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ORIGINAL ARTICLE

A new application of Gompertz function in photohemolysis: the effect of temperature on red blood cell hemolysis photosensitized by protoporphyrin IX

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Abstract Photosensitization by protoporphyrin IX (PpIX) is accelerated at different irradiation temperatures, different dark incubation temperatures (T_{inc}) and different irradiation times. The applicability of Gompertz function to the fractional photohemolysis ratio, a and the rate of fractional photohemolysis, b is found to be the most appropriate model to fit the experimental data with minimum parameters and minimum errors. The reduction in Gompertz parameters, the fractional ratio values of a, and increase in the fractional rate values b, for 20 μM PpIX irradiated with black light at low irradiation temperature 5°C and higher T_{inc} 37°C was noticed. The parameter a has higher values at lower irradiation time and lower irradiation temperatures which indicates a longer photohemolysis process and longer t_{50}. Values of the parameter b were found to be strongly temperature-dependent, and always increase with increasing irradiation time and T_{inc} with lower values at lower irradiation time and lower T_{inc}. There are no significant changes in the lysis of RBCs process at irradiation temperatures equal to or higher than 35°C. Similarly, no significant change on t_{50} at higher irradiation time at T_{inc} 24 and 37°C. In conclusion, Gompertz analysis technique adapts to study the photohemolysis process at different conditions as a best-fit model.

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Abbreviations

BL	Black light
C_s	Total solute concentration
DPH	Delayed photohemolysis
H	Fractional hemolysis
HSA	Human serum albumin
OD	Optical density
PB	Phosphate buffer
PBS	Phosphate buffered saline
PpIX	Protoporphyrin IX
EPP	Erythropoietic porphyria
RBC	Red blood cells
$^1\Delta_g$	Singlet oxygen
t_{irr}	Irradiation time
t	Lysis time (the time measured from start of rupturing the RBCs at dark incubation)
t_{50}	Incubation time for 50% hemolysis
T_{irr}	Irradiation temperature
T_{inc}	Incubation temperature

Theoretical parameters

a	fractional photohemolysis ratio
b	fractional photohemolysis rate

1 Introduction

The present study reports new results on the temperature-dependence of PpIX-photosensitized Delayed photohemolysis (DPH) fitted with Gompertz function.

However, the relationship of hemolysis (H) to specific damage endpoints has not been resolved. The object of the present investigation was to measure the temperature-dependence of DPH photosensitized by PpIX and evaluate the results in terms of the photohemolysis mechanism using Gompertz function, despite the importance of their interaction and photoproducts. The Gompertz function is used to examine the inhibition profile models. It was hypothesized that any inoculum size effect would be manifested with the inflexion point of the function and the slope of the straight lines by fitting the model to the data obtained. Gompertz function is also known as *Gompertz's Law of Mortality* especially for the elderly mortality rates, but its application is not restricted to mortality rates [12, 16]. The rate kinetics has been analyzed with Gompertz function that was originally applied to describe age distribution in human population [2, 12]. Recently Kim et al. used Gompertz function for the growth of *Escherichia coli* under oxidative stress induced by photoexcited TiO_2 [14]. There are different models describing the photohemolysis process. However, no standard methods or precise models were established to express the photohemolysis process. Gompertz function seems to be the most applicable function with only two parameters, while some other models used more parameters without good fitting and appears to be questionable [1, 3]. The relationship between incubation temperature (T_{inc}), irradiation temperature (T_{irr}), irradiation time (t_{irr}), and laysis time (t = the time measured from start of rupturing the RBCs at dark incubation) with these new photohemolysis parameters was studied.

The present paper reports new results on delayed photohemolysis fitted to a new application of Gompertz function module. The Gompertz function is defined as:

$$H = H_0 e^{-cbe^{-bt}},$$

which can be simplified as

$$H = H_0 e^{-ae^{-bt}}, \quad (1)$$

where H is the percentage of hemolysis during the lysis time t (the time measured from start of rupturing the RBCs at dark incubation), H_0 the initial maximum number of cells, normalized to one, a is a fractional hemolysis ratio, and b is the rate of fractional hemolysis change.

The new results with PpIX fitted to Gompertz function show that the DPH rate parameters are similar to previous finding with Hypericin and photofrin with higher accuracy [1].

