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PHOTO BLEACHING OF METHYLENE BLUE WITH GALACTOSE AND D-MANNOSE BY HIGH INTENSITY RADIATIONS

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ABSTRACT

Photo bleaching of methylene blue with two aldohexoses (galactose and D-mannose) in 50% aqueous methanol in presence of acetate buffer has been investigated with steady illumination of high intensity radiation of 661 nm on specially designed optical processor in photochemical research laboratory. High power Hg bulb was used as source of radiation. Quantum yield of reduction of dye showed that configuration of aldoses has some bearing on rate of reduction and depends upon the concentration of reducing sugars. This follows the first order decay kinetics of triplet transient species of the dye with life time of 10.1µs. Transient intermediate of methylene blue rapidly convert into leuco form after abstraction of H ion from hexoses by following step.

$$MB_T^+ + H^+ \rightleftharpoons MBH_T^{++}$$
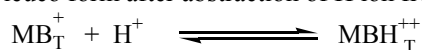
Reduction was carried out at different parameters like concentration of dye, reducing sugars, acidity which was adjusted by Hammmett 's acidity function and temperature. A comparison of reduction of dye by two monosaccharides reflect that quantum yield (Φ) was higher when D-mannose was in the reaction mixture. The values of rate constants were obtained and compared with international data.

Keywords: Aldohexoses, high intensity radiation, configuration

INTRODUCTION

Generally photochemical reductions of methylene blue were carried out with organic reductants. The nature of organic reducing agent were as amines, phenylhydrazines, anethole, Urea, thiourea and their derivatives N-phenylgylcine, nitriloacetic acid, ethylene diamine tetra acetic acid, paraphenyldiamine (PPDA) and mono, di, trimethylamines.

The quantum yield Photoreduction of methylene blue in the pH range 5.0—10.5 with ionic species of N-phenylglycine were reported by Matsumoto (1962). The lifetime of methylene blue was found to be dependent on the pH value, Bonneau et al. (1974) where as the quantum yield was found to be a function of phenylglycine concentration, acidity and independent upon concentration of thionine, Uddin (1996) in absolute methanol. The ground state association on the photo reduction of basic dyes by anionic N-phenylglycines were reported by Gak et al. (1998). Eimer et al. (1993) investigated the quenching of excited singlet and triplet states of methylene blue and the Azurs A, B and C by benozoquinones in methanol and acetonitrile. Rate constants were obtained from fluorescence lifetime for singlet state process. Triplet state processes and transient absorption spectra were investigated by laser flash photolysis. It was obtained from the decay of the triplet at 830 nm as a function of the quinone concentration. It is reported from transient absorption determination that quenching process by an electron transfer reaction takes place from the dye-excited state to quinones. Jonnalagadda and Natter, (1999) reported spectro-photometrically, the kinetics and mechanism of reduction of aqueous toluidine blue by ph $NHNH_2$. The forward rate constants for uncatalysed and acid catalysed reaction were 1.4×10^{-2} mol^{-1}.dm^3 s^{-1} and 60 mol^{-1}.dm^3 s^{-1} An equilibrium state, established between triethylamine and triplet state of methylene blue in absolute methanol was reported by Uddin (2000). Photoreduction of methylene blue via chemical quenching of triplet benzophenone was studied by Sindhu et al. (1987). The kinetics data fit into the integrated rate expression, which were ½ orders with respect to the substrates with no aggregation, but complexation between ground state benzophenone and MB^+ was observed. Jana (2000) showed 1st order kinetics in between mercapto succinic acid by methylene blue in acidic medium. The rate constant showed optimum value with respect to hydrogen ion concentration and the ionic strength of the reaction system.

Faure et al. (1967) observed the production of singlet oxygen by thiazine dye photosensitization. Chu and Ma (1998) observed photodecolorization of various dyes at 253.7nm. Results show that photodecolorization of all selected dyes follows pseudo first order decay and the reaction mechanism is dominated by free radical reaction where acidic photoproducts are formed. Jockusch et al. (1997) observed that Nucleophilic radicals, such as α-amino and ketyl radicals, produced by reaction of benzophenone triplet with amines and alcohols respectively. The effect of the anionic polyelectrolyte Na

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polyphosphate (NaPP) on primary processes and on quantum yield of photoreduction of methylene blue was observed by Fischer and Schiller, (1985). Mowry and Orgen (1999) studied reduction of methylene by ascorbic acid spectrophotometrically at low pH with previous conclusions based upon visual determinations of color loss. Spectrophotometric studies of temporal decay of MB^+ absorbance at 665nm show that the reaction is first order in MB^+, ascorbic acid and HCl. A slower reaction occurs with only MB^+ and ascorbic acid present. Maria *et al.* (1996) investigated the photophysics of the exited states of safranine T and its reaction with aliphatic amines. The fluorescence lifetime of the singlet state was found to be 1.3 μ S at 20° C in water. The objective of present investigation is to study the photo reduction of methylene blue with galactose and D-mannaose on optical processor as a function of quantum yield. A compression in quantum yield of reaction will be related with the configuration of two aldohexoses. The lifetime of triplet transition state will also determined.

MATERIALS AND METHODS

All reagents used were of (merck and BDH) analar grade. Acidity of reaction was determined by Hammett 's acidity function using spectrophotometer.

$$H_o = pK + \log \frac{d_1 - d}{d - d_2}$$

An inert atmosphere is required for reduction of dye for which Feiser's Method is used.

In Feiser's method nitrogen of 99.8% purity was passed through a series of different dreschel bottles containing Feiser's solution, Feiser (1924) and Uddin *et al.* (1998) in 1st two bottles, saturated solution of lead acetate, 1×10^{-1} M sodium hydroxide, silica gel and the solvent of reaction i.e. 50% aqueous methanol respectively.

