KINETICS OF METHYLENE BLUE WITH ORGANIC REDUCTANTS

A THESIS

SUBMITTED FOR THE DEGREE OF

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BY

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DEDICATION

This “Thesis” is dedicated to

my husband

“Syed Saeed Akhter”

and loving kids

“Shees and Ailiya”
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ABSTRACT

The photobleaching of methylene blue with reducing sugars in 50% aqueous methanol using acetate buffer has been investigated with steady - illumination of monochromatic radiations of 661 nm on specially designed optical bench in a dark room. The dye reduction processes were studied at various parameters i) concentrations of dye, ii) concentrations of reductants such as Galactose, D-Mannose, D-(+)-Ribose, Maltose and Lactose, iii) acidity (H₀) of reaction solution iv) temperature as a function of quantum yield (φ). Hammett acidity function (H₀) was used to determine acidity of the reaction solution. The optical method was adopted to evaluate the values of H₀.

Bleaching of the dye simultaneously with demethylation has been observed upon irradiation, color recover as the source of irradiation turned off or exposure to air. Reaction follows second order kinetics with respect to dye concentration and reductant and first order with respect to triplet transient species with life time of 10.1 µs.

A general mechanism has been proposed. Mathematical expressions were established and ratios of rate constants were determined from experimental evidences. The tests were put forward to justify the mechanism. The mechanism was also supported by analysis of reaction mixture by TLC and column chromatic graphic techniques. Visible, Infra red and Mass spectrum of isolated product of the triplet transient showed the high reactivity of the dye triplet species towards different reagents present in the mixture.

The values of ratios of rate constant appearing in general mechanism were verified and it was found that quantum yield was the consequence of triplet
transient species of the dye with solvent and reductants as follows:

\[
\text{HOCH}_2\text{(CHOH)}_4\text{CHO} + 3\text{OH}^- \rightarrow \text{HOCH}_2\text{(CHOH)}_4\text{CO}_2^- + 2\text{H}_2\text{O} + 2\text{e}^-
\]

Two equilibrium states have been established with solvent and dye molecule.

\[
\begin{align*}
\text{MB}_T^+ + \text{H}^+ & \overset{K'}{\rightleftharpoons} \text{MBH}_T^{++} \\
\text{MBH}_T^{++} + \text{RH} & \overset{K''}{\rightleftharpoons} \text{MBH}_T^{++} \cdot \text{RH}
\end{align*}
\]

Where \( K' \) represents the equilibrium between protonated triplet state of methylene blue with solvent molecule and doubly protonated triplet state of the dye and \( K'' \) shows the equilibrium between the doubly protonated state of methylene blue and reductant with the associated complex of the dye with reductant (\( \text{MBH}_T^{++} \cdot \text{RH} \)).

An attempt has also be made to evaluate the values of energy of activation (\( E_a \)) and activation parameters such as enthalpy change of activation (\( \Delta H^* \)), entropy change of activation (\( \Delta S^* \)), free energy change of activation (\( \Delta G^* \)) as a function of quantum yield (\( \phi \)) using Arrhenius and Erying relations. The values of temperature coefficients of the photochemical reactions of methylene blue in presence of monosaccharides i.e. Galactose, D-Mannose, D-(+)-Ribose and disaccharides Maltose and Lactose were also evaluated.
خلاص / نتیجہ

میتیا کھیل میں لیپو میں شارح نیوزی لینڈ کے نیوزی لینڈ کے مبینی میں کھیل نویں والا نہا کر میں کھیلنے کے لئے مسہل کی قدر کا تحلیل کرتی ہے۔ (ii)

یہ مطالعہ کا انتظام کیا ہوا ہے جو ایک سوال کے لئے ہے۔ (iii)

یہ مطالعہ کا انتظام کیا ہوا ہے جو ایک سوال کے لئے ہے۔ (iv)

یہ مطالعہ کا انتظام کیا ہوا ہے جو ایک سوال کے لئے ہے۔
CHAPTER 1
THEORETICAL ASPECTS
1. THEORETICAL ASPECTS

INTRODUCTION

1.1 METHYLENE BLUE

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<th>Structural formula</th>
<th>Common name</th>
<th>Methylene blue [MB]</th>
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<tr>
<td></td>
<td>Suggested name</td>
<td>Methylene blue</td>
</tr>
<tr>
<td></td>
<td>Other names</td>
<td>Swiss blue</td>
</tr>
<tr>
<td></td>
<td>C.I. Number</td>
<td>52015</td>
</tr>
<tr>
<td></td>
<td>C.I. name</td>
<td>Basic blue 9</td>
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<tr>
<td></td>
<td></td>
<td>Solvent blue 8</td>
</tr>
<tr>
<td></td>
<td>Class</td>
<td>Thiazin</td>
</tr>
<tr>
<td></td>
<td>Ionisation</td>
<td>Basic</td>
</tr>
<tr>
<td></td>
<td>Solubility in water</td>
<td>3.55%</td>
</tr>
<tr>
<td></td>
<td>Solubility in ethanol</td>
<td>1.48</td>
</tr>
<tr>
<td></td>
<td>Absorption maximum</td>
<td>668,609 nm</td>
</tr>
<tr>
<td></td>
<td>Colour</td>
<td>Blue</td>
</tr>
<tr>
<td></td>
<td>Empirical formula</td>
<td>C₁₆H₁₄ClN₃S</td>
</tr>
<tr>
<td></td>
<td>Formula weight</td>
<td>319.86</td>
</tr>
</tbody>
</table>

This is a very commonly used dye, usually following “ripening”, a process involving atmospheric oxidation and the production of the three strongly metachromatic dyes Azure A, B and C. A comparison of these dyes shows the relationship clearly.
METHYLENE BLUE

A chemically basic dye, methylene blue is also known as tetramethyl thionine, and there are various grades used, including methylene blue chloride, which is preferred for biological work. A view of methylene blue is given in Figure 1.1 which is taken by polarized light gallery from net. The dye is easily oxidized and often contains azure B and methylene violet, depending on the manufacturer. With 16 carbons, 18 hydrogens, 3 nitrogens, and 1 chlorine atom in each molecule, the molecular weight of methylene blue is 319.86 and its absorption maxima, if pure, reside at 668 and 609 nanometers. Actual staining material labeled as methylene blue is either aged or alkanalized, and the pure substance appears to be a rather poor histological stain. As a green powder, the melting point is 190 degrees Celsius. To an organic chemist, methylene blue is better known as 3,7-bis-(dimethylamino)-phenothiazin-5-ium chloride and to some histologists as Swiss blue.

Widely utilized as a nuclear stain (both DNA and RNA) and as a stain in bacteriology, methylene blue is usually prepared as the double zinc chloride formulation, which is less soluble and easier to isolate. However, this form of the dye is toxic, so medicinal preparations and compounds used with live biological specimens should be zinc-free. The salt, methylene blue thiocyanate, is used as a redox indicator in the sanitary monitoring of milk.

Sometimes marketed under the trade names urolene blue and aniline violet, chemists also refer to the dye compound as methylthionine chloride or tetramethylthionine chloride. In health care, pharmaceutical-grade methylene blue is prescribed to treat methemoglobinemia, a condition also referred to as "blue baby syndrome" where the blood cannot deliver oxygen where it is required in the body. Methemoglobinemia is a symptom sometimes exhibited by babies born in areas with very high levels of nitrate pollution in the drinking water supply, particularly in rural areas that are dependent on well water and where chemical fertilizers are widely applied. Similarly, methylene blue is indicated as a remedy for cyanide poisoning. Methylene blue is sometimes used in surgery as a dye for parts of the body, the medical team wishes to identify.

As a redox indicator, methylene blue solution is colourless until it is oxidized and turns blue. Glucose, a reducing agent, when added in alkaline solution, reduces methylene blue
to its colourless form once again. Veterinarians utilize methylene blue also as an antiseptic. New research reveals that methylene blue can be activated by light to an excited state, which can then activate oxygen, creating oxidizing radicals. These radicals can initiate cross-linking of amino acid residues on proteins, so that methylene blue may be useful for the photochemical welding of tissues.

![View of Methylene Blue](image)

**Figure 1.1: View of Methylene Blue**

### 1.2 BASIS OF COLOUR

Visible light represents a very small part of the electromagnetic spectrum and corresponds roughly with radiation within wavelength range 3800Å - 7800Å (1 Å =10⁻¹⁰ cm). Day light covers this range and extends into ultraviolet region (less than 3800 Å), but its composition varies with the season, time of day and climate conditions. The light from artificial illuminates is nominally white, but varies widely according to the type of lamp. There is a deficiency of blue in the light of tungsten lamps and of red in that of simple discharge lamps. Correction is possible by using fillers to absorb the excess of red or blue.
The absorption of light by coloured substance is due to electronic transitions between different orbital within the molecule and the wavelengths absorbed are determined by energy differences between the orbitals. Every dye or pigment therefore exhibits a pattern of absorption arising from its chemical structure, and this may be represented by an absorption plotted as ordinate against wavelength (or frequency) as abscissa. This graph is characteristics of the colouring matters and may be used for identification purpose. Absorption spectra are normally determined from sample in solution. Dyed fibers and pigments in paint or other media absorb light in a characteristic pattern, and the unabsorbed light is mainly reflected rather than transmitted; in such cases, however, the physical form of particles of colouring matter and substance affect the nature of the reflect light. For examples, see below:

Figure 1.2

Figure 1.2 shows the effect on white light reflected off a solid object.
Figure 1.3 shows the effect on white light transmitted through a solution, or other transparent article.

So what exactly causes an object to be coloured?

1.3 THE EFFECT OF MOLECULAR ENERGY LEVELS

There are several ways in which molecules can appear to be coloured

- Simple molecular excitation, such as in a neon tube, may cause the appearance of colour. This is due to rotation and/or vibration of the molecules.

- Transition metal complexes are coloured due to the distortion of the metal's d-electron shell caused by legends surrounding the metal ion.

- Electronic motion in conjugated organic systems, and charge transfer.

- Colour in crystalline solids arises from band theory - the blurring of many orbitals throughout the solid. Solids are only coloured if the gap between the Highest Occupied Molecular Orbital (HOMO, the Fermi level) and the Lowest Unoccupied Molecular Orbital (LUMO) is small enough.

- Colour due to refraction, scattering, dispersion and diffraction - these are all due to the geometrical and physical dimensions of a solid or a solution.
The first four mechanisms all rely on some form of energy transfer to move either molecules or electrons from their ground state into some excited state. However, only one of these effectively applies to dye molecules, as dye molecules are almost without exception organic conjugated systems. The overlap-lapping π-orbitals effectively mean that no one electron absorbs more energy than another, since all π-electrons in the conjugated system are smeared above and below the molecule. Conjugated organic molecules absorb specific wavelengths of electro-magnetic radiation. If this absorption falls within the visible region, then the light reflected or transmitted is deficient in a particular colour, and the solid (or solution) appears coloured as shown in Figure 1.4:

![Diagram](image)

Figure: 1.4

$$\Delta E = h\nu$$

where $\Delta E$ is the difference between the two electronic levels, $h$ is Planck's constant and $\nu$ is the frequency of the absorbed radiation.

### 1.4 COLOUR VISION

Colour is an interpretation by the brain of sights received via the optic nerve form the dye in response to light with in an approximate wavelength range of 3800-7800 Å.
1.5 AFFECTS ON THE COLOUR QUALITY OF THE DYE

i) Hue

The hue of a dye depends on the wavelength it absorbs. The absorption of the dye depends on its structure. We can see that any change which affects the π-system will affect the hue. A structural change which causes the absorption band to longer wavelengths (i.e. yellow -> orange -> red -> violet -> blue -> green) is called a bathochromic shift. The reverse shift, towards shorter wavelengths is known as a hypsochromic effect.

ii) Chromophores and Auxochromes

The dye molecules contain two groups; the chromophore and the auxochrome. The chromophore is a group of atoms which control the colour of the dye. The auxochrome was a salt-forming group, which helped to improve the colour of the dye [1]. The auxochrome was a salt-forming group, which helped to improve the colour of the dye. This theory was later modified when it was discovered that the chromophore is usually electron-withdrawing, and auxochromes are normally electron-donating. The two groups are connected by a conjugated system.

So how can a dye molecule's hue be altered? A bathochromic shift may be caused by increasing the electron-withdrawing power of the chromophore (X or Y), increasing the electron-donating power of the auxochrome (Y ≠ X) and by increasing the length of the conjugated system connecting the two. (It must be remembered that the system shown below is symmetrical, so the chromophore and auxochrome are interchangeable here, but this is not always the case.)