2 Materials and methods

Red blood cells from fresh citrated bovine blood were isolated by centrifuging repeatedly at 1,000*g* for 10 min at 4°C and re-suspending the cells in pH 7.4, 10 mM phosphate buffer plus 0.9% saline (PBS) until the supernatant was clear. The light scattering optical density of the isolated RBC at 750 nm (OD_{750}) was ≈ 2.0 cm^{-1}, which corresponds to haemocytometer measurements of $(8.95 \pm 0.14) \times 10^7$ cm^{-3}. The di-sodium salt of Protoporphyrin IX (PpIX) from Sigma Chemical Co. was diluted with PBS without any detergents to get the desired concentration. The RBCs were incubated with 10, and 20 μM PpIX in PBS at 37°C for 30 min followed by centrifugation at 1,000*g* for 10 min and re-suspended in PBS. Spectroscopic measurements indicate that most of PpIX bounded to RBCs after 30 min incubation remained bound after centrifugation. The fractional H was scaled to zero at OD_{750} immediately after irradiation and scaled to unity after OD_{750} had attained a constant value. The linearity of the light scattering assay was shown in previous work [1, 3, 20]. This technique was re-evaluated by hemolyzing RBC suspensions in distilled water and measuring the hemoglobin absorption after centrifugation.

2.1 Cells with 10 μM PpIX irradiated with high-pressure Hg–Xe arc lamp

The cells were incubated with 10 μM PpIX and irradiated with Hg–Xe arc lamp at different irradiation time t_{irr} (2–15 min.), different incubation temperatures, T_{inc} (5.0–42°C), and different irradiation temperatures, T_{irr} (5.0–35°C). The photohemolysis light source was a 200-W high-pressure Hg–Xe arc filtered by 2 cm of water and a Corning C.S. No. 0-52 filter ($\lambda > 360$ nm). 6 ml samples were irradiated in a cylindrical glass cuvette [2 cm (length) × 2 cm (diameter)] located in a thermostated holder with stirring and air bubbling.

2.2 Cells with 20 μM PpIX irradiated with black light

Other irradiations were performed using a 15-W Sylvania F15T8/BL ''black light'' (BL) fluorescent lamp. Samples (15 ml) were irradiated at $T_{irr} = 5.0$°C in an uncovered Petri dish 6 cm diameter) located 5.5 cm below the lamp on a rotary table, the cells were irradiated at different t_{irr} (9–14 min) and incubated at $T_{inc.} = 37$°C. The incident fluence rate from 300 to 400 nm was ≈ 2.1 m W cm^{-2}. After irradiation, 3 ml aliquots were incubated in the dark and OD_{750} was followed until hemolysis was complete.

3 Results

3.1 Cells with 10 μM PpIX irradiated with arc lamp

3.1.1 Effect of t_{irr}

Two groups of cells incubated with 10 μM PpIX and irradiated with arc lamp at T_{irr} = 24°C with different irradiation time (t_{irr} = 2–4.5 min). One group incubated at 24°C and one group incubated at 37°C. Typical DPH curves are shown in Fig. 1, where the *points* represent the experimental data while the *solid lines* show their best fit of Gompertz function. Table 1 shows the fitted parameters to Eq. 1 with experimental and theoretical time required for 50% fractional hemolysis. The normalized value of H_0 in all runs was 1.01 ± 0.06. The data of the experimental t_{50} compared with the theoretical data are in good agreement (see Tables 1, 2, 3, 4). The theoretical values of t_{50} was calculated by the following formula

$$t_{50} = \ln(0.693/a)/ - b. \quad (2)$$

The significant decreases in both groups in t_{50} are decrease with increasing T_{inc} and t_{irr}. The lysis time of the group incubated at 37°C was faster than the group incubated at 24°C. There is a significant change in t_{50} at lower irradiation time with slight increment at higher values while the fractional hemolysis rate b, is rapidly increased at higher t_{irr} and slightly changes at lower irradiation time $t_{irr} \leq 3$ min (see Table 1, group II). The fractional hemolysis rate, b, increases with increasing the irradiation time. At lower incubation temperature 24°C the fractional hemolysis ratio, a, has no significant change with changing the irradiation time, while it decreases significantly at lower t_{irr}. In group incubated at T_{inc} = 37°C. Furthermore, the highest value of a with T_{inc} = 24°C is lower than the lowest value of a with T_{inc} = 37°C (see Table 1).