A special optical bench was arranged according to need of the experiments. Mercury Mazda lamp is a convenient lamp to obtain a fine strong beam of light. A parallel beam of light was obtained through convex lenses L_1 and L_2 (Onto a small hole (O) 2mm in a metal plate) and lens L_3 arrangement. Red light was obtained by passing the beam further through a Kodak Wratten filter F. A plain reflecting glass R was used for reflection of a fraction of

the filtered light on to a reference photocell P_2. This light was then passed through reaction cell (C). A magnetic stirrer (M) was used to homogenize the reaction solutions. The photocell P_1 was used to detect the light after passing through the reaction cell. G_1 and G_2 were used to convert the light signal into electronic current measured in μA or mA from photocell P_1 and P_2 respectively.

REACTION CELL

The reaction cell used in the present experiment, which was a modified double walled cell, used by Sawkar (1960), Ahmed (1963), and Getoff *et al.* (1977). It was similar to the cell used by Uddin (2000). In the present system, there is a stirring system to make the reaction mixture homogenous and also it was attached to a thermostatic bath of $\pm$ 0.1°C variation.

EXPERIMENTAL PROCEDURE

30.0 ml of reaction mixture containing known volumes of methylene blue, and the buffer solutions in 50% methanol were pipetted out into the reaction cell. As the same volume of the reductants and buffer were pipetted for the determination of acidity H_o of the reaction mixture in first duplicate part of the reaction. The cell was then fitted with the nitrogen retractable bubbler and the outlet tap. The cell was kept in dark with shutters and well stirred magnetically during the flushing and irradiation periods. Oxygen free nitrogen was bubbled to the reaction vessel through for 40 minutes. After degassing the solution, the bubbler was retracted without allowing air to enter into the vessel, and the flow of nitrogen was maintained during the run. The lamp was on for 30 minutes before exposing the reaction solution to monochromatic light of 661 nm. Photocell responses from the deflection on the scale of the galvanometer were noted with no cell in the beam (D_o), with the cell containing solvent (D_m) and with the cell containing reaction solutions (D_{nt}). Simultaneous observations were taken on the reference galvanometer. The intensity of the light was measured from the ammeter. The acidity of the reaction mixture (a duplicate portion) was measured as described Hammett 's acidity function during the flushing period. The quantum yield was calculated as a function of concentration of reductants, acidity H_o temperature, concentration of dye and temperature by using following formula

INLET

Outlet N_2 to reaction mix

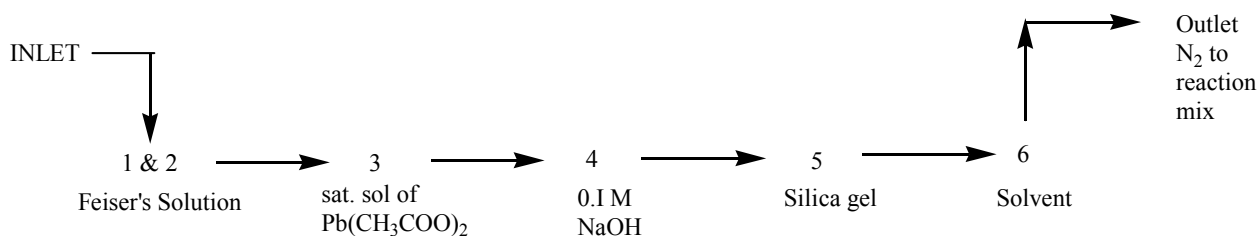| 1 & 2	3	4	5	6
Feiser's Solution	sat. sol of $Pb(CH_3COO)_2$	0.I M NaOH	Silica gel	Solvent

Table 1. Influence of concentration of reductants on quantum yield of methylene blue

RH = Reducing Sugar   BUFFER = Acetate
[MB] = 1×10^{-6} mol dm^{-3} Temp. = 25°C
Solvent = 50% aqueous methanol

| 10^3 . [RH] (mol dm^{-3})	Quantum yield ϕ Galactose	Quantum yield ϕ D-Mannose	10^{-3} . $\frac{1}{[RH]}$ (mol^{-1} dm^3)
[H$_o$] = 4.2		Confidence Interval = 0.11405	
0.8	0.151 ± 0.001	0.179 ± 0.002	1.250
1.6	0.173 ± 0.001	0.192 ± 0.005	0.625
2.0	0.201 ± 0.001	0.224 ± 0.002	0.500
3.0	0.251 ± 0.002	0.276 ± 0.001	0.333
4.0	0.311 ± 0.001	0.326 ± 0.001	0.250
5.0	0.372 ± 0.001	0.399 ± 0.002	0.200
6.0	0.496 ± 0.001	0.499 ± 0.001	0.166
[H$_o$] = 7.7		Confidence Interval = 0.05847	
0.8	0.099 ± 0.008	0.105 ± 0.005	1.250
1.6	0.119 ± 0.001	0.129 ± 0.001	0.625
2.0	0.129 ± 0.008	0.147 ± 0.002	0.500
3.0	0.147 ± 0.003	0.165 ± 0.001	0.333
4.0	0.181 ± 0.001	0.183 ± 0.001	0.250
5.0	0.221 ± 0.001	0.236 ± 0.002	0.200
6.0	0.277 ± 0.003	0.275 ± 0.002	0.166

$$\varphi = \frac{Slope \ x \ V}{I_o (1-\alpha) \varepsilon L}$$

Where L is the length of path traveled by the beam in the cell i.e. the length of the cell is 7.0 cm, V is the volume of the solution is 0.030 dm^3, I$_o$ is the initial intensity of the beam and (1-α) is the fraction of light transmitted through the window were measured during the experiment.

