g > 0$, then the program uses the Henyey-Greenstein scattering function originally introduced by Henyey and Greenstein to mimic the scattering of light from distant stars by galactic dust. The same function approximately mimics the scattering function experimentally observed in biological tissues.
The scattering is accompanied by the change in the direction of the incident photon. In order to find the new direction of propagation, a spherical coordinate system was used. Figure 8.3(a) shows the azimuthal ($\psi$) and the deflection angle ($\theta$) of a dynamically varying spherical coordinate system. A normalized phase function, $p(\theta)$, describes the probability density function for the azimuthal and longitudinal angles for a photon when it is scattered. If the phase function has no azimuthal dependence, then the azimuthal angle $\psi$ is uniformly distributed between 0 and $2\pi$, and may be generated by multiplying a pseudo-random number, $\xi$, uniformly distributed over the interval 0 to 1 by $2\pi$ (i.e., $\psi = 2\pi \xi$). The azimuthal angle $\psi$ for an isotropic distribution is given by

$$\cos \theta = 2\xi - 1, \quad \text{for } g=0 \quad (8.32)$$

Since scattering in tissue is characterized by the Henyey-Greenstein function (Witt 1977), the generating function of the Henyey-Greenstein phase function (Jacques et al 1987) is given by

$$\cos \theta = \frac{1}{2g}\left[1 + g^2 - \left(\frac{1-g^2}{1-g+2g\phi}\right)^2\right] \quad \text{for } g>0 \quad (8.33)$$

The azimuthal angle $\psi$, which is uniformly distributed over the interval 0 to $2\pi$ is given by

$$\psi = 2\pi \phi \quad (8.34)$$

If a photon is scattered at an angle ($\theta, \psi$) from the direction ($\mu_o, \mu_s, \mu_e$) in which it is traveling, then the new direction ($\mu'_o, \mu'_s, \mu'_e$) is specified by

$$\mu'_o = \frac{\sin \theta}{\sqrt{1-\mu'_e^2}} \left(\mu_s \cos \psi - \mu_e \sin \psi\right) + \mu_e \cos \theta \quad (8.35)$$

$$\mu'_s = \frac{\sin \theta}{\sqrt{1-\mu'_e^2}} \left(\mu_s \cos \psi + \mu_e \sin \psi\right) + \mu_e \cos \theta \quad (8.36)$$

$$\mu'_e = -\sin \theta \cdot \cos \psi \cdot \sqrt{1-\mu'_e^2} + \mu_e \cos \theta \quad (8.37)$$
If the angle is too close to the normal (say $|\mu_z| > 0.99999$), the following equations should be used to obtain the new photon direction

$$\mu'_x = \sin \theta \cdot \cos \psi$$  \hspace{1cm} (8.38) \\
$$\mu'_y = \sin \theta \cdot \sin \psi$$  \hspace{1cm} (8.39) \\
$$\mu'_z = \frac{\mu_z \cdot \cos \psi}{|\mu_z|}$$  \hspace{1cm} (8.40)

However, in the present chapter Equations (8.23), (8.24) and (8.25) are used for changing the photon direction by the scattering event.

### 8.3.7 Photon termination

After passing through many scattering and absorption events, the photon will lose its weight and it will never reach zero. Propagating a photon with a minimum weight yields little information. Absorbing or discarding all the remaining weight, after the weight falls below a minimum, skews the absorption profile or violates energy conservation respectively. A technique called roulette is used to terminate a photon once its weight drops below a specified minimum $W \leq W_{\text{threshold}}$. The roulette technique gives such a photon (with weight $W$) one chance in $m$ of surviving with a weight $m(W)$ or else its weight is reduced to zero. The photon weight is updated using the generated random number, $\zeta$, in the interval $(0,1)$ according to the following decision

- if $\zeta \leq 1/m$ then $W \leftarrow mW$
- if $\zeta > 1/m$ then $W = 0$

Thus the photon is killed in an unbiased fashion, without sacrificing energy conservation and without continuing propagation until its weight has reached zero.