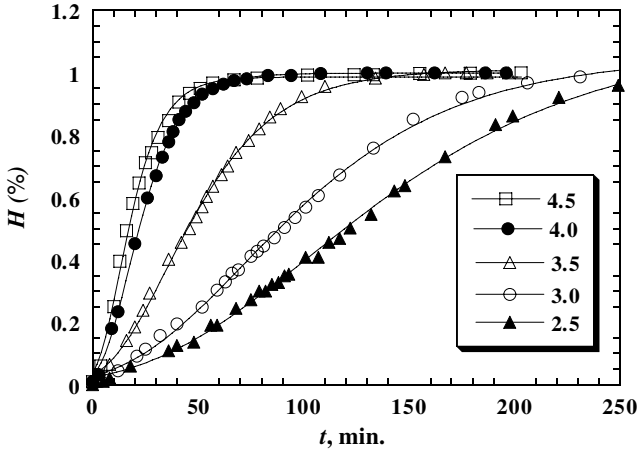


Fig. 1 Photosensitization of DPH by 10 μM PpIX, irradiated with Hg–Xe arc lamp at 24°C and incubated at 24°C. The cell were irradiated for *open circle* 4.5, *open square* 4.0, *open diamond* 3.5, *open triangle* 3.0, and *inverted triangle* 2.5 min.: the *solid* lines are calculated with the Gompertz function using the parameters in Table 1, group I

3.1.2 Effect of T_{inc}

Figure 2 shows the effect of T_{inc} on the cells sensitized with 10 μM PpIX irradiated with Arc lamp for 3 min at T_{irr} = 24°C and incubated immediately after irradiation at different temperatures (T_{inc}) from 5 to 42°C. The fractional photohemolysis rate, b, is much higher at higher T_{inc} in shorter time as compared to those incubated at lower T_{inc} (see Table 2). The hemolysis rate b, and the ratio a, are increasing exponentially with increasing T_{inc}. The apparent activation energy of this process is found to be 6.76 kcal mol^{-1} for T_{inc} 5–24°C and 11.60 kcal mol^{-1} for T_{inc} 37–42°C (Table 2).

3.1.3 Effect of T_{irr}

Two other groups of 10 μM PpIX, each was irradiated with arc lamp at different T_{irr} (5–35°C) and fixed irradiation time (t_{irr} = 15 min.) with fixed T_{inc} 37 and 24°C (see Table 3). The incubation time leading to H = 0.5 measured from start of dark incubation (t_{50}) is another convenient measure of the average DPH rate. The dependence of t_{50} on irradiation temperature is shown in Fig. 3. The value of t_{50} is larger at lower values of T_{irr} and T_{inc}. The plot of $\ln(1/t_{50})$ vs. $1/T_{irr}$ leads to an apparent activation energy equal to 5.06 ± 0.01 kcal mol^{-1} for T_{irr} 5–19°C and 14.80 ± 0.02 for T_{irr} 19–35°C at 24°C incubation temperature and 3.62 ± 0.01 kcal mol^{-1} for T_{irr} 5–19°C and 11.88 ± 0.02 kcal mol^{-1} at 37°C incubation temperature (Fig. 3). The difference between the t_{50}s in both groups are decreasing with increasing T_{irr} (see Fig. 4b). Similarly the difference between the fractional hemolysis ratio, a, in both groups are decreasing with increasing T_{irr} (see Fig. 4a). t_{50} and a are larger at lower T_{irr} and the difference between the two groups decreases with increasing T_{irr} till no significant change between the two parameters as seen in both groups at higher T_{irr}. Both groups show an increase of b and a decrease of a versus T_{irr}. The difference between the fractional ratio a, of both groups are decreasing from 9.11 to 0.56 (see Fig. 4a). Furthermore, the value of a is much higher at lower T_{irr} in both groups. Similarly, the difference between t_{50} of both groups decreases from 37.12 to 1.82 (see Fig. 4b).