RESULTS

The influence of concentration of monosaccharides on quantum yield of photochemical reduction of methylene blue was investigated at two different acidities 4.2 and 7.7 in 50% methanol in presence of acetate buffer. The values of quantum yield (ϕ) at different concentration of reductants are summarized in Table 1 and representative plots of 1/ϕ versus 1/[RH] are shown in Figures 1-2. Table 1 showed that quantum yield of the photo reduction of methylene blue increases with the increase in concentration of reductants. The quantum yield corresponding to maximum concentration of reductants were calculated as 0.496±0.001 and 0.499±0.001 at 4.2 and 0.277±0.003 and0.275±0.002 at H$_o$= 7.7 for Galactose and D-Mannose The plot of ϕ versus H$_o$ (Figures 3 and 4) shows that reaction is greatly influenced by hydrogen ion activity and it was observed that quantum yield (ϕ) decreases as the H$_o$ acidity of reaction

medium increases. The plots of ($\phi_1 - \phi$) / ($\phi - \phi_2$) against 1/h$_o$ are straight lines passing through the origin as shown in Figure 5. ϕ_1 and ϕ_2 are the quantum yield at low and high acidity region respectively.

($\blacklozenge$ H$_o$ = 4.2 , $\blacktriangle$ H$_o$ = 7.7)
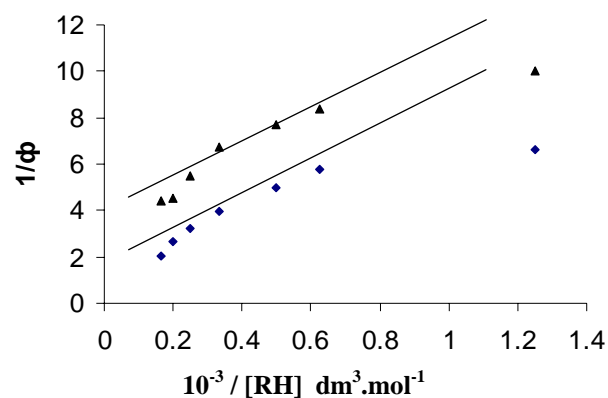
Fig. 1. A plot of 1/ϕ vs. 1/[RH] for the photoreduction of methylene blue with Galactose.

The values of maximum and minimum quantum yield should be regarded as a result of the equilibrium shifting towards high and low acidity regions.

Table 2. Influence of temperature on quantum yield of photochemical reaction of methylene blue with reducing sugars

| [MB] | = | 1×10^{-6} mol dm^{-3} | | [RH] | = | 8×10^{-4} mol dm^{-3} |
| Solvent | = | 50% Methanol | | Buffer | = | Acetate |

Acidity	TEMPERATURE ^{o}C					
[H$_o$]	20	25	30	35	40	45
Confidence Interval = 0.05184						
4.2 ϕ Galactose	0.130± 0.001	0.151± 0.001	0.195± 0.002	0.217± 0.002	0.232± 0.001	0.260± 0.003
4.2 ϕ D-Mannose	0.144± 0.001	0.179± 0.002	0.203± 0.001	0.236± 0.003	0.251± 0.003	0.290± 0.006
Confidence Interval = 0.028800						
7.7 ϕ Galactose	0.065± 0.002	0.099± 0.008	0.108± 0.001	0.118± 0.004	0.130± 0.002	0.144± 0.002
7.7 ϕ D-Mannose	0.086± 0.001	0.105± 0.005	0.139± 0.001	0.150± 0.003	0.173± 0.001	0.195± 0.001

Table 3. Comparison of specific rate constant of reduction of thiazine dye with international data

| Specific rate constants	Galactose	D- mannose	Literature value of reduction of MB with other reductants
k_5 (mol^{-1} dm^3 s^{-1})	5.122×10^4	4.910×10^4	9.5×10^7 [Uddin, 1998] 6.26×10^5 [Uddin, 1996] 3.2×10^8 [Mahmood, 2003] 5.0×10^6 [Uddin, 1998]
k_6 (s^{-1})	9.99×10^4	9.09×10^4	10^5 [Grey, 2002] 4.0×10^4 [Fischer, 2002] 7.0×10^6 [Ohashi, 1973]
k_7 (mol^{-1} dm^3 s^{-1})	9.403×10^5	9.002×10^5	3.0×10^7 [Uddin, 1998] 3.35×10^6 [Uddin, 1996] 10^6 [Ohashi, 1973]
k_9 (mol^{-1} dm^3 s^{-1})	4.99×10^{18}	4.01×10^{18}	7.85×10^{17} [Mahmood, 2003]
k_{10} (s^{-1})	3.747×10^{15}	3.747×10^{15}	2.75×10^{15} [Mahmood, 2003]
k_{12} (s^{-1})	3.0428×10^4	3.00×10^4	9.99×10^3 [Mahmood, 2003]
k_{13} (mol^{-1} dm^3 s^{-1})	7.790×10^{10}	7.1790×10^{10}	10^9 [Grey, 2002]

Equilibrium constants of the proposed mechanism of photoreduction of methylene blue with Reducing sugar

$K^{/}$	0.914×10^{-6}	0.891×10^{-6}	10^{-6} [Mahmood, 2003]
$K^{//}$	1.642×10^{14}	1.241×10^{14}	10^{14} [Uddin, 1998] 1×10^{14} [Mahmood, 2003] 7×10^9 [Bonneau, 1975]

Figure 6 showed that quantum yield is not a function of dye concentration. The plot of ϕ vs [MB] is a straight line which shows that quantum yield is independent upon the varying concentration of dye for reducing sugars. The values of quantum yield for the photo reduction of methylene blue with monosaccharides are 0.151 ± 0.001 for Galactose 0.179±0.002 for D-Mannose.

The variation of quantum yield of photo reduction of cationic dye, methylene blue by reducing sugars studied at ten different acidities (H$_o$) i.e. 4.2, 4.7, 5.3, 6.6, 6.9, 7.0, 7.3, 7.4, 7.6 and 7.7. The data interpretations of quantum yield at different acidities have been revealed that quantum yield of photo reduction of methylene blue is the function of acidity as shown in the Figs. 3 and 4.