![Diagram of chromophore and auxochrome](image)

The position of the chromophore and auxochrome also has an effect. In general, the meta position (M) has the shortest wavelength, and the para (P) and ortho (O) absorption wavelengths are approximately equal, and longer than meta. Again this is due to resonance forms.
iii) **Strength**

The *molar absorption co-efficient* indicates the strength of a dye at low concentrations. It can be calculated using the Beer-Lambert Law

\[ A = \varepsilon c l \]

where \( A \) is the absorbance of the dye at a particular wavelength, \( \varepsilon \) is the molar absorption co-efficient, \( c \) is the concentration of the dye and \( l \) is the path-length through the cell.

iv) **Brightness**

The brightness is best described by the shape of the absorbance band; if the band is narrow and sharp, then the dye is bright, if broad, then the dye is dull. This is due to other wavelengths absorbed by the dye, other than the one which causes the hue. The superposition of these determines how broad an absorption band is.

### 1.6 COLOUR AND CONSTITUTION

All organic dyes decolourize on reduction, and the colour is associated with unsaturation. The colour of organic dye is associated with the presence of certain groups of atom, which are called chromophores. Examples of these include the nitro, nitroso, azo ethylene and carboxyl groups and it will be seen that all are unsaturated. Floye et al [2] gave the name chromogens to compounds containing such groups and showed that although they are coloured, they do not behave as dyes for the natural fibers unless they are also substituted by basic or weakly acidic groups such as –NH₂, –NH(CH₃), –N(CH₃)₂ or –OH. The presence of group of this kind greatly increases the colour-fielding power of a chromophore and for this reason they become known as “AUXOCHROMES”. An auxochrome may also serve to bring about a shift in the main absorption band form the ultra – violet into visible part of the spectrum, so that a colourless substance is transformed into a coloured one.
1.7 DYE

A dye is a coloured substance that can be applied in solution or dispersion to substrate; this giving it a coloured appearance, usually the substrate is a textile fiber but it may be paper, leather, hair, fur, plastic materials wax, a cosmetic base or food stuff. There are two types of dyes.

1. Water-soluble dyes.
2. Water-insoluble dyes.

i) Water soluble dyes:
Water solubility is conferred on many dyes by commonest of these is the sulphonic acid group, this is usually introduced into one or more of the intermediate compounds from which the dye is made but sometimes an insoluble dye is sulphonated.

ii) Water insoluble dyes:
There are various systems whereby water insoluble dyes are applied to textile and other materials, and the dyes concerned may be classified as follows.

iii) Dyes soluble in the substrate:
These include disperse dyes which are prepared as finely divided aqueous solution dyes, such as varnish, transparent liquors, printing ink etc.

iv) Temporarily solubilized dyes:
These dyes are converted into water-soluble forms by chemical modification.

v) Pigments:
Pigments differ from dyes in that they have no affinity for fibers or other substrates. Pigments are applied in the form of suspension in a drying oil or a resinous vehicle in which they are insoluble in a plastic supporting medium.

vi) Oxazine dyes:
Dyes of this class are characterized by the oxazine chromophore.

\[
\begin{array}{c}
\text{O} \\
\text{N} \\
\end{array}
\]

and are therefore, related to the azines, just described any still more closely to the thiazines.
vii) Thiazine Dyes:
These dyes are analogues of the oxazines, and atom of sulphur replaces oxygen in the hetero cyclic ring. Only five of them are known to be still manufactured (Under various names). All but one is blue or green basic or soluble dyes.

1.8 SIGNIFICANCE OF PHOTOCHEMISTRY

Photochemistry is the branch of chemistry that studies reaction driven by molecule that are split or excited by electromagnetic radiation. Many chemicals in the atmosphere absorb light of specific wavelength, usually in the visible and UV range [3-7]. The radiation acts upon the molecules to break bonds between atoms or to excite electrons within the atoms to higher energy bands. Thus excited atoms and radicals (molecules with lone reactive electrons) are formed. These species are especially unstable and reactive and thus are very effective oxidizing and reducing agents for atmospheric chemicals [8-12].

The subject of photochemistry both because of its intrinsic interest and because of contribution it has made to the general problems of reaction kinetics [13 –17].

Reactions of many types e.g. synthesis, decomposition, polymerization and isomeric change can be brought about by exposure to suitable light [18-22]. Some photochemical reactions however differ in one respect from thermal “dark” reaction where as latter always accompanied by decrease in free energy of reaction system. Certain photochemical reactions involve in free energy.

Examples of such reaction are azonization of o-phen, the decomposing of ammonia, the polymerization of anthracene, and one of the most from the biological standpoint, the photosynthetic process occur in plant whereby carbon dioxide and water in the presence of chlorophyll produce carbohydrate and oxygen [23-27].

The importance of photochemistry can be taken in consideration that uphill now on photochemistry results from 36,555 journals and 58,990 websites are available on www.scirus.com network. Photochemical reaction of dyes has 303 journals, 348 websites at www.scirus.com network. Photochemical reduction of methylene blue has resulted from 22 journals, 15 websites at www.scirus.com network.
1.9 PHOTOCHEMICAL REACTION

Reaction proceeds by absorption of light radiation. These belong to the visible and ultraviolet region of the electromagnetic spectrum (2000 to 8000 Å). The reactant molecules absorb photons of light and get excited. These excited molecules then produce the reaction. A reaction which takes place by absorption of the visible and ultraviolet radiation is called photochemical reaction.

1.10 PHOTOCHEMICAL REACTION IN THE LIQUID PHASE

Many substances undergo photochemical reaction when liquefied or dissolved in a solvent. These reactions may be initiated by direct light absorption on the part of reactant, or they may be photosensitized.

The quantum yield of a photochemical process will be less in the liquid phase than for the same reaction in gas phase as shown in Table 2. The reason is that in the liquid phase an active molecule or atom may readily be deactivated by frequent collision with other molecules or by reaction with the solvent, further more, because of the very short mean free path in the liquid phase.

Table 2

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Wavelength λ (Å)</th>
<th>Quantum yield</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gas</td>
<td>Liquid phase</td>
</tr>
<tr>
<td>2NH₃ → N₂ + 3H₂</td>
<td>2100</td>
<td>0.14 - 0.32</td>
<td>0.0 (in liq-NH₃)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NNH₃</td>
</tr>
<tr>
<td>CH₃COOH → CH₄ + CO₂</td>
<td>2300</td>
<td>1</td>
<td>0.45 (in H₂O)</td>
</tr>
<tr>
<td>Cl₂O → Cl₂ + 1/2 O₂</td>
<td>4358</td>
<td>3.2</td>
<td>1.8 (in CCl₄)</td>
</tr>
<tr>
<td>NO₂ → NO + 1/2 O₂</td>
<td>4050</td>
<td>0.5</td>
<td>0.03 (in CCl₄)</td>
</tr>
</tbody>
</table>
1.11 PHOTOCHEMICAL KINETICS

The rate laws which photochemical reactions follow are generally more complex than those for thermal reaction. In the first place, the rate of the primary activating process is controlled by intensity of the activating radiation used, and is proportional to it. The primary process is then followed by one or more reaction whose nature must be known before a rate law for the over all process can be deduced. To obtain accurate knowledge about reaction mechanism it is necessary to measure the rate at which various reactants disappear, the rate at which products are formed, and also the effect of the intensity of light as well as its frequency on these. From the data thus collected it is frequently possible to postulate a mechanism to account for the observed facts. Molecular and atomic spectra of the reactants can help in this connection. For some reaction these spectra allow the definite establishment of the nature of the primary step. Consider a reaction:

\[ A_2 \rightarrow 2A \]

Which proceeds by photochemical activation, the reaction follows the mechanism

\[ a) \quad A_2 + h\nu \rightarrow A_2^* \quad \text{(Activation)} \quad k_1 \]
\[ b) \quad A_2^* \rightarrow 2A \quad \text{(Dissociation)} \quad k_2 \]
\[ c) \quad A_2^* + A_2 \rightarrow 2A_2 \quad \text{(Deactivation)} \quad k_3 \]

The first stage in this sequence is the absorption of a quantum of light by \( A_2 \) with the formation of an activated molecule according to step (a). This activated molecule may undergo now dissociation according to reaction (b) or it may be deactivated by collision with an inactive molecule of \( A_2 \) according to reaction (c). The final product \( A \) is formed only in reaction (b). Consequently the rate of formation of \( A \) must be given by

\[ \frac{dC_A}{dt} = k_2 C_{A_2} \quad \text{1.12.1} \]

For reaction (a) the rate is determined only by the rate at which light is absorbed, follows.

\[ \frac{dC_A^*}{dt} = k_1 I_\alpha \quad \text{1.12.2} \]
The rate of disappearance of $A_2$ is given in turn by the sum of the rates of reaction (b) and (c), namely

$$\frac{-dCA_2}{dt} = k_2CA + k_3CA_2^*CA_2$$

Equating 2 and 3 to obtain the condition for stationary state, we get

$$k_1I_a = k_2CA_2 + k_3CA_2^*CA_2$$

$$CA_2 = \frac{k_1I_a}{(k_2 + k_3CA)}$$

Inserting the value of $CA^*$ in equation (1), the rate of formation of $A$ will

$$\frac{dCA}{dt} = k_2CA_2^* = \frac{k_2k_1I_a}{(k_2 + k_3CA_2^*)}$$

For every two molecules of $A$ formula one molecule of $A_2$ reacts, the photochemical efficiency of the process $\phi$ will be

$$\phi = \frac{1}{2I_a} \cdot \frac{dCA}{dt} = \frac{1}{2} \cdot \frac{[k_2k_1]}{(k_2 + k_3CA_2)}$$

where $k_1$, $k_2$, and $k_3$ represent the rate constants of the steps a, b and c respectively.
CHAPTER 2

LITERATURE SURVEY
2. LITERATURE SURVEY

A comprehensive literature survey on photoreduction of methylene blue reveals that reduction reaction of the dye is being very widely studied. It has been observed that it is easily reduced by reducing agent to colorless leuco methylene blue (LMB) which is hydrogenated form of methylene blue [MB] which can be oxidized back into blue colored methylene blue upon exposure to air.

Photoreduction has been studied on various aspects, like electron transfer reaction, photodegradation, photogalvanic effect, photolysis, photobleaching, dimerization, and solar cells.

Literature Survey gives information about different states of methylene blue, its reactivity and the mechanism of photochemical reduction reaction of the dye with organic and inorganic reducing agents.

2.1 VARIABLE STATES OF METHYLENE BLUE

Singly protonated form of methylene blue is most common and stable form of dye which is 3,7, dimethylamino phenothiazine-5-ium chloride having following structure existing at pH 4.0 [28]

![Methylene Blue Structure 1](image)

The same structure is given by Fischer [29], Kikuchi [30] and Faure [31].

The doubly protonated structure of methylene blue found in dilute acidic solution having structure is given below:

![Methylene Blue Structure 2](image)

Triply protonated form of methylene also exists in strong concentrated acidic solution
An orange color basic dye is observed in highly basic solution of pH ≥ 13 having structure.

The photochemical reduction of methylene blue results in the formation of leuco MBH [32, 33] through the photoreduction of intermediate MBH [34]. Kayser et al [35] studied the complete photoreduction of methylene and gives following mechanism.

2.2 METHYLENE BLUE- SENSITIZED ELECTRON TRANSFER REACTION

Casarotto et al [36] studied that the electron transfer reaction occurs between the triplet state of methylene blue and some terpenes e.g. citronellol, α-pinene and β-pinene. The rate constant K\textsubscript{ET} for these reactions were determined but no linear co-relation between ln K\textsubscript{ET} and ionization potential of the terpenes was found. This finding was explained in terms of steroelectronic effects associated with the terpenes. Yingjin et al [37] observed the photoinduced hydride transfer reaction between methylene blue [MB\textsuperscript{+}] and leuco crystal violet [CVH] spectrophotometrically under conditions of direct excitation of MB\textsuperscript{+} with steady illumination of visible light and of photosensitization by benzophenone (BP) and α-nitronaphthalene (NNP) with UV light. It was observed that hydride transfer reaction in acetonitrile occurs in the triplet state of [MB\textsuperscript{+}]. The quantum yield for the
disappearances of MB\(^+\) in acetonitrile was estimated to be \(1.1 \times 10^{-4}\) and it was independent of CVH concentration.