Table 1 RBCs with 10 μM PpIX irradiated with arc lamp at different irradiation times at fixed incubation and irradiation temperatures

Sample	t_{irr} (min)	t_{50} (min)[a]	t_{50} (min)[b]	a	b (min^{-1})	T_{inc} (°C)	T_{irr} (°C)
Group I	2.5	121.70	130.32	3.857 ± 0.095	0.0132 ± 0.0001	24	24
	3.0	90.00	92.01	3.647 ± 0.099	0.0181 ± 0.0001	24	24
	3.5	45.50	45.38	3.521 ± 0.865	0.0358 ± 0.0006	24	24
	4.0	22.45	21.99	3.594 ± 0.081	0.0748 ± 0.0010	24	24
	4.5	17.49	16.90	3.289 ± 0.184	0.0921 ± 0.0033	24	24
Group II	2.0	84.00	82.13	19.704 ± 1.437	0.0408 ± 0.0001	37	24
	2.5	66.80	64.30	8.230 ± 0.852	0.0385 ± 0.0017	37	24
	3.0	62.00	58.06	6.456 ± 0.571	0.0384 ± 0.0017	37	24
	3.5	28.30	27.27	4.425 ± 0.168	0.0680 ± 0.0015	37	24
	4.0	19.43	18.33	5.260 ± 0.335	0.1106 ± 0.0034	37	24
	4.5	16.00	15.15	4.154 ± 0.117	0.1182 ± 0.0019	37	24

Results are expressed by mean ± SD

[a]Experimental values obtained from data curves at $H = 0.5H_0$

[b]Theoretical values calculated from Eq. 2

Table 2 RBCs with 10 μM PpIX irradiated with arc lamp for 3 min at fixed irradiation temperature and different incubation temperatures

t_{irr} (min)	t_{50} (min)[a]	t_{50} (min)[b]	a	b (min^{-1})	T_{inc} (°C)	T_{irr} (°C)
3	700	823.56	3.454 ± 0.100	0.0020 ± 0.0001	5.0	24
3	320	335.94	5.117 ± 0.162	0.0060 ± 0.0001	24	24
3	120	130.26	9.371 ± 1.589	0.0200 ± 0.0018	37	24
3	89	88.54	23.378 ± 3.470	0.0397 ± 0.0023	42	24

Results are expressed by mean ± SD

[a]Experimental values obtained from data curves at $H = 0.5H_0$

[b]Theoretical values calculated from Eq. 2

Table 3 RBCs with 10 μM PpIX irradiated with arc lamp at fixed irradiation time and fixed incubation temperatures with different irradiation temperatures

Sample	t_{irr} (min)	t_{50} (min)[a]	t_{50} (min)[b]	a	b (min^{-1})	T_{inc} (°C)	T_{irr} (°C)
Group I	15	55.70	56.04	16.201 ± 3.749	0.0562 ± 0.0044	37	5.0
	15	49.00	49.23	9.512 ± 1.841	0.0532 ± 0.0041	37	12
	15	40.67	41.72	4.678 ± 0.436	0.0458 ± 0.0025	37	19
	15	28.30	28.70	4.560 ± 0.463	0.0656 ± 0.0037	37	25
	15	14.15	14.53	4.005 ± 0.251	0.1207 ± 0.0045	37	35
Group II	15	92.83	89.05	7.096 ± 0.866	0.0261 ± 0.0016	24	5.0
	15	76.85	78.61	5.663 ± 0.313	0.0233 ± 0.0013	24	12
	15	59.81	60.99	3.473 ± 0.206	0.0264 ± 0.0013	24	19
	15	37.74	37.65	3.202 ± 0.131	0.0406 ± 0.0013	24	25
	15	16.04	16.35	3.443 ± 0.157	0.0980 ± 0.0030	24	35

Results are expressed by mean ± SD

[a]Experimental values obtained from data curves at $H = 0.5H_0$

[b]Theoretical values calculated from Eq. 2

3.2 Effect of t_{irr} on cells with 20 μM PpIX irradiated with black light

Table 4 shows the photosensitization of 20 μM PpIX DPH irradiated with black light at low irradiation temperature T_{irr} = 5°C and incubated at high temperature T_{inc} = 37°C. The samples were irradiated at different irradiation times. The hemolysis rate b exponentially increases with increasing t_{irr} while the ratio a, is exponentially decreasing. The ratio a, is