The plot of ϕ versus H_o is a typical "S" shaped curves obtained by the photo reduction of methylene blue by reducing sugars showing that value of quantum efficiency is controlled by two equilibrium species of the dye in the excited state and these species are predominant in the extreme region of acidity i.e. from 4.2 to 7.7. Higher values of quantum yield in lower acidity value signify that reduction is more favorable in lower acidic medium due to π - π^* transition, as compared to higher acidic medium. The two-transition state of cationic dye i.e. singlet and triplet are involved in reduction process. But according to mechanism, singlet state could not be involved in the effect of acidity $[H_o]$ because singlet states either converted to excited triplet state or deactivated to ground state by emitting fluorescence radiations. Since fluorescence is assumed to take place from the conversion of singlet state to ground state, therefore the effect of acidity depends upon the triplet state whose lifetime was 10.1μs.
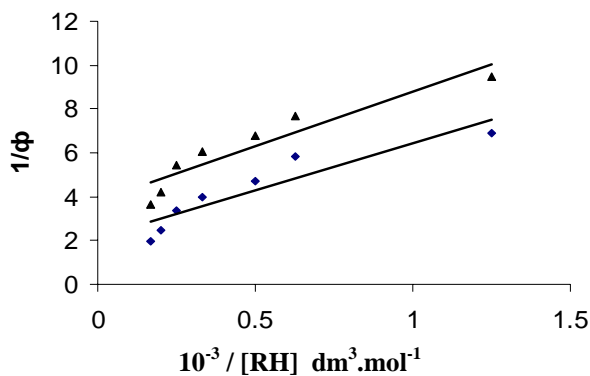
($\blacklozenge$ $H_o = 4.2$, $\blacktriangle$ $H_o = 7.7$)

Fig. 2. A plot of $1/\phi$ vs $1/[RH]$ for the photoreduction of methylene blue with D-Mannose.
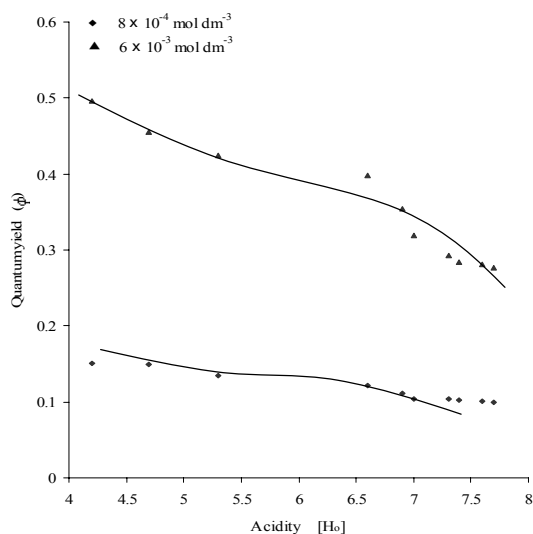
Fig. 3. A plot of ϕ vs $[H_o]$ for the photoreduction of methylene blue with Galactose.
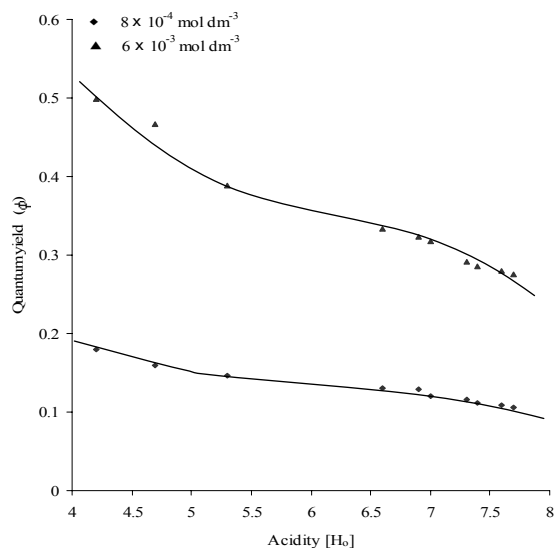
Fig. 4. A plot of (ϕ) verses $[H_o]$ for the photoreduction of methylene blue with D-Mannose.
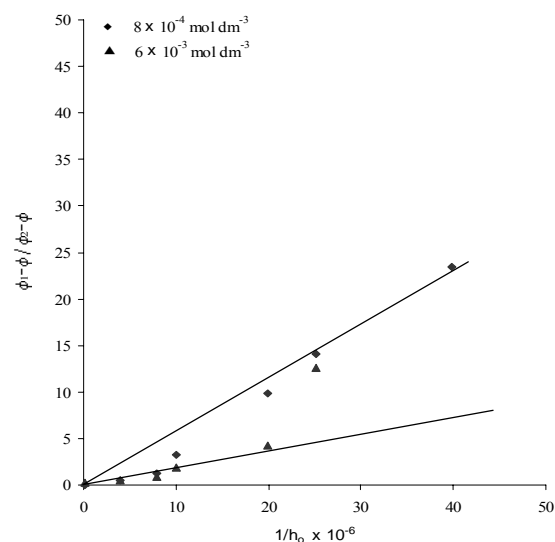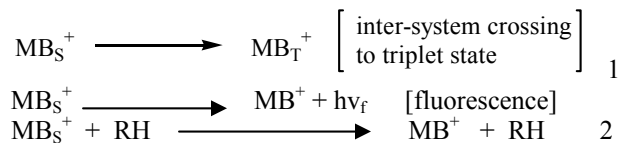

Fig. 5. A plot of $\phi_1-\phi / \phi-\phi_2$ vs $1/h_o \times 10^{-6}$ for the photoreduction of methylene blue with Galactose