### 8.4 SIMULATION RESULTS

The tissue optical properties $\mu_{at} = 1.2 \text{ mm}^{-1}$, $\mu_a = 24.0 \text{ mm}^{-1}$ and $g=0.95$ are considered for the present study from the reported data (Qu et al., 1994). The total absorption coefficient of the tissues prior to the PDT exposure is the sum of the tissue
absorption coefficient ($\mu_{\text{at}}$) and the photosensitizer absorption coefficient ($\mu_{\text{at}}$) at the wavelength of irradiation. In the present study three combinations of the $\mu_{\text{at}}$ and $\mu_{\text{at}}$ were employed to determine the photon transport in the tissues under the photobleaching conditions. The combinations were chosen such that

i. the sensitizer absorption coefficient is comparable to the tissue absorption coefficient ($\mu_{\text{at}} = \mu_{\text{at}}$).

ii. the sensitizer absorption coefficient is 10 fold lesser than the tissue absorption coefficient ($\mu_{\text{at}} < \mu_{\text{at}}$).

iii. The sensitizer absorption coefficient is 10 fold higher than the tissue absorption coefficient ($\mu_{\text{at}} > \mu_{\text{at}}$).

The number of photons ($N_{\text{ph}}$) set for each simulation was 100,000 and the simulations were run using a Pentium III, 650 MHz processor.

The contour of relative photon distribution along the depth of the tissue, for different irradiation geometry (normal incidence, isotropic incidence, normal incidence from a finite diameter source) and for different ratio of tissue to sensitizer absorption coefficient are shown in Figures 8.4 to 8.6 respectively. The columns in the Figures 8.4-8.6 represents the contour plots at different photobleaching rates of the photosensitizer (viz., 0, 0.5, 5, 10 and 20 cm$^2$ J$^{-1}$) and the rows represent the different tissue to sensitizer absorption coefficient ratio (1:1; 0.1:1 and 1:10) respectively. Figure 8.1 shows the contour plots of the relative fluence in the tissues when the incident beam is normal to the surface of the tissue. When the tissue absorption coefficient and the sensitizer absorption coefficient are comparable or equal to each other, the fluence distribution is relatively less for a highly photostable sensitizer ($\beta=0$ cm$^2$ J$^{-1}$). However, under photobleaching conditions ($\beta=0.005, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0$ and $40.0$ cm$^2$ J$^{-1}$), it is observed that the relative fluence distribution is increased. With further increase in the photobleaching rate, the fluence distribution increased to a considerable level and at bleaching rates above 5.0 cm$^2$ J$^{-1}$, the profile of the fluence rate distribution is almost similar. Similar Monte Carlo simulations were run for the conditions where, the sensitizer absorption coefficient is assumed to be 10 fold lesser than the tissue absorption coefficient ($\mu_{\text{at}}$). Since the sensitizer
Figure 8.4 Contour plot of relative fluence rate distribution in tissues (2cm x 2cm), for normal incidence from a point source at different photobleaching rates.
Figure 8.5 Contour plot of relative fluence rate distribution in tissues (2cm x 2cm), for isotropic incidence from a point source at different photobleaching rates.
Figure 8.6 Contour plot of relative fluence rate distribution in tissues (2 cm x 2 cm), for normal incidence from a finite diameter source at different photobleaching rates.
absorption coefficient ($\mu_{at}$) is very low when compared to the tissue absorption coefficient, the variation in photobleaching rate does not influence the relative fluence rate distribution in tissue even at higher bleaching rate. However, when the sensitizer absorption coefficient is assumed to be 10 fold higher than the tissue absorption coefficient, the relative fluence distribution in tissue is very superficial for $\beta=0$ cm$^2$ J$^{-1}$, and it increased with the increase in bleaching rate. Though a marked difference is observed between a bleaching rate of 0.0 cm$^2$ J$^{-1}$ and 0.005 cm$^2$ J$^{-1}$, the contours of fluence rate distributions are not considerably different at bleaching rate above 5.0 cm$^2$ J$^{-1}$.

Similar fluence rate distributions are observed in the case of isotropic incidence. However, the fluence distributions does not penetrate beyond 1cm because of the deflection angle ($\theta$) and the azimuthal angle ($\zeta$) along the z-axis. However, in this case the fluence rate distribution is wider near the surface because of the isotropic nature of the irradiation source.