Table 4 RBCs with 20 μM PpIX irradiated with black light at fixed incubation and irradiation temperatures

T_{irr} (min)	t_{50} (min)[a]	t_{50} (min)[b]	A	b (min^{-1})	T_{inc} (°C)	T_{irr} (°C)
9.0	39.00	38.83	6.184 ± 1.467	0.0564 ± 0.0084	37	5
10	34.33	32.12	4.440 ± 0.594	0.0578 ± 0.0060	37	5
11	23.00	21.57	3.194 ± 0.219	0.0708 ± 0.0046	37	5
12	15.50	14.32	2.877 ± 0.196	0.0994 ± 0.0061	37	5
13	8.88	8.01	2.441 ± 0.338	0.1573 ± 0.0200	37	5
14	3.30	3.51	2.901 ± 0.819	0.4081 ± 0.0995	37	5

Results are expressed by mean ± SD

[a]Experimental values obtained from data curves at $H = 0.5H_0$

[b]Theoretical values calculated from Eq. 2

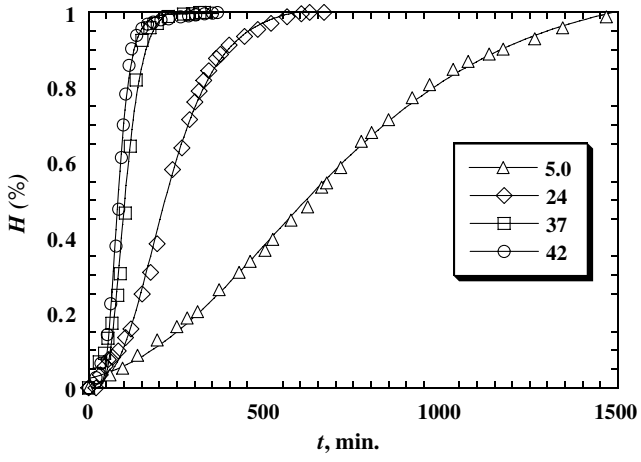


Fig. 2 Photosensitization of DPH by 10 μM PpIX, irradiated with Hg–Xe arc lamp for 3.0 min. at 24°C. The cells were incubated at different temperature: *open circle* 5.0, *open square* 24, *open diamond* 37, and *open triangle* 42°C: The *solid* lines are calculated with the Gompertz function using the parameters in Table 2