Lunak et al [38] studied the methylene blue sensitized hydroxylation of 2-hydroxy benzoic acid by hydrogen peroxide initiated by visible radiations and catalyzed by ferric chloride. Iron (11) was observed to have no appreciable catalytic effect on the photosensitized reaction at concentration as low as \(10^{-8}\) mol. dm\(^{-3}\). The first step of the reaction is the photochemical reduction of iron (111) to iron (11) i.e. the generation of Fentons reagents.

Smith and Hartman [39] observed that excited state of methylene blue bond to dry gelatin undergoes electron transfer quenching reaction with neighboring amino acid residues to give reduced methylene blue free radicals. Fluorescence characteristic of the localized methylene blue singlet state was observed from dyed gelatin, dried at 50\(^\circ\)C i.e. in its random coil form, the triplet state was subjected to rapid quenching by electron/proton transfer with proteins moieties. Dong and Wang [40] studied the heterogeneous electron transfer kinetic parameter of quasi-reversible electrode reaction.

Kuraray [41] observed that a reduction process was claimed in which an aqueous solution containing an electron donor, viologen compound and photosensitizer was placed in a chamber of the cell having a diaphragm made of the modified poly (vinyl alcohol) compound. Absorption measurements of the methylene blue solution in the cell showed that ~90% of the methylene blue was reduced to form leuco dye.

Tamura et al [42-43] and Solar and Getoff [44] studied the quenching rate constant and the radical yield of the electron transfer reaction between methylene blue and ferrocene in acetonitrile by an emission absorption flash photolysis. The rate constants were diffusion controlled, and the radical yield being much lower than unity that decreases strongly when the free enthalpy change \(\Delta H\) (gained in the electron process) became smaller. Chibisov and Zacharova [45] and Rylkov and Cheshevi [46] studied the kinetics and thermodynamic of electron transfer reaction of the dye with metal ions. The free energy
for the self exchange reaction of metal ions as well as the quantum yield for the radical were evaluated. They also discussed the measurement of the frequency factor, transmission coefficient, spin statistical factor and ion radical pair formation in relation to the electron transfer kinetics.

Nosak and Fox [47] and Lunak et al [48] observed the kinetics of electron transfer reaction of the dye. They also observed the dependence of quantum yield on incident pulse width for the reduction of observed dye. They found that for colloidal TiO₂, the difference in quantum yield was caused by absorption of incident pulses. Jockusch et al [49] studied the electron transfer between ketone triplets and organic dyes. In their studies cationic dyes such as phenosafranine, safranine T, Thiopyronine, and methylene blue were found to be efficient quenchers for the triplet states of aromatic ketones. The triplets of benzophenone, xanthone, thioxanthone benzil, and N- methylacridine quenched by these dyes via energy transfer occurred in polar solvents like acetonitrile.

Hatsui et al [50] studied the photooxidation of quadricyclane (1), mechanism explained by photoelectron transfer from quadricyclane (1), to the excited methylene blue molecule. Steiner and Winter [51] observed the photo-reduction of thionine by allylthiourea using flash photolysis. Fry and Hammonds [52] studied the quenching rate of some triplet by ferrocene (F) with a flash photolysis technique and presumed that quenching was due to the formation of a short lived charred transfer complex because they could not observe any transient absorption attributable to the addition of ferrocene (F). Many other Scientists like Bhaumik and Syed [53] and Canadall et al [54] studied that aromatic compounds were quenched by ferrocene as reported by Fry and Hammonds [52].
2.3 **ABSORPTION STUDIES OF METHYLENE BLUE**

The absorption studies of methylene blue were investigated by several workers [55 - 58]. They observed molar absorption coefficient of methylene blue cation, conjugate monoacid. Wildes et al [58] studied principal spectral features, which are summarized in Table 3.

### Table 3

**METHYLENE BLUE CATION CONJUGATES MONOACID**

Values of Molar Absorption Coefficient of Methylene Blue in Different Solvents and Acidic pH

<table>
<thead>
<tr>
<th>Solvent</th>
<th>pH</th>
<th>T-T Absorption</th>
<th>Life time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\lambda_{\text{max}}$ (nm.)</td>
<td>$\varepsilon_{\text{max}}$ (mol$^{-1}$ dm$^3$ cm$^{-1}$)</td>
</tr>
<tr>
<td>Water</td>
<td>Acidic</td>
<td>490</td>
<td>3300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>430</td>
<td>3300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>372</td>
<td>7000</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>Acidic</td>
<td>470</td>
<td>4500</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td>428</td>
<td>6200</td>
</tr>
<tr>
<td>1:1</td>
<td></td>
<td>372</td>
<td>14000</td>
</tr>
</tbody>
</table>
Ohno and Litchen [59] studied the absorption of methylene blue cation in different medium in acidic pH. The values are given in Table 4.

Table : 4

**METHYLENE BLUE CAT ION CONJUGATE MONO ACID**

Values of Molar Absorption Coefficient of Methylene Blue in Different Solvent and Acidic pH

<table>
<thead>
<tr>
<th>Solvent</th>
<th>pH</th>
<th>T-T Absorption</th>
<th>S-S-Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(\lambda_{max})</td>
<td>(\epsilon_{max})</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(nm)</td>
<td>(mol(^{-1}) dm(^3)cm(^{-1}))</td>
</tr>
<tr>
<td>EtOH/Water</td>
<td>2.0</td>
<td>703</td>
<td>18700</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td>680</td>
<td>19000</td>
</tr>
<tr>
<td>(\text{H}_2\text{SO}_4), 0.1N</td>
<td></td>
<td>370</td>
<td>13200</td>
</tr>
<tr>
<td>(1 \times 10^{-2}) N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>Acidic</td>
<td>690</td>
<td>14500</td>
</tr>
<tr>
<td>(\text{H}_2\text{SO}_4)</td>
<td></td>
<td>370</td>
<td>12500</td>
</tr>
<tr>
<td>(1 \times 10^{-2}) N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1 \times 10^{-2}) N HCl in 50% V/Vaq CH(_3)CN sol.</td>
<td>Acidic</td>
<td>670-710</td>
<td>(\geq 16200)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>370</td>
<td>15300</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Absorption studies of methylene blue cation by several investigators are summarized in Table 5.

**Table 5**

**METHYLENE BLUE CATION**

Absorption studies of Methylene blue cation by different workers.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>pH</th>
<th>T-T Absorption</th>
<th>Life time (μs)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>$\varepsilon_{\text{max}}$ (mol$^{-1}$ dm$^3$cm$^{-1}$)</td>
<td></td>
</tr>
<tr>
<td>30% aq. EtOH</td>
<td>8.2</td>
<td>840</td>
<td>20000</td>
<td>36</td>
</tr>
<tr>
<td>Borate buffer</td>
<td></td>
<td>420</td>
<td>9000</td>
<td></td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>--</td>
<td>860</td>
<td>--</td>
<td>14</td>
</tr>
<tr>
<td>Acetonitrile/Water</td>
<td>--</td>
<td>840</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>1:1</td>
<td></td>
<td>750</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>EtOH</td>
<td>--</td>
<td>860</td>
<td>$2^{+}000$</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td>420</td>
<td>14000</td>
<td></td>
</tr>
<tr>
<td>MeOH</td>
<td>--</td>
<td>420</td>
<td>11000</td>
<td>5.9</td>
</tr>
<tr>
<td>MeOH</td>
<td>--</td>
<td>410</td>
<td>22000</td>
<td>11±2</td>
</tr>
<tr>
<td>Water</td>
<td>Neutral</td>
<td>420</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>
Danziger et al [60] reported the molar absorption coefficient in aqueous alkaline solution as 9000 mol\(^{-1}\) dm\(^3\)cm\(^{-1}\) at \(\lambda_{\text{max}} = 420\) nm with life time 30 \(\mu\) s and 18000 mol\(^{-1}\) dm\(^3\)cm\(^{-1}\) at \(\lambda_{\text{max}} = 840\) nm [59]. Kayser and Young [61] determined molar absorption coefficient in methanol as 11000 mol\(^{-1}\) dm\(^3\)cm\(^{-1}\) at \(\lambda_{\text{max}} = 420\) nm with life time 5.9 \(\mu\) s.

Faure et al [62] found molar absorption coefficient as 24000 mol\(^{-1}\) dm\(^3\)cm\(^{-1}\) at \(\lambda_{\text{max}} = 850\) nm in ethanol. Muller and Crothers [63] reported the value of molar absorption coefficient in phosphate buffer \(\varepsilon_{665} = 81600\) mol\(^{-1}\) dm\(^3\)cm\(^{-1}\) while Atherton and Harriman [64] found as \(\varepsilon_{665} = 82000\) mol\(^{-1}\) dm\(^3\)cm\(^{-1}\). Solar et al [65] found the molar absorption coefficient of the protonated form of methylene blue, semiquinone (S) at different absorption bands as summarized in Table 6.

### Table 6

**SPECTRAL STUDIES OF SEMIQUINONE AT DIFFERENT pH**

<table>
<thead>
<tr>
<th>Absorption bands of (S)</th>
<th>(\text{MBH}^{++})</th>
<th>pH=1.0</th>
<th>(\text{MBH}^{+})</th>
<th>pH =4.0</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\lambda_{\text{max}}) (nm)</td>
<td>(\varepsilon_{\text{max}}) (mol(^{-1}) dm(^3)cm(^{-1}))</td>
<td>(\lambda_{\text{max}}) (nm)</td>
<td>(\varepsilon_{\text{max}}) (mol(^{-1}) dm(^3)cm(^{-1}))</td>
</tr>
<tr>
<td>1(^{st})</td>
<td>880</td>
<td>1190</td>
<td>880</td>
<td>2350</td>
</tr>
<tr>
<td>2(^{nd})</td>
<td>380</td>
<td>900</td>
<td>395</td>
<td>910</td>
</tr>
<tr>
<td>3(^{rd})</td>
<td>290</td>
<td>3750</td>
<td>290</td>
<td>3800</td>
</tr>
</tbody>
</table>
Absorption studies of thionine in different solvents were carried out by several investigators [62, 66 - 70] and the results are summarized in the following Table 7.

**Table 7**

**ABSORPTION STUDIES OF THIONINE IN DIFFERENT SOLVENT**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>pH</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt; (nm)</th>
<th>ε&lt;sub&gt;max&lt;/sub&gt; (mol&lt;sup&gt;-1&lt;/sup&gt; dm&lt;sup&gt;3&lt;/sup&gt;cm&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Life time µs</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>~ 0.7</td>
<td>495</td>
<td>5000</td>
<td>--</td>
<td>[66]</td>
</tr>
<tr>
<td>Water</td>
<td>≈ 1</td>
<td>655</td>
<td>15400</td>
<td>--</td>
<td>[67]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>375</td>
<td>14000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>--</td>
<td>650</td>
<td>--</td>
<td>--</td>
<td>[62]</td>
</tr>
<tr>
<td>Water</td>
<td>2.5</td>
<td>645</td>
<td>--</td>
<td>16±2</td>
<td>[68]</td>
</tr>
<tr>
<td>Water 1 x 10&lt;sup&gt;-2&lt;/sup&gt; mol. dm&lt;sup&gt;3&lt;/sup&gt; acid</td>
<td>2</td>
<td>650</td>
<td>16000</td>
<td>--</td>
<td>[69]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>380</td>
<td>14000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water/EtOH 1 x 10&lt;sup&gt;-2&lt;/sup&gt; mol. dm&lt;sup&gt;3&lt;/sup&gt; acid</td>
<td>Acidic</td>
<td>650</td>
<td>20500</td>
<td>--</td>
<td>[69]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>380</td>
<td>16500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water/Acetonitrile 1 x 10&lt;sup&gt;-2&lt;/sup&gt; mol. dm&lt;sup&gt;3&lt;/sup&gt; acid</td>
<td>Acidic</td>
<td>650</td>
<td>20000</td>
<td>--</td>
<td>[70]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>380</td>
<td>15500</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The values of molar absorption coefficient of $10^{-5}$ mol. dm$^3$ solution of dye at different wavelength and pH studied by several workers are summarized in Table 8.