Further to understand the light transport in the tissues and its dependency on the variation in sensitizer concentration due to photirradiation, graphs were drawn between

i. the sensitizer absorption coefficient (concentration) Vs depth and

ii. the relative fluence rate Vs depth under different photobleaching conditions under the following irradiation geometry.

8.4.1 Normal Incidence from a point source

The variation in the fluence rate distribution and the sensitizer absorption coefficient along the depth (z-axis) of tissue under different photobleaching conditions and at different tissue to sensitizer absorption coefficient ($\mu_{at} = \mu_{ab} \cdot \mu_{at} < \mu_{ab} \cdot \mu_{at} > \mu_{at}$), for normal incidence from a point source in planar (1-D), cylindrical (2-D) and spherical (3-D) coordinates are shown in Figures 8.7-8.9 respectively. The column-1 in Figure 8.7-8.9 shows fluence rate distribution per unit area under different bleaching conditions for different sensitizer to tissue absorption coefficient ratios (1:1, 0.1:1, 1:10), and the column-2 represents the corresponding variation in the sensitizer absorption coefficient (concentration) respectively.
Figure 8.7  Relative fluence rate and sensitizer concentration (in planar coordinates) for a normal incidence from a point source at different bleaching rates for 
\( \mu_{as}=\mu_{at} \) (a&b), \( \mu_{as}<\mu_{at} \) (c&d), \( \mu_{as}>\mu_{at} \) (e&f)
Figure 8.8 Relative fluence rate and sensitizing concentration (in cylindrical coordinates) for a normal incidence from a point source at different bleaching rates for $\mu_{at} < \mu_{sat}$ (a&b), $\mu_{at} < \mu_{sat}$ (c&d), $\mu_{at} > \mu_{sat}$ (e&f)
Figure 8.9 Relative fluence rate and sensitizer concentration (in spherical coordinates) for a normal incidence from a point source at different bleaching rates for $\mu_{\text{ref}} = \mu_{\text{ref}}$ (a&b), $\mu_{\text{ref}} < \mu_{\text{ref}}$ (c&d), $\mu_{\text{ref}} > \mu_{\text{ref}}$ (e&f)
In planar coordinates (1-D), when $\mu_0 = \mu_\text{tot}$ and $\beta = 0$ (photostable), the relative fluence rate decreases with the increasing depth for normal incidence from a point source. However, with a bleaching rate of 0.005 cm$^2$ J$^{-1}$, the relative fluence rate near the surface increased to 1.6 fold with respect to that observed with $\beta = 0$ cm$^2$ J$^{-1}$ and decreased gradually with increasing depth. Similar trend of the fluence rate was observed for simulations with different bleaching rate. With increasing bleaching rate, the relative fluence rate near the surface decreased gradually with respect to the bleaching rate of $\beta = 0.005$ cm$^2$ J$^{-1}$ and at higher bleaching rate ($\beta = 40$ cm$^2$ J$^{-1}$) the fluence rate reached almost the value corresponding to the photostable sensitizer ($\beta = 0$ cm$^2$ J$^{-1}$). However, an isosebestic point is observed at a depth of 0.3 cm, at which the relative fluence rate values are the same irrespective of the different bleaching rate. Beyond the isosebestic point, the fluence rate distribution for the sensitizer with low bleaching rate showed lower values when compared to that of high bleaching rate, with very minimal variation near the surface (Figure 8.7-a). When $\mu_0 < \mu_\text{tot}$, the relative fluence rate distribution decreased with increasing depth and it followed the same trend for all the $\beta$ values (Figure 8.7-c). However for $\mu_0 > \mu_\text{tot}$, a marked difference in the relative fluence rate as a function of depth was observed. When the bleaching rate is very minimal of the order of 0.005 cm$^2$ J$^{-1}$ the fluence rate near the surface increased to 5 fold with respect to a photostable sensitizer ($\beta = 0$ cm$^2$ J$^{-1}$). Similar to the observations under the condition $\mu_0 = \mu_\text{tot}$, isosebestic point was observed. But two isosebestic points was observed in this case with one at 0.15 cm corresponding to the low bleaching rates ($\beta = 0.005$ to 2.0 cm$^2$ J$^{-1}$) and the other at 0.3 cm corresponding to high bleaching rates ($\beta = 3.0$ to 40.0 cm$^2$ J$^{-1}$) (Figure 8.7-e). The corresponding variation in the sensitizer absorption coefficient at different sensitizer to tissue absorption coefficient is shown in Figures 8.7 (b),(d) and (f) respectively.