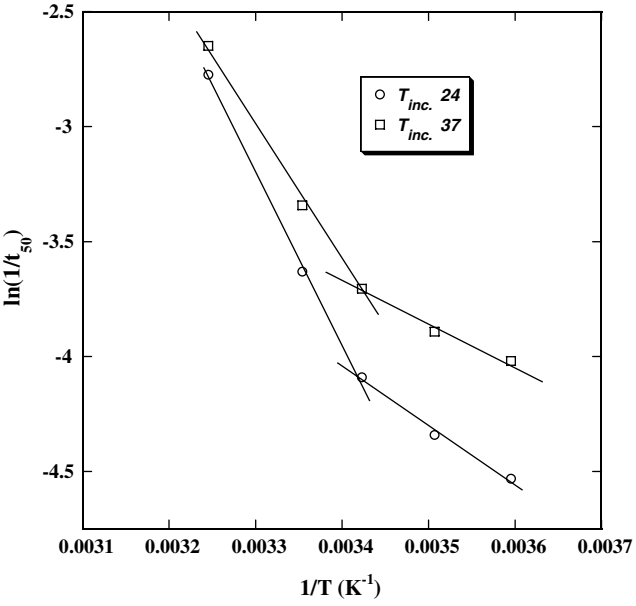


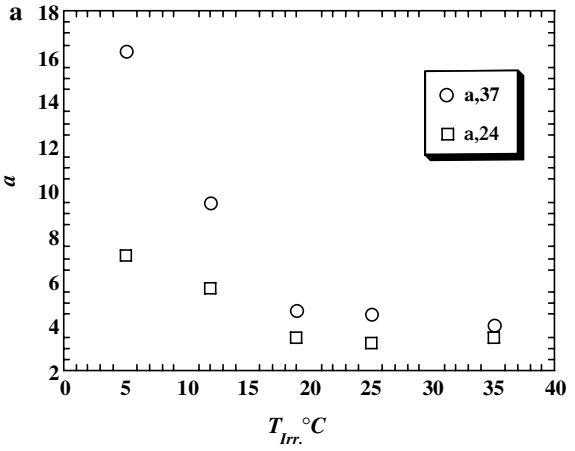
Fig. 3 Arrhenius plot for the dependence of the DPH rate on incubation temperature. The RBCs plus 10 μM PpIX were irradiated with the arc lamp for 15 min at *open circle* 24°C and *open square* 37°C with an apparent activation energy of 5.06 ± 0.01 kcal mol^{-1} for T_{irr} 5–19°C and 14.80 ± 0.02 for T_{irr} 19–35°C at T_{inc} 24°C and 3.62 ± 0.01 kcal mol^{-1} for T_{irr} 5–19°C and 11.88 ± 0.02 kcal mol^{-1} at T_{inc} 37°C

rapidly decrease at low t_{irr} with no significant change at the higher values, while the rate b, is rapidly increasing at higher t_{irr} and gradually increased at lower values. The applicability of Gompertz function Eq. 1 to the fractional photohemolysis damage is the most appropriate model to fit the experimental data with minimum parameters and minimum errors. Similar DPH curves are shown in Fig. 5, cells with 20 μM PpIX irradiated with black light at different t_{irr}. The T_{inc} and T_{irr} was fixed at 37 and 5°C, respectively. The experimental data points were taken immediately at the starting of incubation time after irradiation and the solid lines are the fit of Gompertz function.

The best fitting is confirmed by comparison of the absolute sameness of the experimental data points with the solid lines passing through the experimental data points.

4 Discussion

The Gompertz function parameters a, b, and the time required to attain $H = 0.5$ (t_{50}) are useful measures of the average DPH rate. Protoporphyrin IX is one of the most widely investigated photosensitizing agents of red blood cell (RBC) hemolysis. Post-irradiation or ''DPH'' has been investigated in most experiments at different temperatures. The generally accepted colloid-osmotic mechanism postulates that photochemical damage to the RBC membrane leads to cation efflux, followed by cell swelling and rupture [7, 18]. The



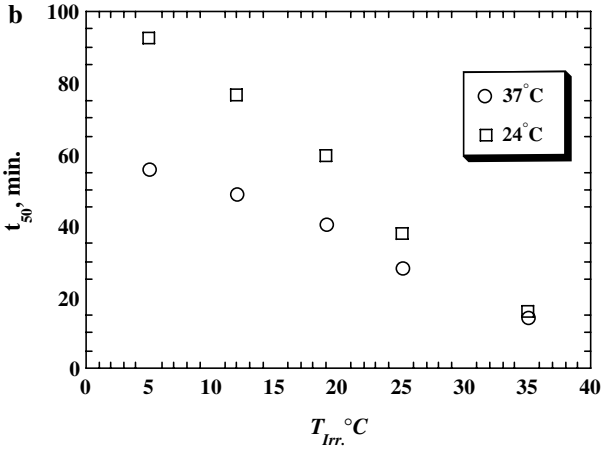


Fig. 4 **a** The variation of fractional hemolysis ratio (*a*) with irradiation temperatures of 10 μM PpIX irradiated with arc lamp for 15 min. and incubated at *open circle* 37°C and *open square* 24°C, Table 3 **b**The variation of the time required to 50% hemolysis (t_{50}) with irradiation temperatures of 10 μM PpIX irradiated with arc lamp for 15 min. and incubated at *open circle* 37°C and *open square* 24°C, Table 3