**Table 8**

**VARIATION IN ABSORPTION OF METHYLENE BLUE WITH pH**

<table>
<thead>
<tr>
<th>pH</th>
<th>1$^{st}$ Band</th>
<th>2$^{nd}$ Band</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>$\varepsilon_{\text{max}}$ (mol$^{-1}$ dm$^3$ cm$^{-1}$)</td>
<td>$\lambda_{\text{max}}$ (nm)</td>
</tr>
<tr>
<td>0.65</td>
<td>880</td>
<td>1270</td>
<td>375</td>
</tr>
<tr>
<td>2.0</td>
<td>Orange red</td>
<td>8800</td>
<td>380</td>
</tr>
<tr>
<td>2.10</td>
<td>665</td>
<td>74000</td>
<td>370</td>
</tr>
<tr>
<td>3.40</td>
<td>880</td>
<td>8800</td>
<td>410</td>
</tr>
<tr>
<td>6.00</td>
<td>880</td>
<td>3300</td>
<td>--</td>
</tr>
<tr>
<td>7.00</td>
<td>665</td>
<td>1700</td>
<td>--</td>
</tr>
<tr>
<td>8.20</td>
<td>840</td>
<td>35000</td>
<td>665</td>
</tr>
<tr>
<td>10.20</td>
<td>--</td>
<td>20000</td>
<td>500</td>
</tr>
<tr>
<td>7.00</td>
<td>656</td>
<td>53500</td>
<td>--</td>
</tr>
</tbody>
</table>
2.4 REDUCTION STATES OF METHYLENE BLUE

Reduction state of methylene blue is color less state of the dye where hydrogen atom from reducing agent or from medium attacks to the nitrogen atom of the ring [80-82]. Keene et al [83] explained the mechanism of the reaction. Two processes take place one irreversible and other irreversible; the first one predominated in the presence of organic reducing agent. In neutral solution of methylene blue, the solvated electron reacts with MBH⁺ ( \( k = 2.5 \times 10^{10} \) mol dm⁻³ ) resulting the production of semiquinone (\( \lambda_{max}=420 \) nm, \( \epsilon_{420}=1040 \) mol⁻¹ dm⁻³ em⁻¹). Solar et al [65] observed that semiquinone dissimulates to leuco methylene blue with a rate constant as \( 2K = 3 \times 10^9 \) mol dm⁻³ s⁻¹.

REDUCTION PROCESS OF THE DYE

The photochemical reduction of a dye has been described by several workers (\( \lambda_{max}=490-668 \)) [84-88]. The proposed scheme of the reduction process is described as follows.

\[
\begin{align*}
D^+ & \xrightleftharpoons[k_1]{K_1} D^{*+} \\
& \xrightarrow[k_2]{hv} \xrightarrow[k_{1[R]}]{K_2} M \rightarrow \text{Reduction Product}
\end{align*}
\]

where \( D^+ \) is the dye cation in ground state and \( D^{*+} \) is that in first electronically excited singlet state. \( D^{*+} \) is a second metastable excited state. \( [R] \) is the concentration of electron donor and \( M \) is monomer of half reduced dye. \( K_1 \) and \( K_2 \) are the rate constants for dissociation process and \( k_r \) is the rate constant for reduction process and \( k_d \) is the rate constant of semi reduced dye.

Imamura and Koizumi [84] and Linqvist [89] proposed another mechanism, according to which the first triplet state \( D^1 \) goes to ground state by \( D^1 + D^1 \rightarrow 2D \) process or
by monomolecular process but as the ground state, dye accumulates the \( \text{D}^\prime + \text{D} \rightarrow 2\text{D} \) process and begins to make greater contributions. It produces semiquinone and half oxidized state X. The whole process can be summarized as follows.

\[
\begin{align*}
\text{D}^\prime & \xrightarrow{k_1} \text{D} & \quad & 1 \\
\text{D}^\prime + \text{D} & \xrightarrow{k_2} 2\text{D} & \quad & 2 \\
\text{D}^\prime + \text{D} & \xrightarrow{k_3} \text{X} & \quad & 3 \\
\text{X} & \xrightarrow{k_4} \text{leuco dye} & \quad & 4
\end{align*}
\]

where \( k_1 \rightarrow k_4 \) are the rate constant for different processes of the reduction of methylene blue.

General mechanism for photoreduction of methylene blue is given as follows:

\[
\begin{align*}
\text{D} + \text{hv} & \rightarrow \text{D}_s & \quad & 5 \\
\text{D}_s & = \text{Singlet state of the dye} \\
\text{D} & = \text{ground state of the dye} \\
\text{D}_s & \rightarrow \text{D} + \text{hv}_f & \text{Fluorescence} & \quad & 6 \\
\text{D}_s & \rightarrow \text{D} + \text{Q} \quad \text{(heat)} & \text{Internal conversion} & \quad & 7 \\
\text{D}_s & \rightarrow \text{D}_T & \text{Transition to triplet state} & \quad & 8 \\
\text{D}_T & \rightarrow \text{D} + \text{hv}_T & \text{Phosphorescence} & \quad & 9 \\
\text{D}_T + \text{AH}_2 & \rightarrow \text{DH} + \text{AH} & \quad & 10 \\
\end{align*}
\]

Formation of free radical \( \text{AH}_2 \) is reducing agent

\[
\begin{align*}
\text{D}_s + \text{AH}_2 & \rightarrow \text{DH} + \text{AH} & \quad & 11
\end{align*}
\]

Formation of free radical electron transfer

\[
\begin{align*}
2\text{DH} & \rightarrow \text{DH}_2 + \text{D} & \text{Semiquinone formation} & \quad & 12 \\
2\text{AH} & \rightarrow \text{Product} & \text{Disappearance of free radical} & \quad & 13
\end{align*}
\]

Waltz and Munster [90] proposed spatial pattern formation in methylene blue reactions system under basic condition. Usui and Koizumi [91] studied kinetics of photoreduction of methylene blue and found that in the borate buffer solution the radiation proceeds approximately as of the first order with respect to the dye, while in phosphate buffer...
solution the reaction is a simple second order with respect to the dye. This was due to some difference between the reaction mechanisms of these two cases.

Ross and Calvin [92] showed that reaction of methylene blue was endothermic by 50 kcal/mol at pH = 1.0. Somer and Temizer [93] investigated reduction of methylene blue by visible light in absence of reducing and proposed following mechanism involving two excited species.

\[
\begin{align*}
\text{MB} & \xrightarrow{\text{hv}} \text{MB}^* & \text{Excited singlet state} & \text{1} \\
\text{MB}^* & \rightarrow \text{MB}^1 & \text{Excited triplet state} & \text{2} \\
\text{MB}^1 + \text{MB}^1 & \rightarrow \text{MB} + \text{MB}^* & \text{3} \\
\text{MB}^1 & \rightarrow \text{MB} & \text{4} \\
\text{MB}^* & \rightarrow \text{M}3 & \text{5} \\
\text{MB} + \text{MB}^1 + \text{H}_2\text{O} & \rightarrow \text{S} & \text{6} \\
2\text{S} + \text{H}^+ & \rightarrow \text{LMB} + 3\text{MB} + \text{H}_2\text{O}_2 & \text{7} \\
\text{LMB} + \text{H}_2\text{O}_2 & \rightarrow \text{MB} + 2\text{H}_2\text{O} + \text{H}^+ & \text{8}
\end{align*}
\]

where MB* is the first excited state of single methylene blue, MB1 is the corresponding triplet state and S is hypothesized long lived intermediate and LMB is the leuco methylene blue. Usui et al [94], Imamura and Koizumi [84] and Usui et al [95] studied that methylene blue shows photoreduction in buffer solution by visible light without any reducing agent and observed that quantum yield of photoreduction reaches as 10^-2 in the phosphate buffer.

2.5 PHOTO REDUCTION OF METHYLENE BLUE BY LIGHT

Usui and Koizumi [91] studied photoreduction of methylene blue and thionine in ethanol. They observed that as initial concentration of dye was varied in the region 10^-6 – 10^-5 mol dm^-3 for methylene blue and thionine, the over all second order rate constant (k)
decreased. They also showed a linear relationship between the values of quantum yield ($\phi$) and mole fraction of ethanol. Somer and Teanizer [93] observed the photoreduction of methylene blue and thionine in water by red light. Reaction energy consideration requires two photons to reduce each methylene blue molecule. They also studied the influence of light intensity and methylene blue concentration on the rate constant, and proposed two photon mechanisms involving a long-lived dimer intermediate molecule. Usui and Koizumi [91] found that only thiazine dyes such as methylene blue, methylene green, toluidine blue, thionine and new methylene blue show photoreduction by absorption of photon of light, no other dye like phenoxazine dye and indophenone show photoreduction by absorption of light without reducing agent.

2.6 PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE BY EDTA

Kosui et al [96] and Bonneau [97] observed the photoreduction of methylene blue with EDTA. They observed an unusual sigmoidal dependence on concentration of EDTA, and found that the maximum quantum yield at high concentration of EDTA ($\sim 0.1 \text{ mol dm}^{-3}$) exceeded to the value (0.156) provided from the equation representing the quantum yield in aqueous solution

$$\phi = \frac{\phi_H + k_r(\mu) \text{ [EDTA]}}{k_d + k_s(\mu) \text{ [EDTA]}}$$

where $k_r(\mu)$ and $k_s(\mu)$ are the reactive and overall rate constant for the reaction between triplet MB with EDTA respectively and $k_d$ is the rate constant for the product, $\phi_H$ = intersystem crossing probability of methylene blue.

Oster and Wootherspoon [98] studied the photoreduction in the presence of EDTA. Bonneau [97] and Wildes et al [58] studied the photoreduction of thiazine dye by EDTA. The apparent reactivity of thiazine dyes in their triplet states has been studied in aqueous solution in the pH range 4-8. The trianionic species of ethylene diamine tetra acetic acid (EDTA) was used as electron donor. The pH dependence was found to be related to the change in the degree of protonation of the triplet excited dye. The apparent reactivity and life time of two differently protonated form of thionine, azur B, and methylene blue were
determined by classical and dye laser flash techniques. Kaneko and Yamada [99] studied the effect of addition of EDTA in phenothiazine and phenothiazine derivatives in presence of reducing agent by visible light of 400 -800 nm. Wave length dependence and the optimum conditions for the photo potential induced in a thionine -Fe^{2+} systems were studied. The photopotential decreases proportionally with the increasing amount of EDTA added to the thionine Fe^{2+} systems. In case of methylene blue ion (+2) system, the addition of small amount of EDTA causes an increase in its photo potential. It reached its maximum when the molar ratio of EDTA/MB =3 when the molar ratio Fe^{2+}/MB was 500, the photopotential of which was ~3.4 as high as that without addition of EDTA. Merkel and Nickerson [100] studied the photochemistry of riboflavin and noted that methylene blue is photo reduced with EDTA. Oster and Wootherspoon [98] investigated the role of EDTA as an electron donor in the photoreduction of methylene blue.

2.7 PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE WITH INORGANIC REDUCTANTS

Jain et al [101] studied the photocatalytic effect of MB on Na_{2}CO_{3} and K_{2}CO_{3}. The photocatalytic formation of formic acid and formaldehyde were also measured spectrophotometrically. The effect of variation of various parameters like pH, amount of photocatalyst (MB), concentration of Na_{2}CO_{3} and K_{2}CO_{3}, light intensity etc. on the yield of photo product were also investigated. Lakshami et al [102] studied the photocatalytic degradation of methylene blue assisted by TiO_{2} in aqueous solution. The rate constants for this heterogeneous photocatalysis were evaluated as a function of the concentration of the dye, the amount of TiO_{2} and the pH. Kirjak [103] observed the electroreduction of bismith (III) and tellunium (IV) for chloride and tartrate solution of thiazine dyes. The influences of thionine, Azur A and methylene blue on the electroreduction were also discussed.

Gessner et al [104] studied the variation of the electronic spectra of basic dye methylene blue and neutral reductant with time. Harmatz and Blauer [105] reported reaction of methylene blue (oniform) excited by visible light. Methylene blue causes oxidation of sodium ascorbate, ferrocytochrome C and EDTA. All investigations were carried out
spectrophotometrically at 25 °C, in dilute and deserted aqueous solution. Murthy et al [106] observed the electrochemical reduction of thionine and methylene blue in the presence of iron (Fe^{3+}), and examined in terms of catalytic current due to homogenous chemical reaction between leuco dye and Fe^{3+}. Xiong and Jen [107] studied the trace amount of manganese in water by the use of methylene blue in presence of acetate buffer solution. The rate of decolorization depends on the concentration of Mn (I). Kamburova [108] studied the interaction of Cr (IV) and the thiazine dye (MB). A sensitive and selective method for the determination of micro quantities of Cr (VI) in soils and alloys was suggested. Zhu et al [109] studied the polarographic characteristics of tellurium (IV) and basic dye at mercury electrode. He found that the mechanism of the electrode process depends on the concentration of Te (IV). Tanaka et al [110] studied the kinetics of thionine reduction by several Fe (II) chelates in presence of nitrogenated system at pH 7.0. The oxidation of leuco thionine (II) by Fe (II) chelate was also examined. The physicochemical studies of some metal complexes such as vanadium (V), niobium (Nb) and tantalum (Ta) with dyes such as Erichrome cyanine-R and methylene blue have been studied by Awadallah et al [111]. The complexion processes of metal with methylene blue were investigated by conductometric and spectrophotometric methods. The measurements were used to investigate stoichiometry, formation constants and thermodynamics parameters like enthalpy change (ΔH°), free energy change (ΔG°) and entropy change (ΔS°) of the complexes. The effect of pH on absorption spectra of complexes, time and temperature on the stability of metal chelates were also studied.