The relative fluence rate distribution for a normal incidence from a point source, in cylindrical coordinates (2-D) also decreased with increasing depth in the tissue. When $\mu_0 = \mu_\text{tot}$, the relative fluence rate distribution is more for a highly photolabile sensitizer ($\beta = 40.0$ cm$^2$ J$^{-1}$) at deeper layers. However when $\mu_0 < \mu_\text{tot}$, no significant different could be observed under different photobleaching conditions. At higher values of $\mu_0$, i.e., $(\mu_0 > \mu_\text{tot})$, a marked difference is observed in the fluence rate distribution along the depth of the tissue. The fluence rate
distribution near the surface increased for a photolabile sensitizer with respect to a photostable sensitizer. Similar to the observation in planar coordinates, the fluence rate distribution near the surface decreased with increasing photobleaching rate and at deeper layers the relative fluence rate for a highly photolabile sensitizer ($\beta=40.0 \text{ cm}^2 \text{ J}^{-1}$) is higher than that for the photostable sensitizer ($\beta=0.0 \text{ cm}^2 \text{ J}^{-1}$). Two isosebestic points was observed with one at 0.15 cm corresponding to the low bleaching rates ($\beta=0.005$ to 2.0 cm$^2$ J$^{-1}$) and the other at 0.3 cm corresponding to high bleaching rates ($\beta=3.0$ to 40.0 cm$^2$ J$^{-1}$) (Figure 8.8(e)). The variation in the sensitizer absorption coefficient at different ratio of sensitizer to tissue absorption coefficient showed a marked difference with respect to that observed in the case of planar coordinates. In spherical coordinates (3-D), an almost similar result with that of cylindrical coordinate was observed.

8.4.2 Isotropic incidence from a point source

For an isotropic point source, the variation in the fluence rate distribution and the sensitizer absorption coefficient along the depth of the tissue under different photobleaching conditions and at different tissue to sensitizer absorption coefficient ($\mu_{at}=\mu_{as}$, $\mu_{at}<\mu_{as}$ and $\mu_{at}>\mu_{as}$), in planar (1-D), cylindrical (2-D) and spherical (3-D) coordinates are shown in Figures 8.10-8.12 respectively. In planar coordinates, when $\mu_{at}=\mu_{as}$, the relative fluence decreased almost like an exponential decay curve. With the increasing bleaching rate, the relative fluence rates are observed to be higher at deeper layers with respect to a photostable sensitizer (Figure 8.10-a). Similar trend was observed for $\mu_{at}<\mu_{as}$, but no considerable differences were observed under different photobleaching conditions (Figure 8.8-c). At $\mu_{at}>\mu_{as}$, the observations are similar to that of the normal incidence (Figure 8.10-e). The corresponding variations in the sensitizer absorption coefficient under different photobleaching conditions are shown in Figures 8.10 (b), (d) and (f) respectively. The relative fluence rate distribution and the variation in the sensitizer absorption coefficient, in cylindrical and spherical coordinates showed a similar profile as observed in the case of normal incidence from a point source.