Deactivation of two excited state and association with reductant molecule take place according to following mechanism

$$MB_S^+ \longrightarrow MB_T^+ \quad \left[\begin{array}{l} \text{inter-system crossing} \\ \text{to triplet state} \end{array}\right] \quad 1$$

$$MB_S^+ \longrightarrow MB^+ + h\nu_f \quad [\text{fluorescence}]$$
$$MB_S^+ + RH \longrightarrow MB^+ + RH \quad 2$$

Triplet excited state of the MB may interact with solvent molecule or equilibrium established
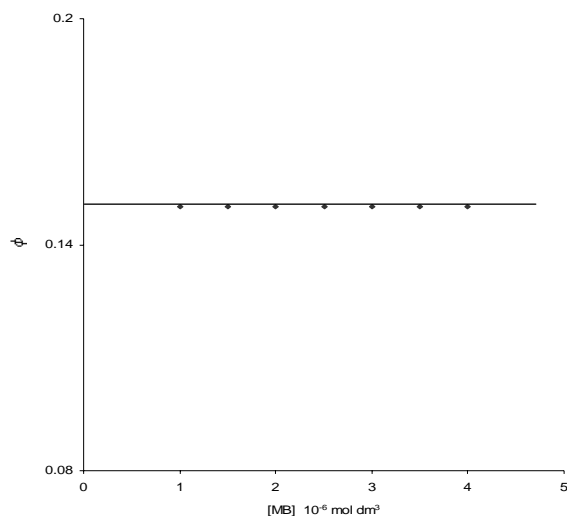
$$MB_T^+ + H^+ \rightleftharpoons MBH_T^{++} \quad 3$$
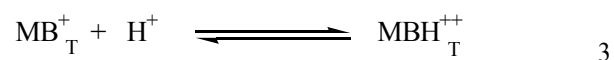
Fig. 6. A plot of ϕ vs [MB] for photochemical reduction of methylene blue with reducing sugar at $H_o = 4.2$

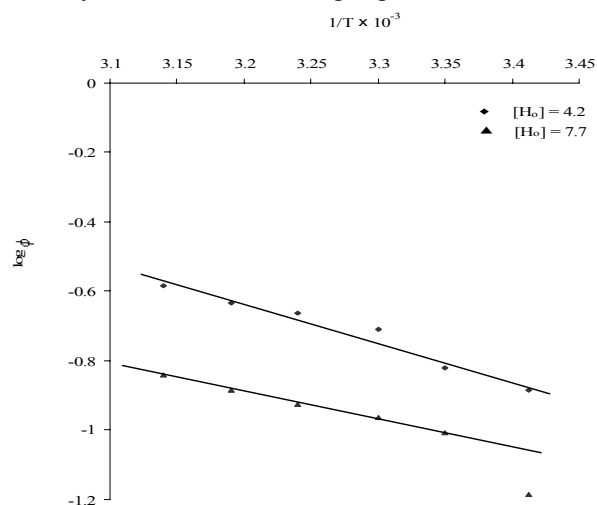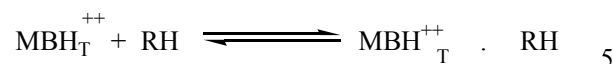Fig. 8. A plot of log ϕ versus 1/T (K^{-1}) for D-Mannose at $H_o = 4.2$ and 7.7

Triplet excited state of the dye may associate with the reductant (galactose or D-mannose) molecule

$$MB_T^+ + RH \longrightarrow MB_T^+ \cdot RH \qquad 7$$

The associated complex may convert into leuco form by dissociation.

The influence of temperature from 20° C to 45° C on quantum yield of photo reduction of methylene blue at concentration of dye 1×10^{-6} mol dm^{-3} and reductant concentration 8×10^{-4} mol dm^{-3} showed that quantum yield in inert reaction mixture along with reducing sugar is effected by change in temperature. The results are tabulated in the Table 2.and the plot of log Φ vs 1/T are shown in Figs. 7-8. The average value of temperature coefficient is found to be 1.150 and 1.184 at two different acidities i.e. 4.2 and 7.7 for Galactose and D mannose and found that it increases gradually as temperature rises, the difference in values could be correlated with structural difference of OH$^-$ group of reducing sugars, Bertolotti *et al.* (1999). The lower value of quantum yield at $[H_o] = 7.7$ is also in accordance with the other studies, Adamcikova and Parlikova (1998).

Fig. 7. A plot of log ϕ versus 1/T (K^{-1}) for Galactose at $H_o = 4.2$ and 7.7

$$MB_T^+ + RH \longrightarrow MBH_T^+ + R \text{ (reduction)} \qquad 4$$

where RH is reducing sugar.

It is supposed that MBH_T^{++} is associated with sugar molecule resulting in the following equilibrium.

$$MBH_T^{++} + RH \rightleftharpoons MBH_T^{++} \cdot RH \qquad 5$$

The effect of acidity on triplet state may be due to the acid base equilibria or kinetic effect. Equation no (5) shows that increase in acidity will cause a decrease in quantum yield, which is in agreement with the result, Uddin and Hasnain (2002) as observed in the present work.

