Kinetic Measurements for Photodecomposition of Bilirubin

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In present studies, chloroform solution of bilirubin was exposed to direct sunlight, diffused sunlight, ultraviolet, white, tungsten, blue, green and red lights to observe the degradation process. Multiple wavelength scans ranging from 200-800 nm were obtained after appropriate intervals of time for degradation of bilirubin in all the mentioned lights. It has been observed that the degradation process obeys first order reaction rate. Rates of reactions for different lights are calculated by plotting graph between $\ln(A_t - A_\infty)$ versus time. Half lives for the photodecomposition of bilirubin were also calculated. A new kinetic model for the rate determination of decomposition reaction is proposed which fits the experimental data in excellent way giving correlation coefficients up to 0.999. Appearance of peaks at other wavelengths in multiple scan indicate the formation of intermediates before complete degradation.

Key Words: Photodecomposition, Bilirubin decay, Kinetic model.

INTRODUCTION

Bilirubin is a compound of biological importance acquired chiefly from porphyrin, found in the red blood cell¹. Usually 0.1-1.5 mg of bilirubin is present in 100 mL of human plasma². Bilirubin causes the faint yellow colour of blood plasma and serum. Jaundice results from increased concentration of circulation bilirubin, usually more than 2 mg per 100 mL. Accumulation of toxic yellow metabolite, bilirubin, may cause certain diseases particularly related to liver, like haemolytic disorders and disturbance in hepatobiliary system³. Being a toxic substance, in severe hyperbilirubinemia, sufficient pigment may partition into brain to cause irreversible damage, even death⁴. Hyperbilirubinemia and jaundice are found in newly born babies because of late functioning of liver. Such infants are exposed to direct with sunlight or fluorescent light bulbs and the concentration of plasma bilirubin is significantly reduced^{5,6}. Decrease in concentration is due to photodegradation of bilirubin through a complex series of oxidation reactions. Biliverdin appears to be an early intermediate while final products are highly polar water soluble diazo compounds^{7,8}. It was found that products formed when bilirubin was exposed to light, were similar to that of dipyrrole⁹. Decomposition of bilirubin in the presence of tin and zinc metalloporphyrins was investigated¹⁰. Tin porphyrins increased the degradation upto 35 %. Combined dose of riboflavin and metalloporphyrins for photodecomposition of bilirubin leads to reduction of toxic effects associated with phototherapy¹¹.

Bilirubin is insoluble in aqueous solutions but soluble in dilute alkalis because of its weak acidity. Conversion of bilirubin to biliverdin was studied in sodium hydroxide solution under solar light at different temperatures¹². It can also be dissolved in organic solvents where it exercises more conformational freedom and can form inter and intramolecular hydrogen bonding¹³. Effect of γ -rays for radio-degradation of bilirubin in chloroform with variation in concentration was determined in air, N₂ and O₂¹⁴.

Mild treatment of jaundice affected babies has been used for over 40 years; initially with blue and later with white light. Phototherapy is deemed to be secure as well as efficacious¹⁵. Kinetics of bilirubin photodegradation with application to amniotic fluid was studied and order of reaction was determined¹⁶. Nanoparticle-sensitized photodecomposition and its therapeutic application have been investigated recently¹⁷.

In our present studies, solution of bilirubin in chloroform was exposed to different UV-visible lights and determined the quantitative change in concentration spectrophotometrically.

EXPERIMENTAL

Bilirubin was obtained from BDH (England) and chloroform from Merck (Germany). Chloroform was purified before use as it normally contains around 1 % ethyl alcohol, added as stabilizer. CH₃Cl was shaken five times with half of its volume of water, dried over anhydrous calcium chloride for 24 h and then distilled (b.p. 61 °C/760 mm Hg). The solvent was kept

in dark in order to avoid the formation of phosgene¹⁸. Bilirubin was used as such without any purification. Spectra were recorded on UV visible double beam spectrophotometer, model Optizem 2120 UV plus with multiple scanning facility.

Stock solution of bilirubin in chloroform (10 mg/250 mL, 68.5 $\mu\text{mol L}^{-1}$) was prepared and kept in a flask already covered with black paper. A dilute solution of bilirubin (20.55 $\mu\text{mol L}^{-1}$) was used to determine λ_{max} (Fig. 1).

Fig. 1. Electronic absorption spectrum of bilirubin to determine λ_{max}

Absorbance of different concentrations of bilirubin were recorded at 456 nm (λ_{max}) to determine extinction coefficient of the solution ($\epsilon = 56300 \text{ L mol}^{-1} \text{ cm}^{-1}$). Stability of bilirubin in chloroform was measured by keeping the solution (20.55 μmol) in a flask completely wrapped with black paper to avoid any deterioration by light. The sample was placed in dark for 72 h. Spectra were recorded after every 24 h showing no change in its absorbance.

Different light sources were employed in this study for the irradiation of bilirubin *i.e.*, direct sunlight, diffused sunlight, white, tungsten, UV, blue, green and red lights.

In case of direct sunlight, sample of bilirubin solution was exposed to sunlight at 1 pm when the temperature was $32 \pm 1^\circ\text{C}$. Recording the spectra was continued after successive 6 min intervals till the completion of reaction. Decrease in absorbance with the passage of time was observed and colour of the sample changed from yellow to green and then to light green very rapidly (Fig. 2).

Same procedure was adopted for diffused sunlight when the sample was kept in the room at 1 pm (temperature $32 \pm 1^\circ\text{C}$). Temperature was maintained by keeping the flask in water bath adjusted at 32°C . The spectra were recorded after successive intervals of 2 h. The sample was monitored for 26 h till there was no further decrease in absorbance indicating the completion of reaction (Fig. 3).