8.4.3 Normal incidence from a finite beam diameter source

For an irradiation source geometry of finite diameter with normal incidence, the variation in the fluence rate distribution and the sensitizer absorption coefficient along the depth
Figure 8.10 Relative fluence rate and sensitizer concentration (in planar coordinates) for an isotropic point source at different bleaching rates for $\mu_{st}=\mu_{at}$ (a&b), $\mu_{st}<\mu_{at}$ (c&d), $\mu_{st} > \mu_{at}$ (e&f).
Figure 8.11 Relative fluence rate and sensitizer concentration (in cylindrical coordinates) for an isotropic point source at different bleaching rates for $\mu_{sat} = \mu_{at}$ (a&b), $\mu_{sat} < \mu_{at}$ (c&d), $\mu_{sat} > \mu_{at}$ (e&f)
Figure 8.12 Relative fluence rate and sensitizer concentration (in spherical coordinates) for an isotropic point source at different bleaching rates for $\mu_{as} = \mu_{at}$ (a&b), $\mu_{as} < \mu_{at}$ (c&d), $\mu_{as} > \mu_{at}$ (e&f)
of the tissue under different photobleaching conditions and at different tissue to sensitizer absorption coefficient ($\mu_{at}=\mu_{ab}$, $\mu_{at}<\mu_{ab}$, $\mu_{at}>\mu_{at}$) in planar (1-D), cylindrical (2-D) and spherical (3-D) coordinates are shown in Figures 8.13-8.15 respectively. The relative fluence rate distribution and the variation in the sensitizer absorption coefficient in planar coordinates (Figure 8.14 (a)-(f)), for the normal incidence with a finite diameter source (2mm) displayed a similar profile as observed in the case of normal and isotropic incidence from a point source. In cylindrical and spherical coordinates, the fluence rate distribution was same for a depth up to 2 mm and then it starts decreasing with increasing depth. However, it is observed that at deeper layers, the relative fluence rate distribution for a highly photolabile sensitizer ($\beta=40.0$ cm$^2$ J$^{-1}$) is more when compared to a photostable sensitizer ($\beta=0.0$ cm$^2$ J$^{-1}$), as in the case of normal and isotropic incidence from a point source. The variation in the sensitizer absorption coefficient under different photobleaching conditions showed a steady value up to 2 mm and varied with further increase in the depth ($\beta=40.0$ cm$^2$ J$^{-1}$)

8.4.4 Number of photons absorbed by the sensitizer

When the exposure to the tissue is assumed as 1 second, the simulated relative fluence rate per watt of the incident power will be the fluence distribution (J/cm$^2$). For a specific wavelength of irradiation, the number photons per joule of energy delivered is calculated using the relation $N_{ph} = \frac{\lambda}{hC_0}$, where $\lambda$ is the wavelength of the laser beam in nm, h is the Plank’s constant (6.626 x 10$^{-34}$ Js) and $C_0$ is the speed of the light (2.998 x 10$^8$ m/s) (Jacques 1999). Hence the number of photons per joule of energy delivered for 630 nm irradiation is estimated as $N_{ph(630)} = 3.171 x 10^{18}$. With this calculated value, the number of photons absorbed by the sensitizer per unit volume (cm$^{-3}$) is calculated as

$$\text{Photons absorbed by sensitizer /cm}^3 = \text{Relative fluence (} \text{cm}^{-2} \text{)} \times N_{ph} \times \mu_{at} \text{ (cm}^{-1}$$