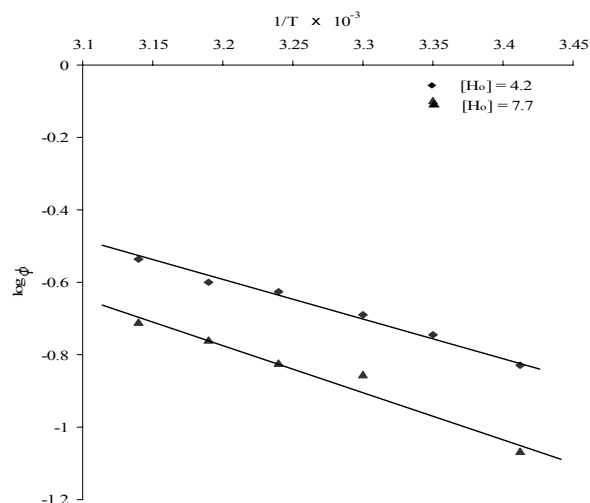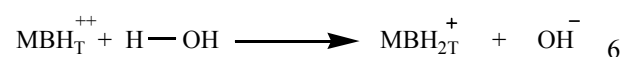If effect in acidity [H$_o$] be represented by the reaction

$$MBH_T^{++} + H-OH \longrightarrow MBH_{2T}^+ + OH^- \qquad 6$$

DISCUSSION

Methylene blue is a basic member of thiazine dye family, which is famous for its photo reduction process by visible light in absence of reducing agent in suitable buffer solution, Somer and Green (1973). The present work was performed to study the photo reduction of methylene blue by monochromatic radiations at wavelength of 661 nm with reducing sugars like monosaccharides. This is strikingly visible example of the conversion of light to chemical energy.

The reaction was observed under different parameters like effect of concentration of dye, reducing sugars, acidity of the reaction mixture and temperature. It is revealed that quantum yield ϕ depends upon acidity of reaction mixture, concentration of reductants and temperature of reaction. It was also found that reduction process is independent upon the concentration of dye. A comparison of the value of quantum yield of reduction shows that configuration of the aldoses has some bearing on rate of reduction and it was higher for D- mannose as compared with galactose (Tables 1 and 2).

Absorption of radiation at wavelength of 661 nm results in photo bleaching of the dye molecule, indicated that the photons cause the methylene blue to break the double bonds and the molecules turn colorless. The reductant is necessary for the photo bleaching process of methylene blue because it is involved, Mahmood and Kok (2003) in the oxidation-reduction part of bleaching.

In the oxidized form of the dye, there is a conjugation of double bonds across the three rings. The electronic excitation energy for this molecule is within the visible region of the spectrum. In the reduced form, the double bond on the nitrogen in the middle ring is no longer present, and conjugation is restricted to the two outer rings. Any electronic transitions (such as π bonding to π^* antibonding) now have energies in the visible region. The use of reductants shows that the bleaching process requires the presence of reducing sugars according to following equations (see on the bottom of this page).

The reduction process shows that quantum yield is governed by the step where H^+ from aldehydic group of reducing agent abstracted by N atom present in the middle ring of the dye.
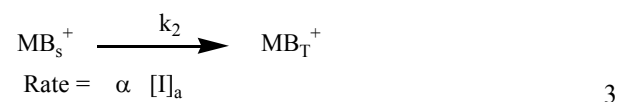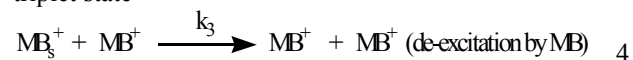
Mechanism of Reduction

$$MB^+ + h\nu \longrightarrow MB_S^+$$
$$\text{Rate} = [I]_a \tag{1}$$
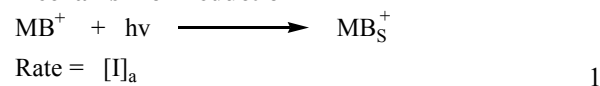
where I_a is the intensity of light and MB^+ is the singlet excited state after absorbing I_a of light.

$$MB^+ \xrightarrow{k_1} MB^+ + h\nu_f \quad \text{(fluorensces)}$$
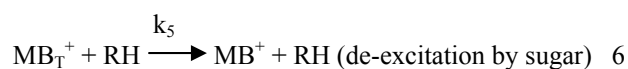$$\text{Rate} = (1-\alpha)[I]_a \tag{2}$$
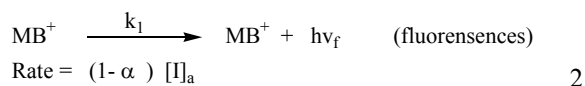
α = fraction of light absorbed

$$MB_s^+ \xrightarrow{k_2} MB_T^+$$
$$\text{Rate} = \alpha[I]_a \tag{3}$$

k_2 = rate constant for intersystem crossing of singlet to triplet state

$$MB_s^+ + MB^+ \xrightarrow{k_3} MB^+ + MB^+ \text{ (de-excitation by MB)} \tag{4}$$

k_3 = quenching rate constant for singlet state with unexcited molecule.

$$MB_s^+ + RH \xrightarrow{k_4} MB^+ + RH \text{ (de-excitation by sugar)} \tag{5}$$

k_4 = quenching rate constant for singlet state with reductant molecule.

$$MB_T^+ + RH \xrightarrow{k_5} MB^+ + RH \text{ (de-excitation by sugar)} \tag{6}$$
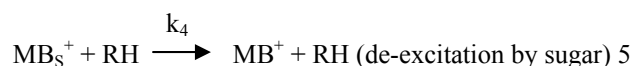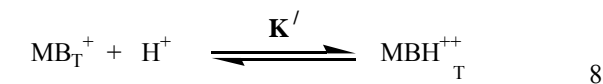
k_5 = quenching rate constant for triplet state with reductant molecule
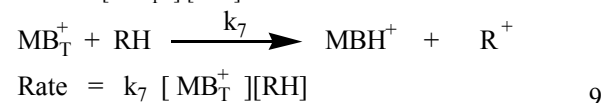$$\text{Rate} = k_5[MB_T^+][RH]$$

$$MB_T^+ \xrightarrow{k_6} MB^+ \tag{7}$$
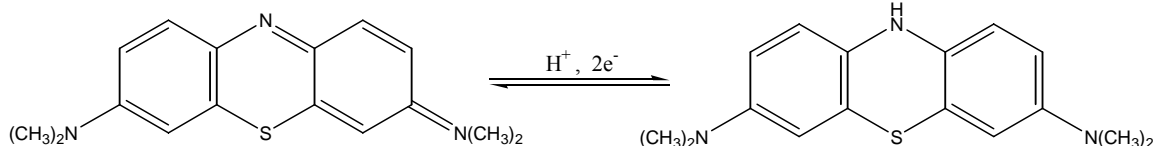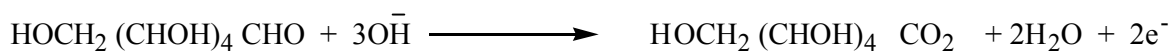$$\text{Rate} = k_6[MB_T^+]$$

k_6 = intersystem crossing of the singly charged triplet state to the ground state.