Raju and Raju [112] and Raju et al [113] observed that phenothiazine dyes were reduced by Fe^{2+} as a reductant in buffer solution in presence of sodium oxalate of concentration ranging 0.06-0.12 mol dm^{-3}. The dyes reduced to their colorless leuco bases in a 2e⁻ reduction with Fe^{2+}. The function of pH and oxalate ion in the reaction medium was also discussed. A more extensive study on the redox potential of Fe (III) / (II) iron couple in buffer medium was also reported by Raju and Raju [112]. Zhang and Shen [114] discussed the photocatalytic reduction of methylene blue by cadmium sulphide CdS mono particles mediated in reverse micelles. Bunce [115], Ohashi et al [116] and Freeman et al [117] reported that photochemistry of aromatic halides have been actively investigated due to their role as an environmental pollutants or photo ability as mean of their efficient
conversion of less toxic substance. Photoability enhanced in presences of electron transfer agents like amines and dienes.

Sharma et al [118] investigated the photocatalytic bleaching of the dye spectrophotometrically with zinc oxide powder. Quitevis et al [119] reported the photoreduction of methylene blue on cadmium sulfide powder and reduction of the surface bound methylene blue has been investigated using both picoseconds multiple light scattering transients and transient band gap emission measurements. A comparison of the bleaching kinetics for adsorbed dye with near-IR absorption band gas emission transient from the semiconductor indicate that interfacial electron transfer is driven by mobil conduction band carriers with little participation by the larger lived weakly trapped carriers.

Fretwell and Douglas [120] reported that photoreduction of methylene blue is independent of film thickness of T O₂ and appears to be limited by the surface area of the film. Hence quantum yield for methylene blue reduction (φM BR) fall significantly from 1.7 to 0.5% as film thickness and hence photo absorption efficiency is increased. Kundu et al [121] studied the increased encounter probability between ammonia and an immobilized cationic dye in non-ionic micelles demonstrating the reduction capability of ammonia while present even at ppm levels. Catalytic amounts of silver nanoparticles (7nm in size) have been shown to enhance the reaction rate of the reduction reactions, explained by the strong affinity between Ag nanoparticles and the nitrogen atom of the donor ammonia molecule.
2.8 PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE WITH ORGANIC REDUCTANTS

Generally photochemical reductions of methylene blue were carried out with organic reductants [122-132]. The nature of organic reducing agent were as amines, phenylhydrazines, anethole, urea, thiourea and their derivatives N-phenylglycine, nitriloacetic acid, ethylene diamine tetra acetic acid, paraphenyldiamine (PPDA) and mono, di, trimethylene amines.

Matsumoto [133] found the effect of pH on quantum yield of photoreduction of methylene blue in the pH range 5.0—10.5. He also noted the effect of buffer solutions and to a possible change in the ionic species of N-phenylglycine with the pH value. N-Phenylglycine behaved as a monobasic acid of \( \text{PK}_a \) 4.3 to base titration, so that it existed solely as univalent anion \( \text{C}_6\text{H}_5\text{NH.CH}_2\text{COO} \) in this pH range. The lifetime of methylene blue was found to be dependent on the pH value [134]. The dependence of the lifetime on pH is summarized in Table 9.

<table>
<thead>
<tr>
<th>Dyes</th>
<th>Life time (μs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH = 4.5</td>
</tr>
<tr>
<td>Thionine</td>
<td>8.5</td>
</tr>
<tr>
<td>Azur B</td>
<td>5.0</td>
</tr>
<tr>
<td>Methyle blue</td>
<td>4.0</td>
</tr>
</tbody>
</table>

The effect of acidity and concentration of phenylglycine and thionine on the quantum yield of the photochemical reduction were studied by Uddin [135] using absolute methanol as nonaqueous medium. The quantum yield was found to be a function of reductant concentration, acidity and independent of concentration of thionine. Hasan and Al Amro [136] studied the kinetics of photosensitized reaction of methylene blue with pre
irradiated cyclohexanone and cyclo-pentanone. According to their investigation, reaction followed 2\(^{nd}\) order kinetics and the rate of reaction was directly proportional to the time of irradiation. The mechanism possibilities were also suggested. Eimer et al [137] studied light induced reaction of methylene blue and related phenothiazinium dyes biological substrates. They reported the properties of excited state of dyes and their reaction with nucleic acid.

Bertolotti et al [138] investigated the quenching of excited singlet and triplet states of methylene blue and the Azurs A, B and C by benzoquinones in methanol and acetonitrile. Rate constants were obtained from fluorescence life time for singlet state process. Singlet quenching rate constants are nearly diffusion controlled in both solvents. 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 die excited state to quinones. Jonnalagadda and Natter [139] reported spectrophotometrically the kinetics and mechanism of reduction of aqueous toluidine blue by \(\text{C}_6\text{H}_3\text{NHNH}_2\). The forward rate constant for uncatalysed and acid catalysed reaction were \(1.4 \times 10^{-2} \text{ mol}^{-1}\text{.dm}^3\text{ s}^{-1}\) and 60 \(\text{mol}^{-1}\text{.dm}^3\text{ s}^{-1}\). Uddin [140] observed photo reduction of methylene blue by triethylenediamine by special type of optical arrangement in absolute methanol. It was found that an equilibrium was established between associated complex and triplet state of methylene blue with reductant. Photo-reduction of methylene blue via chemical quenching of triplet benzophenone was studied by Sindhu et al [141]. The kinetics data fit into the integrated rate expression, which was \(1/2\) order with respect to the substrates. The dye does not show any aggregation, but complexation between ground state benzophenone and MB\(^+\) was observed. Mishra and Chansoria [142] studied the oxidation of mercapto succinic acid by methylene blue in acidic medium. The reaction investigated at various ionic strengths and H\(^+\) ion concentration. Reaction showed 1\(^{st}\) order kinetics with substrate and second order kinetics in methylene blue. The rate constant showed optimum value with respect to hydrogen ion concentration and the ionic strength of the reaction system. Activation parameters have been evaluated and possible reaction scheme has been proposed.
Bonneau and Dubien [57] observed the production of singlet oxygen by thiazine dye photosensitization. For methylene blue in aerated solution, the production of $^1O_2^*$ is approximately five times more efficient in basic than in acidic medium. This was shown to be related to the $pK_a$ of the triplet dyes, by evaluating the yields of $^1O_2^*$ from the lifetime and the quenching rate constants for the two ionic species of sensitizer triplets measured by laser flash photolysis. Moreover changes in the quenching rate constant of the thiazine triple states can be correlated with their triplet energies. Chu and Ma [143] observed photodecolorization of various dyes at 253.7 nm. 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 [49] observed that nucleophilic radicals, such as $\alpha$-amino and ketyl radicals, produced by reaction of benzophenone triplet with amines and alcohols respectively.

Neuman et al [144] observed the effect of ground state association on the photoreduction of basic dyes by anionic N-phenylglycines and their neutral esters. Fischer and Schiller [145] observed the effect of the anionic polyelectrolyte Na polyphosphate (NaPP) on primary processes and on quantum yield of photoreduction of methylene blue. Flash photolysis showed that the bimolecular rate constants of electron transfer physical deactivation were influenced by the fixation of the dye on the polyelectrolyte. Mowry and Ogren [146] studied reduction of methylene by ascorbic acid spectrophotometrically at low pH with previous conclusions based upon visual determinations of colour loss. Spectrophotometric studies of temporal decay of MB$^+$ absorbance at 665 nm 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 [147] 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 $\mu$S at 20°C in water.
2.9 PHOTO GALVANIC EFFECT

Bayer et al [148] reported the photogalvanic effect in electrochemical cell employing aqueous methylene blue and Fe (II)/(III) couple electrolyte and nickel mesh electrodes. Five different standard hydrogen cell configurations were setup by modifying the electrolyte long term open circuit voltage measurements conducted in order to test the stability of the cells. Light on-off reproducibility experiments were also carried out during lengthy cell operation. Gokalp and Cussler [149] investigated that short lived reaction intermediate can be separated and prevented from reacting further by using a membrane reactor. They described such a reactor for the thionine ferrous ion solar cell. Hamdi et al [150] studied the photogalvanic effect of 7 complexes of V(III) tris (β-diketonate), V(acetylacetonate), V(trifluoroacetylacetonate), V(5-Meo-benzoyl trifluoroacetonate), and V(thenoyl trifluorocetionate), in new methylene blue in aqueous MeCN solution in a photogalvanic cell of 70 cm² capacity with identical platinum electrode of 0.25 cm² using visible light of intensity 80mW/cm² at 25°. The sun light engineering efficiency (SEE) was determined for each complex; the best (SEE) was obtained when V(111) (acetylacetonate) was used at a concentration of 1 x 10⁻⁴ mol dm⁻³ with new methylene blue concentration of 1 x 10⁻⁵ mol dm⁻³ at pH 4 in 40% MeCN solution. The effect of incident light intensity on the photogalvanic performance and the action spectrum of the present system were also investigated. According to the experimental results, a mechanism of the oxidation-reduction reaction between the dye and V(III) complexes was proposed. Gangotri et al. [151] discussed an H-shaped photogalvanic cell with methylene blue as photose isitizer, EDTA reducing agent and NaOH aqueous electrolyte containing cetylphridinium chloride surfactant micelles. The cell had an SCE electrode in one arm of the cell and a pt electrode in the other arm. When the pt electrode was illuminated with a W lamp (200W), a photogalvanic and photocurrent of 877.0 mV and 140.0 μA respectively were generated.

Pezza et al [152] studied the photogalvanic effect in the system containing basic dyes [such as thionine, safarineT, and Acridine orange] and a reducing agent Fe⁺⁺, EDTA,
Triethanolamine and allythiourea photovoltages and photocurrents generated by photogalvanic cell were measured in presences of various reducing agents. The results suggested that the photogalvanic system containing aminothioip, reducing agents yield higher values of photovoltages and photocurrent than those employing Fe$^{2+}$ as reducing agent. The energetics and feasibility in their system were also discussed. Thiazine family dyes especially thionine and methylene blue have become more important in recent years, especially as sensitizers in photogalvanic cells for photoelectrochemical utilization of solar energy as discussed earlier [100, 153-156]. Huwane et al [157] studied the optimization and characteristics of dry film merizable recording material. Diffraction efficiencies of 55% with the energetic sensitivity of $60 \text{mJ/cm}^2$ have been obtained in the photosensitive films of 150-$\mu$m thickness with a spatial frequency of 2750 lines/mm.

Asok and Jana [158] discussed, from early development to recently developed stages of solar cells considering the effect of different parameters. Primitive photoelectrochemical (PEC) cell and PEC cell with Fe-thionine and different dyes-reducing agent have been reviewed. Solar cells with phenazine dyes give more output in solar cell. They also reported the utilization of surfactant media for storage of solar energy and production of hydrogen from solar cell using dyes.