The calculated values of the number of photons absorbed by sensitizer when $\mu_{at}=\mu_{ab}$, $\mu_{at}<\mu_{at}$ and $\mu_{at}>\mu_{at}$, for normal incidence from a point source, in spherical, cylindrical and planar geometry are shown in Figure 8.16(a-c),(d-f) and (g-i) respectively.
Figure 8.13 Relative fluence rate and sensitizer concentration (planar coordinates) for an finite diameter source at different bleaching rates for $\mu_{at} = \mu_{at}$ (a&b), $\mu_{at} < \mu_{at}$ (c&d), $\mu_{at} > \mu_{at}$ (e&f)
Figure 8.14 Relative fluence rate and sensitizer concentration (cylindrical coordinates) for an finite diameter source at different bleaching rates for $\mu_{at} = \mu_{at}$ (a&b), $\mu_{at} < \mu_{at}$ (c&d), $\mu_{at} > \mu_{at}$ (e&f)
Figure 8.15  Relative fluence rate and sensitizer concentration (spherical coordinates) for an finite diameter source at different bleaching rates for $\mu_{as}=\mu_{at}$ (a&b), $\mu_{as}<\mu_{at}$ (c&d), $\mu_{as}>\mu_{at}$ (e&f)
Figure 8.16 Number of photons absorbed by the sensitizer after normal incidence from a point source under different bleaching conditions, for $\mu_0 = \mu_{at}$, $\mu_0 < \mu_{at}$, $\mu_0 > \mu_{at}$ in (a,b,c) spherical, (d,e,f) cylindrical and (g,h,i) planar coordinates.
For the normal incidence from a point source, the number of photons absorbed by a photostable sensitizer showed a gradual decrease with the increasing depth, with almost a similar profile when $\mu_{at}=\mu_{at}$, $\mu_{at}<\mu_{at}$ and $\mu_{at}>\mu_{at}$ (Figure 8.16(a-c)). In the case of photolabile sensitizer, the number of photons absorbed by the sensitizer is minimum near the surface of the tissues with respect to the photostable sensitizer. With the increasing photobleaching rate a considerable number of photons are absorbed only beyond a particular depth. In spherical and cylindrical coordinates, the number of photons absorbed by the sensitizer under various photobleaching conditions and at various sensitizer to tissues absorption coefficient is almost the same with very minimal difference between them. Further it is observed that the number of photons absorbed by the sensitizer is very negligible for photolabile sensitizer with a bleaching rate of 40.0 J cm$^{-2}$, up to a depth of 2.5 cm and 2.0 cm for spherical and cylindrical coordinates respectively. However, it is interesting to note that the number of photons absorbed by the photolabile sensitizer increases with depth and it is almost equivalent at higher depths beyond 1 cm. In the case of $\mu_{at}>\mu_{at}$ the number of photons absorbed by the sensitizer is higher for the photolabile sensizers than that of the photostable sensitizer at depth > 1 cm. In planar geometry, the number of photons absorbed increased gradually and at depths greater than 1.5 it is almost equal for all the bleaching rates, for the conditions of $\mu_{at}=\mu_{at}$ and $\mu_{at}<\mu_{at}$. An almost similar trend was observed both the cases except that the number of photons absorbed is relatively less in the case of $\mu_{at}>\mu_{at}$ when compared to $\mu_{at}=\mu_{at}$ (Figure 8.16 (g) and (h)). However, when $\mu_{at} > \mu_{at}$, the number of photons absorbed by the sensitizer was higher near the surface for $\beta=0.005, 0.5$ and 1.0 cm$^2$ J$^{-1}$ respectively, when compared to a photostable sensitizer ($\beta=0$ cm$^2$ J$^{-1}$) and it decreased gradually with the increasing depth (Figure 8.17(i)). Further it is observed that the number of photons absorbed are considerably less for these bleaching rates when compared to $\beta=0$ cm$^2$ J$^{-1}$ beyond the depth of 0.2 cm. With further increase in bleaching rate ($\beta=5, 10, 20$ and 40 cm$^2$ J$^{-1}$) the number of photons absorbed by the sensitizer near the surface is less with respect to $\beta=0$ cm$^2$ J$^{-1}$. It is interesting to note that the number of photons absorbed by the sensitizer increased up to a certain depth (depending on the bleaching rate) and decreased gradually for $\beta=5, 10, 20$ and 40 cm$^2$ J$^{-1}$.

The number of photons absorbed by sensitizer for an isotropic incidence from a point source, under the conditions $\mu_{at}=\mu_{at}$, $\mu_{at}<\mu_{at}$ and $\mu_{at}>\mu_{at}$ in spherical, cylindrical and planar
geometry are shown in Figure 8.17(a-c), (d-f) and (g-i) respectively. The number of photons absorbed by the sensitizer in spherical and cylindrical coordinates for $\mu_a=\mu_{ab}$ $\mu_a<\mu_{at}$ and $\mu_a>\mu_{at}$ are the same when compared to that observed in the case of normal incidence from a point source. However, a considerable difference was observed in planar coordinates for $\mu_a=\mu_{ab}$ $\mu_a<\mu_{at}$. In planar coordinates, the number of photons absorbed by the photolabile sensitizer increased, with increasing depth, in a steeper manner when compared to a gradual increase observed for normal incidence from a point source. At $\mu_a>\mu_{at}$ the number of photons absorbed by the sensitzers under different photobleaching conditions are similar to that observed for a normal incidence from a point source.