$$MB_T^+ + H^+ \underset{}{\overset{K'}{\rightleftharpoons}} MBH_T^{++} \tag{8}$$

$$K' = \frac{[MBH_T^{++}]}{[MB_T^+][H^+]}$$
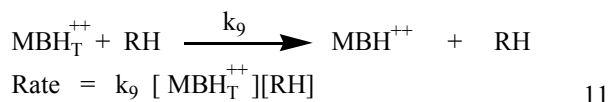
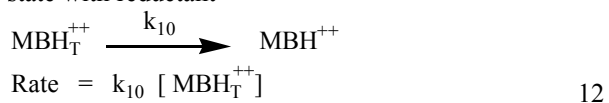$$MB_T^+ + RH \xrightarrow{k_7} MBH^+ + R^+$$
$$\text{Rate} = k_7[MB_T^+][RH] \tag{9}$$

k_7 = quenching rate constant for reaction of singly charged triplet excited state with reductant to form semi reduced dye.

$$MBH_T^{++} + RH \xrightarrow{k_8} MBH_2^{++} + R \tag{10}$$

$$HOCH_2(CHOH)_4 CHO + 3O\overline{H} \longrightarrow HOCH_2(CHOH)_4\ CO_2 + 2H_2O + 2e^-$$

$$MBH_T^{++} + RH \xrightarrow{k_9} MBH^{++} + RH$$
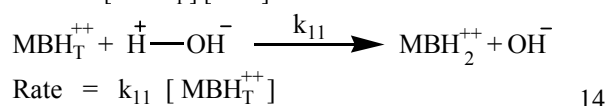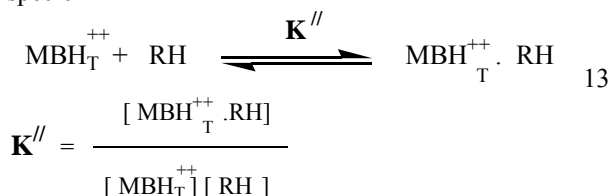
$$Rate = k_9 [MBH_T^{++}][RH] \qquad 11$$
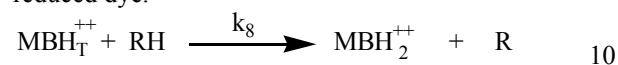
k_9 = quenching rate constant for doubly charged triplet state with reductant

$$MBH_T^{++} \xrightarrow{k_{10}} MBH^{++}$$

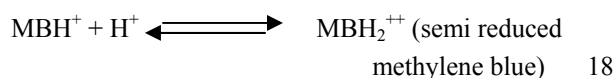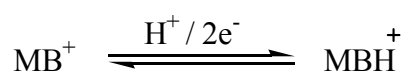$$Rate = k_{10} [MBH_T^{++}] \qquad 12$$

k_{10} = internal conversion of the doubly charged triplet specie

$$MBH_T^{++} + RH \underset{}{\overset{K^{//}}{\rightleftharpoons}} MBH_T^{++}. RH \qquad 13$$

$$K^{//} = \frac{[MBH_T^{++}.RH]}{[MBH_T^{++}][RH]}$$

$$MBH_T^{++} + \overset{+}{H}\text{—}OH^- \xrightarrow{k_{11}} MBH_2^{++} + OH^-$$

$$Rate = k_{11} [MBH_T^{++}] \qquad 14$$

k_{11} = rate constant for the reaction with solvent molecule and OH is solvent at high acidity region

$$MBH_T^{++}. RH \xrightarrow{k_{12}} MB^+ + H^+ + RH$$

$$Rate = k_{12} [MBH_T^{++}. RH] \qquad 15$$

k_{12} = rate constant for dissociation process

$$MBH_T^{++}. RH + MB^+ \xrightarrow{k_{13}} MBH_2^{++} + R + MB^+ \qquad 16$$

$$MBH_T^{++}. RH + MB^+ + e^- \xrightarrow{k_{14}} MBH^{++} + RH + MB^+ \qquad 17$$

$$RH + 3OH^- \longrightarrow RCOOH + 2OH + 2e^-$$

$$MB^+ \underset{}{\overset{H^+/2e^-}{\rightleftharpoons}} MBH^+$$

$$MBH^+ + H^+ \rightleftharpoons MBH_2^{++} \text{ (semi reduced}$$
$$\text{methylene blue)} \qquad 18$$

$$2MBH_2^{++} \xrightarrow{k_{15}} MBH_3^{++} + H^+ + MB^+ \qquad 19$$

$$R + OH^- \longrightarrow \text{aldonic acid}$$
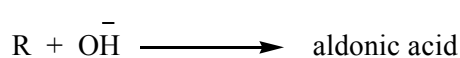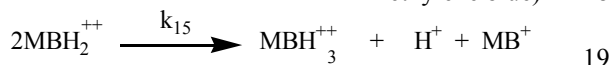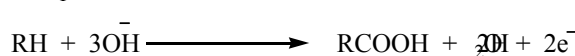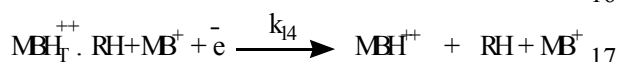
Rate constants were determined by adding and integrating the rate equations .The values compared with International data and tabulated in Table 3 show that reduction of methylene blue follows the same pattern for the formation of leuco dye.