Sharmila et al [159] investigated a new photoelectrochemical cell consisting of phenosafrain, a cationic phenazine dye and ethylene diamine tetra acetic acid in surfactant solution, separated from a saturated aqueous solution of iodine by a pyrex sintered glass membrane. The different surfactants used were cetyltrimethyl ammonium bromide (CTAB cationic), sodium laurylsulphate (SLS; anionic) and Triton X-100 (neutral). The result showed that the electrical performance of the cell was decreased in SLS, increased slightly in Triton X-100 and increased appreciably in CTAB as compared with the cell and without surfactant.
2.10 LASER PHOTOLYSIS

Jia et al [160] studied pulse radiolysis of methylene blue and toluidene blue with poly(vinylalcohol). The transient spectra obtained between 400 and 800 nm and are assigned to the semiquinone radical anions, decayed by a second order process. Further more methylene blue in poly (vinyl alcohol) film were bleached by γ-radiolysis and the change in absorbance was found to be linear with the dose. Kamat and Lichtin [161] have measured the electron transfer yield by monitoring semi reduced thionine by laser flash photolysis method. It was concluded that the rate constant for quenching and the rate constant for electron transfer depend upon various parameters such as nature of solvents, nature of anions present and the ionic strength of the media. Similarly the photoreduction of methylene blue by a ruby laser light was studied [162, 163]. The kinetics treatment and the measurements of the apparent activation energy showed that under the high intensity radiation, the excited triplet state of methylene blue plays a significant role in its photoreduction in the presence of p-chlorophenylglycine. Moreover, photosgalvanic conversion in iron thiazine cell was also discussed by Kamat and Lichtin [163]. Dose response characteristics of polyvinylalcohol film in the presence of methylene blue or methyl orange were studied by Chung et al [164]. The short lived intermediate products from these dyes, their semiquinones, have been successfully investigated by flash photolysis in the presence of various electron donors like Fe²⁺, allylthiourea and diphenylamine etc. In these system reductive quenching of the excited dye, predominantly occurs through triplet state reported by Kamat and Lichtin [163], Havemann and Pietsch [165], Kelly and Rodgers [33], Vogelmann et al [166], Steiner et al [167]. Kikuchi e: al [168] studied that extent of reduction of the excited dye depends on several factors e.g. the free enthalpy changes involved and the oxidation potential of the reductant electron donor, concentration of the counter ion, nature of solvent and pH of the solution [169] and [64]. The improved pulse radiolysis technique investigated by Getoff and Schenck [170] with extended simulation computation had allowed better investigation in the ultraviolet absorption range of both thionine and methylene blue, where there is a strong super imposition of several transients and where bleaching occurs. Getoff and Schenck [170] also reported that a third absorption band arises due to semiquinone formation. Gerald and
Marco [171] reported spectra as well as temporal characteristics of the transient absorption produced by flash photolysis of solution of methylene blue in ethanol. A transient species with an absorption maximum at 380 nm and life time of 4 μs was observed in acidic solutions. The rate constants for the reaction of this species with oxygen and other quenchers were determined. This suggested that the transient species is a semi reduced methylene blue free radical [165, 172-175].

Kamat and Lichtin [163] investigated flash photolysis (694.3 nm) of MB⁺ in alkaline water at a pH 8.2 and neutral acetonitrile system, in addition to the formation of the triplet dye. This triplet dye has a small absorption band at 520 nm. It was assigned to semi oxidized methylene blue (radical cation) MB2⁺ and it was concluded that MB2⁺ formations is the result of an electron ejection process from the excited dye molecule. The reactivity of thiazine dye, methylene blue, thionine and Azur B in aqueous solution in their triplet state was studied by Bonneau et al [176]. The apparent reactivity and life time of two differently protonated forms of methylene blue, thionine and Azur B were studied using dye laser flash photolysis.

Buchweiser et al [177] studied the fluorescence quenching of both protonated and unprotonated forms of thionine and neutral red by several electron donors. The difference between the pKₐ of the singlet state and those corresponding to semi reduced radicals were established. Atherton and Harriman [64] studied the role of MB⁺ excited states in fast H-atom abstraction reaction by pulse laser techniques. The photoreduction of methylene blue has been extensively studied by continuous irradiation and by the flash photolysis [89, 178] at various temperatures. Sindhu et al [141], Zhong and Zhang [179] and Takana et al [180] determined the quantum yield for the reduction of methylene blue by photolysis technique. They observed that quantum yield for the reduction of dye in the absence of reducing agent was negligibly small and the triplet state of methylene blue has no longer life time as to be detected by photolysis technique.

Photoreduction of thionine by reductant such as allylthiourea was investigated by Steiner and Winter [51] using flash photolysis and continuous illumination technique. The quantum yield of photoreduction was increased by addition of azulene. During photolysis,
(thionine-azulene system) thionine was reported to be converted into its photoreduced form i.e. leuco thionine. Similarly azulene reacts with the basic form of the thionine triplet to produce the semithionine radicals and then reduced to leuco thionine by addition of allylthiourea. In such cases the quantum yield was found to be considerably higher as compared to the thionine and allylthiourea system. Gak and Nadtochenko [181] investigated the quenching of triplet excited dye eosine Y and methylene blue by hydrogen peroxide by laser and steady state photolysis. The apparent activation energy of the reduction of methylene blue was obtained by varying the laser light intensity [55]. It was found that the photoreduction through both the lowest triplet state and excited triplet state equally contribute to the apparent activation energy. In this connection, the activation energy extrapolated to zero light intensity should correspond to the activation energy for the lowest triplet states. The activation energy was also found to be decreased with an increase in dose intensity. The quenching rate constants and the radical yields of the electron transfer reaction between triplet methylene blue and ferrocenes have been determined with acetonitrile by an emission absorption flash technique [182]. The rate constants are found to be diffusion controlled and the radical yields, being much lower than unity that decreased strongly when the free enthalpy, (ΔH°) and free energy (ΔG°) (gained in the electron transfer process) become smaller.

2.11 PHOTOBLEACHING OF THIAZINE DYE

Photochemical bleaching of thiazine dye were studied by several works such as Imamura and Koizumi [183], Oster and Adelman [184], Oster and Wootherspoon [185], Vonnach and Getoff [186], Matsumoto [133] and Muneer et al [187] and others investigated the decolourisation of methylene blue by ultraviolet irradiated TiO₂ in the gas phase. Ahmed [188], Uddin [140] reported that aqueous thionine, a member of thiazine dye did not give correct result due to unstability and diversions process. Saleem and Maazawi [189] observed the kinetics and mechanism of color removal of methylene blue with hydrogen peroxide in presence of catalyst alumina surface. They reported that reaction is first order with respect to methylene blue. Mahmood and Kok [190] et al reported photobleaching of methylene blue embedded in the solid matrix PMMA (poly methyl methacrylate), PVA poly (vinyl alcohol) and gelatin by photoacoustic technique. Photoacoustic signals were measured as a function of time, chopping frequency and the beam power. Experimental
results indicate that photobleaching rate is proportional to the incident laser power (260 - 300 mW) and decreased with increasing the concentration of dopant molecules. Methylene blue embedded in PVA shows the highest photobleaching rate than followed by gelatin and PMMA. The increased of photobleaching rate in PVA is also noticed when the beam power is increased. The thermal diffusivity values obtained for bleached PMMA, PVA and gelatin were $1.46 \times 10^{-3}$ cm$^2$/s, $0.83 \times 10^{-3}$ cm$^2$/s and $1.10 \times 10^{-3}$ cm$^2$/s respectively. Spectrophotometric and Raman spectroscopy were used to confirm the photoacoustic results. Bleaching of methylene blue was observed by alkaline glucose [178]. Tanielian et al [191] reported that methylene blue was sensitized by photooxidation of (polycis-1,4-butyadiene) in C$_6$H$_6$ / MeOH. Here a decrease in concentration of methylene blue and molecular weight of poly butadiene exhibited an induction period followed by increased rate. The results were consistent with sensitized photolysis of OOH groups which yielded radical species leading to bleaching and chain scission.

Epling and Lin [192] reported that titanium dioxide was shown to be generally effective as a catalyst for photobleaching of many organic dyes in aqueous solution, using visible light. However results from study of LF dyes indicate that photobleaching rates differ significantly from families of dyes with different functionalities and are dependent on the light source and crystalline form of TiO$_2$ used. Kinetics analysis indicates that the dye photobleaching rates follow pseudo-first-order kinetics. Natural sunlight was effectively used to photobleach some of the dyes.

### 2.12 POLYMERIZATION OF METHYlene BLUE

Danziger et al [60] observed dimerization of methylene blue in water, with the wave length of ~550 nm and half time = ~2 µs. Photo polymerization process was studied by Shepp et al [193] and Chaberek and Alle [194]. They reported that for photo polymerization process, the initiating radical is semi methylene blue. The basic reaction mechanism for methylene blue triethanolamine, methylene blue acrylamide, thionine triethanolamine and thionine acrylamide systems [193] are identical with that described for thionine nitropropiombonamide acrylamide. In these reactions the rate of polymerization depends on concentration of the reductant as well as on the presence of
available oxygen. The polymerization process decreases with the increase in oxygen level. Vedenee et al [195] reported kinetic measurements of acrylamide polymerization in water ethylene glycol. Their binary mixture and water-glycerol binary mixture were performed dilatometrically. The reaction was photoinitiated by irradiation of methylene blue (DCl) in the presence of triethanolamine as a reducing agent. The application of a moderate external magnetic field (0.1T) led to increase the process of polymerization in ethylene glycol or glycerol-containing solutions having δ = 3.0-17.0. The photoreduction of DCl in viscous homogeneous polymer-containing solution was studied by flash photolysis. The application of a magnetic field resulted in the increases of the concentration of transient free radicals, which initiates the process of polymerization. Magnetic field effects are described within the frame of the hyperfine coupling mechanism. Despite having high dynamic viscosities, polyacrylamide solutions are characterized by low micro viscosity and exhibit a relatively small magnetic field effect. Diffusion rates of low molecular weight compounds in polymeric solution were also discussed in such studies.

Mazöhe et al [196] found an electron transfer photosensitization system for polymerization. In this investigation methylene blue triphenylalkyl borate was used. The spectral properties along with the photochemical behaviors and application for polymerization were studied kinetically. The photo induced electron transfer of MB in the tight ion pair state which predominantly exists in non-polar solutions, was found to be greater than that observed in the free solvated ion state in polar solvents. Flow injection technique was used for the radiation of methylene blue at 666 nm by Leon and Catapano [197]. The carrier stream was 1.7 µg ml⁻¹ methylene blue in phosphate-HCl buffer solution at pH 3.2. The reactor was irradiated with a 500 W halogen lamp to facilitate the development of the photochemical reaction. The system allowed determination of ascorbic acid in the range of 0.18-6.12 µg mol⁻¹ with relative standard deviation of 0.09 and 0.31% for 1.97 and 4.92 µg mol⁻¹. The proposed method was applied successfully to the determination of ascorbic acid in vitamin C tablets. Mathews [198] determined the extent of degradation, monitored at 660 nm following the rate of mineralization of methylene blue. The photoinitiation mechanism and photodegradation of methylene blue by solar light and TiO₂ as semiconductor were investigated by Nogueria and Jardim [199]
and Lakshami et al [102]. The rate constant $k$ and half life of methylene blue were also calculated. Photocatalytic bleaching of crystal violet by methylene blue was studied by Rao et al [200]. Photocatalytic degradation of xylidine ponceau and orange-G was investigated by Sharma et al [201].

The photochemical reduction of dye in solid polymer solution under the influence of laser radiation was studied by Sizykh and Tarakanova [202]. A high triplet quantum yield and intersystem crossing yield provide the proof of linear dependence of the dye photoreduction rate and the intensity of laser used. Kim et al [203] studied the generation of functional images by photoinduced chemical transformation of organic dyes in polymer film. Fecher et al [204] proposed a chemical model of the polymerization of acrylamide in presence of sulfide, methylene blue and molecular oxygen. The experimental process of pattern formation becomes light sensitive if methylene blue is added.
2.13 AIM OF THE PRESENT WORK

Methylene blue is very important member of thiazine family and most commonly used in dying, printing, leather, building of tissues, staining of DNA and other important biological purposes. In this thesis kinetics of reduction of methylene blue in 50% aqueous methanol was discussed. The reduction of methylene blue plays an important role in dying.

The present research deals with photochemical reduction of methylene blue by reducing sugars like, Galactose, D-Mannose, Maltose, Lactose and Ribose. The aim of study on these reductants is to get some more knowledge about photoreduction in the presence of reducing sugars as these reductants have not been studied yet. The research area of this thesis concerns the effect of concentration of dye, reductants, temperature and acidities of the reaction mixture on the quantum yield of photochemical reaction of methylene blue.

The values of temperature coefficients of the photochemical reactions of methylene blue with monosaccharides and disaccharides will be evaluated. Arrhenius and Eyring relation will be used to determine the thermodynamics parameters like energy of activation ($E_a$), enthalpy change of activation ($\Delta H^*$), entropy change of activation ($\Delta S^*$) and free energy change of activation ($\Delta G^*$), in 50% aqueous methanol. A reaction mechanism will be proposed to understand the kinetics of photochemical reduction of methylene blue with reducing sugars such as Galactose, D-Mannose, D-(+)-Ribose, Maltose and Lactose. The values of rate constants of different steps will be evaluated from the proposed mechanism and tests would be put forward to justify the mechanism. An attempt will also be made to analyse the photochemical product by TLC (thin layer chromatography) and column chromatographic techniques for the justification of the proposed mechanism.
CHAPTER 3

EXPERIMENTAL ASPECTS
3. EXPERIMENTAL ASPECTS

INTRODUCTION

The photo bleaching of methylene blue with inorganic and organic reducing agent was widely studied [205-210] by visible light and spectrophotometrically. The present investigation was carried out to study the photoreduction of methylene blue with reducing sugars by monochromatic radiation of 661 nm in 50% aqueous methanol using acetate buffer on specially designed optical bench. The photoreduction of methylene blue with reducing sugars was accomplished through the interaction of triplet exited state of the dye and reductants. The acidity (H₄) of the reaction mixtures were adjusted by spectrophotometric means. The quantum yield (ϕ) at different parameters were determined in an inert atmosphere by bubbling nitrogen gas through reaction mixture. In the last TLC in different solvent system were used to check the completion of the bleaching process and mechanism was drawn from the isolated photochemical products:

That’s why the present investigation consists of three parts.