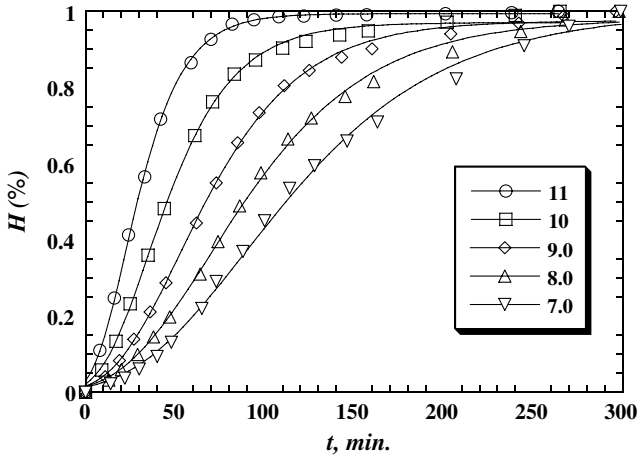


Fig. 5 Photosensitization of DPH by 20 μM PpIX, irradiated with *Black* light at 24°C and incubated at 37°C. The cells were irradiated at different times: *open circle* 11, *open square* 10, *open diamond* 9, *open triangle* 8, and *inverted triangle* 7 min. The *solid* lines are fitted with the Gompertz function using the parameters in Table 4

experiments were performed with bovine RBC, sensitized with PpIX as investigated with some other photodynamic agents [1, 3]. Studies on individual PpIX-photosensitized hemolysis with emphasis on RBC having the hereditary disease erythropoietic porphyria (EPP) have shown abnormally high PpIX levels in RBC, feces, and plasma, accompanied by enhanced photosensitization of skin to sunlight, and a high risk of hepatic failure. Suspensions of EPP cells undergo rapid photohemolysis in albumin-free media, inhibited in the presence of human serum albumin (HSA) [4, 6, 11]. Based on in vitro fluorescence results, Brun and Sabdberg attribute the skin photosensitivity of EPP patients to in vivo light induced transfer of PpIX from RBC to serum proteins followed by transfer of PpIX to membranes of endothelical cells [5, 6]. Their findings are compared to normal human RBC incorporating PpIX in the present work. Colloid-osmotic hemolysis in this temperature range was promoted by photodynamic treatments with PpIX and eosin isothiocyanate, and was further accelerated by hyperthermia after the photodynamic treatments. The anion transport protein (band 3) is the common target for photodynamic and hyperthermic hemolysis. The major role of the band 3 protein in photohemolysis was deduced in other studies showing that the DPH rate photosensitized by eosin isothiocyanate covalently coupled at the band 3 protein was 50–100 times faster than for uncoupled eosin Y [17]. Photosensitization of ghosts in the presence of PpIX led to a temperature-dependent cross-linking of membrane proteins during illumination with activation energy of 11.3 kcal mol^{-1} at different temperatures [9]. It has been shown experimentally that RBC depleted of cholesterol (20–30% depletion) were more susceptible to PpIX-photosensitized hemolysis than untreated cells. These results rule out the key role of cholesterol damage in DPH [10]. The limited available evidence does not implicate any other membrane constituent other than the band 3 protein as the primary photochemical target for photosensitized hemolysis.

The rate of DPH was known to be depending on the incident fluence, photosensitizer concentration (C_s), T_{irr}, and T_{inc}. The present results delineate the temperature-dependence effects of t_{50} and their correlation with new Gompertz function parameters *a* and *b*. In one set of measurements, a short irradiation time at

24°C was followed by a prolonged dark incubation at temperatures from 5 to 42°C (see results of Table 2). The accelerating effect of higher T_{inc} is attributed to thermal activation of the colloid-osmotic lysis. In the other set of measurements, irradiated with temperatures ranging from 5 to 37°C were followed by dark incubation at 24 and 37°C (see results of Table 3). These irradiations were necessarily longer to achieve practical t_{50} values at T_{irr} = 5°C and may have been affected by photobleaching. The increase of the hemolysis rate with T_{irr} could be explained by more efficient reactions of $^1\Delta_g$ with membrane targets, due to higher membrane fluidity and/or changes in the band 3 protein conformation. This explanation is congruous with the similar increment in the protein cross-linking found by Dubbelman et al. [9] with red cell membranes illuminated in the presence of protoporphyrin at 0°C and incubated in the dark at 37°C. The effects of T_{irr} and T_{inc} on consistent DPH parameters are not independent. The activation energy drowns from Tables 2 and 3 are characterized by two different values near 24°C. At higher T_{irr} and T_{inc} values > 24°C the activation energies are almost the same in all groups. At lower temperatures < 24°C the activation energy (6.76 ± 0.01 kcal mol^{-1}) drown from Table 2 is almost twice of that drown from Table 3 group I (3.62 ± 0.01 kcal mol^{-1}) and this can be attributed to lower values of T_{inc}. The rate parameter b in all groups are increases with increasing the T_{irr} and T_{inc} while the ratio parameter a decreases with increasing T_{irr} in both groups of Table 3 and increases with increasing T_{inc} Table 2. The data in Fig. 4a,b show that the accelerating effect of higher T_{inc} and t_{irr} is saturated at T_{irr} > 20°C. This effect can be explained by less than maximum pre-hemolytic photochemical damage at lower values of T_{irr}, in which case higher T_{inc} was required to attain a given fractional hemolysis rate b. Then, if membrane damage saturates at the same t_{irr} and higher T_{irr}, a given b, would be attained at a lower T_{inc}.