REFERENCES

Adamcikova, L. and Parlikova, K. 1998. The Blue Bottle Experiment - Simple Demonstration of Self Organization. Journal of Chemical Education. 73(12):1580.

Ahmed, MG. 1963. Quantum efficiencies for some photochemical reaction of thiazine dyes. Ph.D. dissertation. University of London.

Bertolotti, G., Sonia, G. and Previtali. 1999. The excited state quenching of phenothiazine dyes by P-henzoquinones in polar solvents. Dyes and Pigment. 41(1-2):55-61.

Bonneau, R. and Pereyer, J. 1975. Mechanism of Photoreduction of Thiazine Dye by EDTA. A Studied by Flash Photolysis III. Consequences of a newly found pK_T of Thionine on the Mechanism in Basic Solution. Photochemistry and Photobiology. 21(13): 173-177.

Bonneau, R., Pereyer, J. and Dubien, JJ. 1974. Photochemical Determination of a New Acid Base Equilibrium of Thionine in its Triplet State. Application to the Photoreactivity of Thiazine Dyes in Aqueous Solutions. Journal of Molecular Photochemistry. 6: 245-252.

Chu, W. and Ma, CW. 1998. Reaction kinetics of UV-decolorization for dye. Hong Kong Poly Technical University. 37(5):961-974.

Eimer, M., Tuite, M. and Kelly, JM. 1993. Photochemical interaction of methylcne blue and analogies with DNA and other biological substrates. Photochemistry and Photobiology. (21):103-124.

Faure, J., Bonneau, R., Joussot, R. and Dubien, JJ. 1967. Etude en spectrascopic par éclair des colorants thiaziniques en solution aqueouse. Photochemistry and Photobiology. 6: 331-339.

Fieser, LF. 1924. A New Absorbant for Oxygen in Gas Analysis. Journal of American Chemical Society. 46:2639-2647, 214.

Fischer, T. and Schiller, K. 1985. Investigation on the influence of anionic polyelectrolyte on photophysical primary process of methylene blue reductants. Journal of Inf. Rec. 13(4):265-73.

Gak, VYu., Nadtochenko, VA. and Kini, J. 1998. Triplet Excited Dye Molecule (Eosin and Methylene Blue), Quenching by H_2O_2 in Aqueous Solution. Photochemistry and Photobiology A. Chemistry V(116) : 57-62.

Getoff, N., Harting, KJ., Kittle, G., Peschek, GA. and Solar, S. 1977. Hydrogen as Energy Carrier, Production, Storage and Transport Springer Verlagc. New York, USA.

Gray, A., Epling. and Chitsan, Lin. 2002. Photoassisted Bleaching of dyes utilizing TiO_2 and visible light. Chemosphere. 46(4):561-570.

Jana, AK. 2000. Solar cells based on dyes. Journal of Photochemistry and Photobiology. 132 (1-2):1-7.

Jockusch, H., Timpe, J., Schnabel, WJ. 1997. Photoreduction of organic dyes in Ketone amine systems. Journal of Physical Chemistry. A 101 (23):440.

Jonnalagadda, S. and Natter, B. 1999. Kinetics of Reduction of Toluidine Blue with Sulfite. Kinetic Salt Effect in Elucidation of Mechanism. International Journal of Chemistry. 31(2): 83-88.

Mahmood, W. and Kok, C. 2003. Study of photochemical bleaching of methylene blue doped in PMMA, PVA and Gelatin using photoacoustic Technique. Journal of Nonlinear optical Physics and Metallurgy. 12 (1):91-100.

Maria, CV., Previtali, EM., Marcelo, M., Gehlen, H. and Neumann, MG.1996. The interaction of aliphatic amines with Safranine-T in aqueous solution. Photochemistry and Photobiology. A. 94 (2-3):237.

Matsumoto, S. 1962. Photoreduction of Methylene Blue by Some Derivatives of N-Phenyl Glycine. Relation Between the Structure and the Reactivity of the Electron Donors. Buletin of Chemical Society of Japan. 35:1860-1864.

Mowry, S. and Ogren, PJ. 1999. Kinetics of Methylene Blue Reduction by Ascorbic Acid. Journal of Chemical Education. 76(7):970-976.

Ohashi, M., Tsujimol, K. and Seki, K. 1973. Photo-reduction of 4-Chlorobiphenyl by Aliphatic Amines. Journal of Chemical Society and Chemical Community. 384.

Sawkar, PD. 1960. The Kinetics of Photochemical reduction of thiazine dyes by some organic reducing agents. Ph.D. Dissertation, University of London.

Sindhu, KS., Dahmiwal, NR. and Pandav, BV. 1987. Quenching of triplet dyes by 1,4- diazabicyclo [2, 2, 2] octane in Photoxy gentian"; Indian Journal of Chemistry. 26 A: 281.

Somer ,G. and Green, ME. 1973. Photochemical reduction of methylene blue by water. Photochemistry and Photobiology. 17: l79-l90.

Uddin, F. 2000. Kinetics of Photochemical Reaction of Thionine with Thiourea. European Journal of Organic Chemistry. 7:1345-1351.

Uddin, F. 1996. Photochemical Reduction of Thionine by N-Phenyl Glycine in Methanol. Arabian Journal of Science and Engineering. 21(3):407-425.

Uddin, F. Adhami, IM. and Yousufzai, MAK. 1998. Photoreduction of Methylene Blue by Triethyl Amine. Saudi Journal of Chemical Society. 2(1):47-59.

Uddin, F. and Hasnain, QZ. 2002. Photochemical Reduction of Thionine with Dimethylamine. Kuwait Journal of Science and Engineering. 29 (2):67-85.