1) Measurement of acidity.
2) Determination of quantum yield (ϕ) at different parameters.
3) Isolation of photochemical product by column chromatographic technique and determination of their kinetics and mechanism.

3.1 PREPARATION OF EXPERIMENT

Preparation of experiment involves the following steps.

1. Preparation of Photochemical Research laboratory.
2. Reactions Cell
3. Materials and Methodology.
4. Photochemical Reduction Reaction.
3.2 PREPARATION OF PHOTOCHEMICAL RESEARCH LABORATORY

Photochemical reactions were carried out in a dark room. Therefore for photochemical reduction of methylene blue by carbohydrate family, a special type of dark room with four blacken walls was prepared in which all the facilities for the reaction were available. Photochemical reactions proceeds by absorption of suitable radiation at particular wavelength and require following arrangement for the reaction in the dark room.

1) Radiation source
2) Monochromator
3) Sample cell
4) Radiation detector
5) Signal recorder

3.2.1 RADIATION SOURCES
A suitable radiation source for the reaction must have following characteristics.
1) The sources should be fixed at their place and stable throughout the reaction.
2) Having a beam of sufficient intensity.
3) Should provide continuous radiation in the required region of studies.
4) The source of radiations must emit a detectable signal throughout the region.

3.2.2 MONOCHROMATOR
A monochromator is required for suitable monochromatic radiation from polychromatic radiation. A monochromator is a device that resolves radiation into its components wavelength and permits the isolation of any desired portion of the spectrum.

3.2.3 DETECTORS
A detector has an ability to produce an electric signal when it is struck by photons. The response of most detectors depends on the wavelength of incident photon.

Detectors are of two types
i) Heat detectors  ii) Photon detectors
i) **Heat detectors:**

Heat detectors are thermocouple, thermistor, bolometer, thermophile etc. used over a wide range of wavelengths but have relatively low sensitivity and slow response.

ii) **Photon detectors:**

Photomultiplier, barrier layer cell, PbS cell, photographic plate etc are photon detectors. In general photon detectors are faster and more sensitive. Radiation detectors are shown in Figure 3.1

---

**Figure 3.1: Radiation Detectors**

(a) Thermopile (b) Photo cell
3.2.4 PHOTON MULTIPLIERS

Photon multipliers operate on the principal of photon amplification. A photon strikes cathode causing emission of electrons which are multiplied by striking a series of anodes resulting in electron multiplication.

The radiant energy thus converted into electric energy can easily be measured.

3.2.5 PHOTO CELL

The radiant energy generates a current at the interface of a semi conductor and is used for the detection and measurement of radiations in the visible region.

3.3 CIRCUIT FOR LIGHT SOURCE

The light source for the reaction was a 250 watts, 3 pin, 200/250 volts prefocus high pressure mercury ME/D compact Mazda box type lamp (Code No. 94001) manufactured by GEC lamps and lighting Ltd. The voltage was kept constant within 0.01v by using a 250 watts constant voltage transformer manufactured by Hanovia.

The light output of a mercury lamp depends on three factors. 1) Current, 2) Voltage and 3) pressure of Hg vapours, the latter depending on the temperature of the arc tube (usually 300 – 400 °C). Control of the heat loss of the lamp is important in maintaining constant output as control of the current [146].

3.4 THE OPTICAL BENCH ARRANGEMENT

A special optical bench was arranged according to need of the experiments as shown in Figure 3.2. 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 L₁ and L₂ ( Onto a small hole (O) 2mm in a metal plate) and lens L₃ 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 P₂. This light was then passed through reaction cell (C). A magnetic stirrer (M) was used to homogenize the reaction solutions. The photocell P₁ was used to detect the light after passing through the reaction cell. G₁ and G₂ were used to convert the light signal into electronic current measured in μA or mA from photocell P₁ and P₂ respectively.
Figure 3.2: Optical bench arrangement
3.5 LIGHT FILTERS

A Kodak Wratten optical filter No. 15 was used in order to obtain a desired spectral light of wavelength 661 nm as suggested by Sommer and Kramer [211] and Steiner et al [212]. A filter box was constructed and in the middle of this box, the red Kodak Wratten filter was arranged in such a way that maximum light passes through the filter.

3.6 REACTION CELL

The reaction cell used in the present experiment, which was a modified cell double walled cell, used by Sawkar [80], Ahmed [188], and Getoff et al [213]. It was similar to the cell used by Uddin [140]. In the cell of Getoff et al., there was no arrangement to homogenize the reaction mixture while the other made a stirring arrangement to homogenize the mixture. In Sawkar and Ahmed's arrangement, there was heating coil system to raise the temperature while in Vonnach et al's [186] arrangement, the cell was attached with a thermostatic bath. 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 ± 0.1°C variation.

The cell was a double walled cylindrical glass tube of 7.0 cm in length, 2.5 cm in diameter with flanged ends as shown in Figure 3. A complete sealed needle in a glass tube was placed onto a depression of about 1.5 cm in diameter half way along the lower length of the cell. This set-up served as a magnetic stirrer (M). Two B 10/19 joints were attached to the cell for the intake of oxygen free nitrogen through a retractable tube and an exit with an ordinary tap fitted to it respectively as shown in Figure 3.3. Glass windows were fixed to the flanged ends of the reaction cell with a paste of Araldite and hardner. Two joints (W) attached to the cell was connected to the thermostatic bath for the circulation of water in the outer part of the cell.
Figure 3.3: Reaction Cell
3.7 THERMOSTATIC BATH

In order to maintain the temperature of the reaction mixture, a thermostatic bath of type T-52, manufactured by Haake, Karlsruhe of Germany, connected to the reaction cell was used as described above. The temperature was observed by a thermometer of least count 0.1°C placed in the thermostatic bath. The water bath was provided by the water flowing pump and was connected with reaction cell through rubber tubing so that temperature controlled water was flowing in a cyclic manner.

3.8 STIRRING SYSTEM

A magnetic stirrer made of a small horse shoe magnet placed on electric motor was used to stirr the solution in the reaction cell.

3.9 MATERIALS AND METHOD

REAGENTS

All reagents given below are of Merck and B.D.H grade

1) Nitrogen Gas
   2) Galactose
   3) Maltose
   4) Lactose
   5) D-Mannose
   6) D- (+)-Ribose
   7) Absolute Methanol
   8) Acridine
   9) Di-Thionite
   10) Sodium Anthraquinone-2- Sulphonate
   11) Sodium Hydroxide
   12) Hydrochloric Acid
   13) Lead acetate
   14) Oxalic acid
   15) Sodium acetate

\[ \text{Merck} \hspace{1cm} \text{C}_6\text{H}_{12}\text{O}_6 \]
\[ \text{Merck} \hspace{1cm} \text{C}_{12}\text{H}_{22}\text{O}_{11} \]
\[ \text{Merck} \hspace{1cm} \text{C}_{12}\text{H}_{22}\text{O}_{11} \]
\[ \text{Merck} \hspace{1cm} \text{C}_6\text{H}_{12}\text{O}_6 \]
\[ \text{Merck} \hspace{1cm} \text{C}_3\text{H}_{16}\text{O}_5 \]
\[ \text{Merck} \hspace{1cm} \text{CH}_3\text{OH} \]
\[ \text{Merck} \hspace{1cm} \text{Na}_2\text{S}_2\text{O}_4 \]
\[ \text{Merck} \hspace{1cm} \text{C}_{10}\text{H}_7\text{NaO}_3\text{S} \]
\[ \text{Merck} \hspace{1cm} \text{NaOH} \]
\[ \text{Merck} \hspace{1cm} \text{ICl} \]
\[ \text{Merck} \hspace{1cm} \text{Pb(CH}_3\text{COO)}_2 \]
\[ \text{Merck} \hspace{1cm} (\text{COOH)}_2, \text{2H}_2\text{O} \]
\[ \text{Merck} \hspace{1cm} \text{NaCH}_3\text{COO} \]
16) Sodium chloride  
 Merck  
 NaCl

17) Sulphuric acid  
 Merck  
 H₂SO₄

18) Phenolphthalein  
 Merck

19) Methylene blue  
 Fluka  
 C₁₆H₁₈ClN₃S·2H₂O

20) Acetic acid  
 Merck  
 CH₃COOH

21) Ethyl acetate, Haxane, Ceric sulphate, Silica gel

3.10 GLASS WARES

All the glass wares used for photochemical reduction of methylene blue by carbohydrates were of Pyrex ‘A’ Grade quality. Glass wares were thoroughly washed, cleaned and dried in an oven (w.t.c. binder, 7200, Tuttlinger / Germany Type E 28 No. 89248) at temperature 150°C for 2 hours before starting reaction kinetics.

3.11 DOUBLE DISTILLED WATER

Freshly prepared double distilled water was used for the preparation of 50% aqueous methanol having conductivity of 0.06 × 10⁻⁶ S/cm.

3.12 SOLVENT

50% aqueous methanol was prepared by v/v ratio for preparation of stock solutions.

3.13 PREPARATION OF STOCK SOLUTIONS

i) Stock Solution of Methylene Blue

3,7, Phenothiazine, 5, ium, 3,7, bis (dimethyl amine) chloride commonly known as methylene blue or swiss blue (CAS NO.61-73-4) having molecular weight 355.089 gm/mol, λₘₐₓ 661nm in 50% aqueous methanol of analar grade manufactured by Fluka was used and the structural formula is given as

![Methylene Blue Structure](image)
Stock solution of concentration $1 \times 10^{-4}$ mol dm$^{-3}$ in 500 dm$^3$ of 50% aqueous methanol was prepared. Methylene blue was dissolved in 50% aqueous methanol by using magnetic stirrer and preserved in well stoppered polythene bottle as suggested by Sawkar [80].

ii) **Stock Solution of Acridine**

Stock solution of acridine of concentration $1.0 \times 10^{-3}$ mol.dm$^{-3}$ was prepared by dissolving a known weight of pure acridine in 50% aqueous methanol. This solution was used to determine acidity of the reacting solutions. The molecular weight and $\lambda_{max}$ are 265.36 gm and 489 nm respectively.

iii) **Stock Solution of Hydrochloric Acid**

Hydrochloric acid solution of concentration $1.0 \times 10^{-2}$ mol.dm$^{-3}$ was prepared by passing HCl gas through 50% aqueous methanol. The hydrochloric acid gas was produced by the reaction of sulphuric acid on sodium chloride crystals. The hydrochloric acid solution was standardized by titrating against a standard solution of sodium carbonate.

iv) **Stock Solution of Sodium Hydroxide**

The stock solution of sodium hydroxide was prepared by dissolving a known weight of sodium hydroxide in 50% aqueous methanol. This solution was standardized before each experiments by titrating it with standard oxalic acid of concentration $1.0 \times 10^{-2}$ mol.dm$^{-3}$ in 50% aqueous methanol. The concentration of sodium hydroxide was also kept as $1.0 \times 10^{-2}$ mol.dm$^{-3}$.

v) **Stock Solution of Oxalic Acid**

A known weight of oxalic acid was dissolved in 50% aqueous methanol to prepare a stock solution of concentration $1.0 \times 0.0^{-2}$ mol.dm$^{-3}$.

vi) **Stock Solution of Sodium Acetate**

The concentration of stock solution of sodium acetate was kept as $1.0 \times 10^{-1}$ mol.dm$^{-3}$. For this purpose, a known amount of sodium acetate was dissolved in 50% aqueous methanol. This solution of anhydrous material of sodium acetate was used for the preparation of buffer solutions.

vii) **Stock Solution of Acetic Acid**

Stock solution of acetic acid of concentration $1.0 \times 10^{-1}$ mol dm$^{-3}$ was prepared by dissolving 5.88 cm$^3$ in 1 dm$^3$ of 50% aqueous methanol.
viii) Buffer Solution
Buffer solution was prepared by mixing sodium acetate and acetic acid in 0.1:0.1 ratios.

ix) Stock Solution of Galactose and D-Mannose
The stock solution of concentration of $1 \times 10^{-1}$ mol dm$^{-3}$ was prepared by dissolving
4.5 gms of monosaccharide in 250 cm$^3$ of 50% aqueous methanol.

x) Stock Solution of Maltose and Lactose
The stock solution of concentration $1 \times 10^{-1}$ mol dm$^{-3}$ was prepared by dissolving 9 gms
of disaccharides in 250 cm$^3$ of 50% aqueous methanol.

xi) Stock Solution of D-(+)-Ribose
Stock Solution of D-(+)-ribose of concentration $1 \times 10^{-1}$ mol dm$^{-3}$ was prepared by
dissolving 3.75 gms of pentose in 250 cm$^3$ of 50% aqueous methanol.

xii) Nitrogen Gas
Nitrogen gas used in this work was manufactured by Pakistan Oxygen Ltd. It was of
99.8 % purity. Traces of oxygen from nitrogen were also removed by the method
described by Feiser [214, 215] as discussed in section 3.14.