For white, tungsten, UV, blue, green and red lights samples were placed in a compartment of hardboard at a temperature of $32 \pm 1^\circ\text{C}$. The light sources were mounted at the roof of the

Fig. 2. Electronic visible spectra of bilirubin solution when exposed to direct sunlight

Fig. 3. Electronic visible spectra of bilirubin solution when exposed to diffused sunlight

compartment. Energy saver bulb was used as source of white light and tungsten bulb for tungsten light. For the illumination of ultraviolet light, UV rod was mounted in the hardboard compartment and for blue, green and red lights, ten LEDs of 3 watts each were used.

The decomposition reaction in white light was completed in 36 h while for tungsten, UV, green, blue and red lights, the durations of reaction were 67, 120, 178, 216 and 436 h, respectively.

RESULTS AND DISCUSSION

Bilirubin was dissolved in purified chloroform and exposed to direct sunlight, diffused sunlight, white, tungsten, UV, blue, green and red lights and noted the decrease in absorbance with time at 456 nm where the solution absorbed maximum. Absorbance decreased exponentially with time (Fig. 4).

Assuming that photodecomposition of bilirubin follows first order kinetics (eqn. 1), graphs were plotted between $\ln(A_t - A_\infty)$ versus time where A_t is the absorbance at any time and A_∞ is the absorbance when the reaction is complete. Linear plots verify the first order reaction mechanism (Fig. 5).

$$\ln(A_t - A_\infty) = \ln(A_0 - A_\infty) - kt \quad (1)$$

Rate constants were obtained from slopes of the graphs for each reaction. Correlation coefficients of the plots lie

Fig. 4. Absorbance *versus* time (h) when bilirubin exposed to blue light Fig. 5. $\ln(A_t - A_\infty)$ *versus* time (h) when bilirubin exposed to blue light

between 0.730-0.970 which are acceptable for calculating results. However these values are improved by reorganizing the eqn. 1.

$$\ln(A_t - A_\infty) + t = \ln(A_0 - A_\infty) - kt + t$$

$$\ln(A_t - A_\infty) + t = \ln(A_0 - A_\infty) + t(1 - k) \quad (2)$$

Plot of $\ln(A_t - A_\infty) + t$ *versus* t shows a linear relationship with better correlation coefficients (Fig. 6). From slope $(1 - k)$, rate constants have been calculated which are not very much different from the values obtained from eqn. 1 (Table-1).

The rate of decomposition of bilirubin is the fastest in sunlight, followed by white light, diffused sunlight, tungsten, UV, green, blue and red lights.

Fig. 6. $\ln(A_t - A_\infty) + t$ *versus* time (h) when bilirubin exposed to blue light

The decomposition reaction of bilirubin, in all different light sources, indicates that perhaps the decay paths and intermediate products are similar. However, the rates of the reaction are different. The exact cause of this difference in the rate of reaction is not clear but probably the difference in energy associated in different light sources may be one of the factors. If we analyze the rates of reaction (Table-1), it is found that the rate of reaction in direct sunlight is the highest. This can be interpreted due to the presence of high intensity source of light and also the radiations of high energy. But if high energy is the main cause, then effect of UV radiations should be the next high rate source of light but according to our data, rate of reaction under UV light is even lower than white light, diffused sunlight and tungsten light. Lower rate of decomposition of bilirubin under UV radiations may be interpreted due to the formation of some other product which may be produced with a slower rate. Lowest rate of decay under red light can easily be argued due to the low energy radiations. White light however show higher decomposition rate. This may be the reason that newly born babies are exposed to white tube light for their treatment.

Half lives of all the radiations were calculated (Table-1) from the rate constants data assuming that all the reactions proceed through first order kinetics. Half life for the direct sunlight is just 16.5 min while that of red light which show lowest decomposition rate is 5 days, 15 h and 14 min which is *ca.* 50 times less than that of sunlight. White light takes 3 h and 53 min to decompose half of the bilirubin into product. Its higher rate of decomposition may convince the physician to use it for jaundice treatment.

TABLE-1

Light source	Rate constant	R ² for eqn. 1	R ² for eqn. 2	Half life
Direct sunlight	$5.9 \times 10^{-2} \text{ min}^{-1}$	0.958	0.999	16.50 min
White light	$2.9 \times 10^{-3} \text{ min}^{-1}$	0.91	0.995	233.64 min (3 h and 53.64 min)
Diffused sunlight	$2.5 \times 10^{-3} \text{ min}^{-1}$	0.937	0.997	468.34 min (7 h and 48.34 min)
Tungsten light	$1.16 \times 10^{-3} \text{ min}^{-1}$	0.940	0.996	594.12 min (9 h and 54.12 min)
UV light	$9.8 \times 10^{-4} \text{ min}^{-1}$	0.879	0.997	704.89 min (11h and 44.89 min)
Green light	$6.0 \times 10^{-4} \text{ min}^{-1}$	0.730	0.999	1155.24 min (19 h and 15.24 min.)
Blue light	$3.6 \times 10^{-4} \text{ min}^{-1}$	0.970	0.998	1890.40 min (31 h and 30.40 min) (1 day, 7 h and 30.40 min)
Red light	$8.5 \times 10^{-5} \text{ min}^{-1}$	0.848	0.971	8114.89 min (135 h and 14.89 min) (5 days, 15 h and 14.89 min)

Conclusion

Photodecomposition of bilirubin obeys first order kinetics. Rate of bilirubin decay is the highest in direct sunlight while it is the slowest in red light. Bilirubin is decomposed very rapidly in white light. This may be the reason to expose jaundice baby patients in white light. Although green light is less energetic than blue light, yet rate of photodecomposition in former is higher than in latter. Half life in direct sunlight is just 16.5 min while in red light it is greater than 5 days. The suggested new kinetic model fits the data in a better way than the classical first order equation.

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