The results of Deuticke et al. [8] provide some information about the properties of K^+ leaks in RBC membranes. Based on the effects of non-electrolytes on K^+ efflux from RBC photosensitized by aluminum chlorotetrasulfophthalocyanine, they estimated the formation of less than one membrane leak per cell, whose size increased with increasing time of irradiation starting at ~0.46 nm. Their analysis suggests that destabilization of the cell membrane requires only one leak. The present analysis is the first application using variable temperature data on photohemolysis. Gompertz function has been used in some other fields such as agricultural research work to analyze the growth of the plants and the xylem development [19, 21]. The DPH curves were fitted with the Gompertz parameters (Tables 1, 2, 3, 4). The Solid lines in Figs. 1, 2, and 5 exemplify fitting of the model to the hemolysis data points. The decrease of fitting parameter a with increasing T_{irr} (5–24°C) at constant T_{inc} and constant t_{irr} is consistent for both sets of runs at fixed incubation temperatures, while there is no significant change at higher T_{irr} (24 and 37°C). The fractional hemolysis rate b is the rate at which photochemical damage is converted to lysis. The values of the parameter b almost remain constant (very low increment) at T_{irr} (5 and 19°C) and slightly increase with increasing T_{irr} (24 and 37°C) at higher T_{inc} = 37°C. The b values were found to be strongly temperature-dependent. Same result found with Moan et al. [15] at activation energy about 17 kcal mol^{-1}. Furthermore, the values of b increases with increasing t_{irr}, T_{irr}, and T_{inc}. A speculative connection to colloid-osmotic lysis is that a and b are related to the rate of damaged band 3 protein sites that act in concert to form a K^+ leak. An additional consideration is that $^1\Delta_g$ generated by bound PpIX may react with membrane targets via the external medium. Furthermore, the key features of this model are that cumulative photochemical damage is required for efficient hemolysis and that cooperative interactions take place during the dark incubation stage with higher T_{inc}. At larger T_{irr} and t_{irr} doses, discrepancies between the different studies are considerable (see Tables 1, 3). The difference between the range values of a (4–3) at T_{inc} = 24°C and (20–4) at T_{inc} = 37°C in Table 1 and a (6–3) in Table 4 are due to different irradiation energy sources; with low energy source (BL) higher drug concentration with higher t_{irr} is required. Despite the differences between the parameters in both tables, a and b are consistent with the general findings; a is decreases and b increases with increasing t_{irr}. The general conclusion drown from this results is that at higher t_{irr} and T_{inc} higher hemolysis rate takes place. In the basis of Helmink et al. [13] calculation, using the same form of Gompertz, for the time required to 98% hemolysis was in good agreement with the experiment values, 9.7% ± 2.8 at 24°C and 11.93% ± 2.5 at 37°C for the data shown in Table 1. These results showed that the Gompertz analysis technique adapts to study the effects of t_{irr}, T_{irr}, and T_{inc} on the photohemolysis process at different conditions as a best-fit model with minimum parameters and minimum errors. The notable rapid increase in the rate b and reached its maximum of 0.4 at higher irradiation time (see Table 4) suggests that additional membrane targets are accessible to $^1\Delta_g$ generated in the external medium or might be attributed to membrane rupture which combined

the effects of cell swelling induced by damage to the anion transport protein and thermally-activated photochemical damage to structural membrane proteins.

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