Similarly, the number of photons absorbed by the sensitizer when irradiated with a finite diameter beam, for $\mu_a=\mu_{ab}$ $\mu_a<\mu_{at}$ and $\mu_a>\mu_{at}$ in spherical, cylindrical and planar coordinates are shown in Figure 8.18 (a-c), (d-f) and (g-i) respectively. In planar coordinates, the observations are similar to that of the results observed with normal and isotropic incidence from a point source. In spherical and cylindrical coordinates, the number of photons absorbed by the sensitizer under various photobleaching conditions and at different sensitizer to tissues absorption coefficient does not vary markedly up to a depth of 2 mm (diameter of the irradiation source). At depths greater than 2 mm, the number of photons absorbed increased and then decreases with further increase in the depth. When $\mu_a>\mu_{at}$ the number of photons absorbed by the photolabile sensitizer is more at deeper layers of the tissue for spherical and cylindrical coordinates. In cylindrical coordinates under the condition $\mu_a>\mu_{at}$ the number of photons absorbed by the photolabile sensitizer does not decrease considerably near the surface (Figure 8.17(f)) as observed in spherical coordinates. This observed difference might be due to the very high absorption coefficient of the sensitizer when compared to the tissue absorption coefficient.

8.5 DISCUSSION

The influence of photobleaching rate on the relative fluence distribution in tissues was studied and analyzed based on the proposed theoretical model for photobleaching during PDT, using Monte Carlo simulations. From the results, it is clear that the relative fluence rate distribution is very much dependent on the sensitizer concentration (absorption coefficient) in
Figure 8.17 Number of photons absorbed by the sensitizer after isotropic incidence from a point source under different bleaching conditions, for $\mu_{as}=\mu_{at}$, $\mu_{as}<\mu_{at}$, $\mu_{as}>\mu_{at}$ in (a,b,c) spherical, (d,e,f) cylindrical and (g,h,i) planar coordinates.
Figure 8.18 Number of photons absorbed by the sensitizer after normal incidence from a finite diameter source under different bleaching conditions, for $\mu_{\text{sat}} = \mu_{\text{at}}$, $\mu_{\text{sat}} < \mu_{\text{at}}$, $\mu_{\text{sat}} > \mu_{\text{at}}$ in (a,b,c) spherical, (d,e,f) cylindrical and (g,h,i) planar coordinates.
tissues for all the three irradiation geometry (normal incidence from a point source, isotropic point source and normal incidence from a finite beam diameter).

In the case of normal incidence from a point source under the condition $\mu_{as}=\mu_{at}$, $\mu_{as}<\mu_{at}$, $\mu_{as}>\mu_{at}$, the relative fluence rate distribution showed some variations near the surface with an increased fluence rate for lower photobleaching rate, in all the three coordinates (spherical, cylindrical and planar). This increase in the fluence rate near the surface for lower photobleaching rate may be attributed to the variation in sensitizer concentration, resulting in the net variation in absorption coefficient ($\mu_{as}+\mu_{at}$). With increase in bleaching rate the relative fluence rate near the surface decreased with respect to that of the distribution for $\beta=40.0$ cm$^2$ J$^{-1}$. This observed decrease in relative fluence may be due to the fact that at higher bleaching rate the number of photons required to produce photobleaching will be less and hence the fluence rate distribution at the point of interest (near the surface) will be less when compared to very low photobleaching rate of $\beta=0.005$ cm$^2$ J$^{-1}$. Further it is observed that, at comparable tissue and sensitizer absorption coefficients, there is a critical depth of 0.3 cm, at which the relative fluence rate is equal in planar coordinates, irrespective of the photobleaching rate. The observation of the isosebestic points clearly indicates that for very superficial lesions the bleaching of the sensitizer is favorable because of the increased fluence rate distribution below 0.3 cm. For deep lesions beyond 0.3 cm, the fluence rate distribution for the photostable sensitizer and the photolabile sensitizer with high bleaching rate may be considered favorable, for the parameters used in the present study.

Similar, results were obtained for isotropic incidence from a point source and normal incidence from a finite diameter source, indicating that along the depth of interest, the change in the concentration of sensitizer is independent of the diameter of irradiation source, in planar coordinates. However one could observe some difference in the variation in absorption coefficient of the sensitizer at various photobleaching conditions. In the case of spherical and cylindrical coordinates, the fluence rate distribution for normal incidence from a finite diameter beam showed similar trend beyond the depth of 2.0 mm. The equal values of the relative fluence rate up to 2.0 mm indicates that, irradiation with a finite diameter source will be more ideal for the treatment of superficial lesions depending on the diameter of irradiation source.
In spite of the striking similarity observed between three coordinates and the irradiation geometry, there is marked difference observed under the conditions \( \mu_{at}=\mu_{at}, \mu_{ar}<\mu_{at}, \mu_{ar}>\mu_{at} \), indicating that the fluence rate distribution in tissues is very much dependent on sensitizer absorption coefficient. Further it is worth to mention that similar trend was observed for all three irradiation geometry at higher absorption coefficient of the sensitizer. This clearly indicates that the light transport in tissues is independent of irradiation geometry when the sensitizer absorption coefficient (concentration) is very high when compared to tissue absorption coefficient, in planar coordinates. The simulation results also suggest that at higher sensitizer absorption coefficient and photobleaching rate, the fluence rate distribution is relatively higher at deeper layers of tissues, which may be utilized effectively for PDT Dosimetry.

In PDT, the depth of necrosis is dependent on threshold number of photons required to produce the damage. Based on this assumption, the number of photons absorbed by sensitizer under different conditions of absorption coefficient and photobleaching rates in three different coordinates was estimated. From the results it is observed that under photobleaching conditions, the number of photons absorbed by the sensitizer increases with depth and then slowly decreases with further increase in depth. From the results, one can determine the depth of necrosis for different bleaching conditions. For example, consider that the threshold number of photons required to produce the tissue necrosis is \( 1 \times 10^{18} \). In the case of \( \mu_{at}=\mu_{at}, \mu_{ar}<\mu_{at}, \mu_{ar}>\mu_{at} \) for all the irradiation geometry and three coordinates the photostable sensitizer are likely to produce more damage than the photolabile sensitizer. If the photosensitizer is having photobleaching property, the treatment efficacy will be certainly reduced with respect to a photostable sensitizer. However with high photobleaching rate the tissue necrosis can be achieved at deeper layers than with low photobleaching rate because of more number of photons absorbed at deeper layers. Further from the results of the relative fluence rate distribution it is observed that there is a marked difference in fluence rate distribution when \( \mu_{ar}=\mu_{at} \). However at higher \( \mu_{ar} \), the depth of necrosis will be more for photostable sensitizer than for the photolabile sensitizer, because more number of photons are absorbed. As the current clinically approved sensitizer are more photolabile, the estimation of the tissue necrosis corresponding to the photobleaching property of the sensitizer is mandatory to improve the treatment efficacy. In the case of less photobleaching rate the depth of necrosis is expected to be less because of the less number of
photons absorbed beyond a certain depth. Hence it is more ideal for the treatment of superficial lesions. With increasing photobleaching rate the number of photons absorbed with increasing bleaching rate. At very high photobleaching rate of 40 cm$^2$J$^{-1}$, the threshold number of photons (appro $1 \times 10^{18}$) absorbed by the sensitizer is higher in the region between 0.3 cm to 1.45 cm (this could be seen clearly in planar coordinates (Figures 3.13(i), 3.14(i) and 3.15(i)). This clearly indicates that the tissue necrosis could be achieved only at this region without causing any adverse damage to the tissues at the superficial layers. Hence, one could suggest that for lesions below skin surface, sensitizers with higher absorption coefficient and with more photobleaching property can be used effectively to produce the selective tissue necrosis.

In conclusion, the present study suggests the possibility of enhancing the efficacy of PDT at higher sensitizer concentration and photobleaching rate. Further it is worth to mention that the results presented are based on theoretical simulations made using the experimentally derived optical properties. In this regard, more experimental measurements could be made to verify the proposed theoretical model, as it might really improve the treatment efficacy.