### 3.14 DEOXYGENATING SYSTEM

Nitrogen gas may contain traces of oxygen and for reaction mixture, a completely
deoxygenated system was required. Oxygen gas was removed by Feiser’s Method:
In Feiser’s method nitrogen of 99.8% purity was passed through a series of different
dreschel bottles containing Feiser’s solution [214, 215] in first two bottles, saturated
solution of lead acetate, $1 \times 10^{-1}$ sodium hydroxide, silica gel and the solvent of reaction
i.e. 50% aqueous methanol respectively.

```
INLET -----> 3 -----> 4 -----> 5 -----> 6
Feiser’s Solution sat. sol of Pd(CH$_3$COO)$_2$ 0.1 M NaOH Silica gel Solvent
```

Outlet N$_2$ to reaction mix

Preparation of Feiser’s and other solutions in dreschel bottles.
FEISER'S SOLUTION:

Feiser's solution contained Sodium hydroxide (62.5 g), sodium anthraquinone-2-Sulphonate (7.5 g), Sodium hydrosulphite (dithionite) (75 g) and 500 cm³ deaerated hot water. The solution was stirred gently. The solution was transferred into the two dreschel bottles separately as quickly as possible in order to remove the traces of (i) hydrogen sulphide formed by the decomposition of dithionite (2) and acidic vapors respectively. If any trace of oxygen was present in the nitrogen gas than it was removed by further passing through saturated lead acetate, sodium hydroxide, silica gel and solvent.

3.15 MEASUREMENT OF LIGHT INTENSITY

A photocell [216] was connected in series with an ammeter to measure the intensity of light beam. The current measured by ammeter after passing the light is directly proportional to the amount of light striking at cathode i.e.

\[ I = K I_0 \]

\[ I_0 = \frac{1}{K} \]

where \( K \) is photocell constant (\( K = 20 \)), and \( I_0 \) is the amount of light (measured in lumen). Then \( I_0 = 1/20 \) the equation predicts that the current is a function of amount of light. The response of the cell was recorded on the ammeter.

The energy of photon is given by:

\[ E = N_A \frac{h}{\lambda} \]

\[ = N_A \frac{h}{C/\lambda} \]

where \( N_A = \text{Avogadro's number} = 6.023 \times 10^\text{23} \)

\( h = \text{Planck's constant} = 6.26 \times 10^{-27} \text{ ergs. s.} \)

\( \lambda = \text{wave length of light} = 661 \text{ nm} \) [for present work]

\( \nu = \text{frequency of light used} \)

\( C = \text{velocity of light} = 2.998 \times 10^{10} \text{ cm/s.} \)

The intensity of light is given as:

\[ I_0 = \frac{1}{K} = \frac{x}{K} \text{ lumen} \]

Where \( x \) = current measured by galvanometer.
From calibration point of view, the effective value of current \( x \) was determined with the help of following expression.

\[
I = x = x_2 - x_1
\]

Where \( x_1 \) = current measure by galvanometer when no light striking the photocell.

Where \( x \) is effective value of the current recorded on the ammeter.

\[
I_0 = \frac{x}{20} \text{ lumen (because } K = 20) \]

Since one lumen = \( \frac{1}{680} \) light watts

\[
= 0.00147 \text{ light watts}
\]

\[
I_0 = \frac{x}{20} \times \frac{0.00147}{2.066 \times 10^5} \text{ Einst/s}
\]

Therefore

\[
I_0 = 3.559 \times 10^{-6} \text{ Einst/s}
\]

### 3.16 MEASUREMENT OF THE ACIDITY OF THE SOLUTION

The Photoreduction of thiazine dye family in presence of reductant depends upon the acidity or pH of the solution. Sawkar [80] made first attempt to determine the acidity for the reduction of thionine dye. The state of the dye was determined and either by adding bromophenol blue, thymol blue bromocresol to the buffer solution using spectro photometer. It was observed that due to their acidic nature, these indicators cause a detectable change in the pH of the alcoholic buffer solution, containing sodium hydroxide and hydrochloric acid.

Ahmed [188] used calibrated pH meter to note the pH of the solution but the response of glass electrode in mixed solvent system was not reliable. Glass electrode showed different pH values, if it is placed two or three days in alcoholic water system. Therefore the value of acidity may not be correct. Ahmed [188] determined the acidity of solution by pH meter, using glass electrode, but the acidity scale on which measurements were expressed was based on “Hammett acidity function” \([H_0]\) [217] with alcoholic acridine solution as standard. He chose a basic indicator because thionine is a base. Hydrogen ion activity was determined in terms of Hammett and Deyrap [217] acidity function \([H_0]\) when

\[
H_0 = - \log h_o
\]
\[ h_0 = \frac{f_{H^+} f_B}{f_{BH^+}} \]  \hspace{1cm} (3.16.2)

Where \( f_B \), \( f_{BH^+} \) and \( f_{H^+} \) are the activity coefficients of a base indicator \( B \), its protonated form \( BH^+ \) and the hydrogen \( H^+ \). The dissociation of the base of \( B \) may be represented as follows:

\[ B + H^+ \rightleftharpoons BH^+ \]  \hspace{1cm} (3.16.3)

\[ K_{BH^+} = \frac{[B][H^+]}{[BH^+]} \times \frac{f_{H^+} f_{BH^+}}{f_{BH^+}} \]  \hspace{1cm} (3.16.4)

\[ K_{BH^+} = \frac{[B]}{[BH^+]} \times \frac{[H^+] f_{H^+} f_B}{f_{BH^+}} \]  \hspace{1cm} (3.16.5)

\[ \log K_{(BH^+)} = \log \left( \frac{[B]}{[BH^+]} \right) + \log \left( \frac{[H^+] f_{H^+} f_B}{f_{BH^+}} \right) \]  \hspace{1cm} (3.16.5)

as

\[ h_0 = \frac{f_{H^+} f_B}{f_{BH^+}} \]

\[ \log K_{BH} = \log \left( \frac{[B]}{[BH^+]} \right) + \log h \]  \hspace{1cm} (3.16.6)

\[ \log \frac{1}{K_{DH^+}} = \log \left( \frac{[B]}{[BH^+]} \right) - \log (-\log h_0) \]  \hspace{1cm} (3.16.7)

\[ -pK = \log \left( \frac{[B]}{[BH^+]} \right) - [H_0] \]  \hspace{1cm} (3.16.8)

\[ [H_0] = pK + \log \left( \frac{[B]}{[BH^+]} \right) \]  \hspace{1cm} (3.16.9)

If the absorption of the indicator in extreme acidic and basic solutions are \( d_1 \) and \( d_2 \) then for a solution of intermediate acidity, the absorption could be represented as:

\[ \frac{[B]}{[BH^+]} = \frac{d_1 - d}{d - d_2} \]
Therefore

\[ H_0 = pK + \log \frac{d_1 - d}{d - d_2} \]

Uddin et al [215] adopted an optical method using acridine as basic indicator for the determination of acidity of reaction solution. In the present work same method as described by Uddin et al [215] was used and acidity was found to be in the range of 4.2 and 7.7. For the present work, Weller's [218] value of pK for aqueous solution of acridine was taken as 5.45 ± 0.05. For the measurement of acidity (H_o) of the reaction solution, an acridine solution of \(1 \times 10^{-4}\) mol dm\(^{-3}\) concentration was prepared in 50% aqueous methanol. \(1 \times 10^{-2}\) mol dm\(^{-3}\) of hydrochloric acid and sodium hydroxide was used. The optical density was determined to obtain the values of acidity (H_o).

### 3.17 MEASUREMENT OF ABSORPTION COEFFICIENT

The measurement of absorption coefficient of methylene blue in 50% aqueous methanol is given below: For a solution of methylene blue of concentration \(1 \times 10^{-6}\) mol dm\(^{-3}\) in 50% aqueous methanol, optical densities were recorded at different wavelengths. A plot was shown in figure 4.3. The absorption coefficient \(\varepsilon\) was determined using the formula:

\[
\text{O.D.} = \varepsilon \cdot c \cdot d
\]

where

- O.D. = optical density
- c = concentration of the solution
- \(\varepsilon\) = molar absorption coefficient
- d = diameter of the cell

\[
\text{O.D}_{(\text{max})} = 0.082
\]

\[
d = 1 \text{ cm}
\]

\[
c = 1 \times 10^{-6} \text{ mol.dm}^{-3}
\]

\[
\varepsilon = \frac{0.082}{1 \times 10^{-6}} \times 1
\]

\[
= 8.2 \times 10^{4} \text{ mol.dm}^{-3} \text{ cm}^{-1}
\]
3.18 THE EXPERIMENTAL PROCEDURE

30.0 ml of reaction mixture containing known volumes of methylene blue, and the buffer solutions in 50% aqueous methanol were pipetted out into the reaction cell. The same volume of the reductants and buffer were pipetted for the determination of acidity H₀ of the reaction mixture in first duplicate part of the reaction solution. 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 Åm. Photocell responses from the deflection on the scale of the galvanometer were noted with no cell in the beam (D₀), with the cell containing solvent (Dₑ) and with the cell containing reaction solutions (Dₙ). 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 in section 3.16 during the flushing period. The quantum yield was calculated as a function of concentration of reductants, acidity (H₀), temperature, concentration of dye.

3.19 ANALYSIS OF PHOTOCHEMICAL REACTION MIXTURE

Analysis of photochemical reaction mixture was carried out by TLC and column chromatographic techniques. Isolated product was then subjected to further Mass spectrophotograph, Infra red spectroscopy for determination of kinetics and establishing the reaction mechanism. The irradiated reaction mixture of methylene blue and reducing sugars were used to check the completion of reaction by taking TLC of the mixture in different solvent systems. The TLC of reaction mixture and product were run in pure hexane, ethyl acetate and butanol acetic acid: water (BWA, 12:3:5). In pure hexane and pure ethyl acetate only one spot of the product was visible by spraying with ceric sulphate solution. When TLC was checked in BWA (12:3:5) system, 4 spots were
visualized by spraying same reagent. Then solvent of reaction mixture was evaporated on rotary evaporator (Buchi Germany) and residue was subjected to silica gel (230 – 400) and loaded on pencil silica gel column. Column was run with hexane, then fractions were collected in 35 vials and TLC was performed with same solvent.

The TLC showed no compound in hexane by spraying same reagent followed by heating. The column was again eluted by hexane in chloroform system and finally with pure chloroform but TLC of collected fraction showed no compound with same profile. The column was run with 1% methanol and polarity of the mobile system increased from 1 – 4% of methanol. TLC of 1 – 3% of methanol showed no compound. But fractions which were collected by 4% methanol shows single spot and remaining compound from the vials are collected by evaporating the solvent at room temperature from vials. All the vials then mixed into one another and again purity was checked by same methanol. Finally five compounds separated were subjected to spectrophotometric techniques for establishing reaction kinetics and mechanism.
CHAPTER 4

RESULTS
4. RESULTS

4.1 OPTICAL ABSORPTION OF METHYLENE BLUE

Methylene blue can be activated by light to an excited state which in turn activates oxygen to yield radicals. Such radicals can cause cross linking of amino acid residues on proteins and hence achieve some degree of cross linking. From Beer's law, the absorbance is proportional to the concentration of the species

\[ A(\gamma) = b \varepsilon(\gamma)c \]

where \( A(\gamma) \) is the absorbance at some wavelength \( \gamma \) and \( \varepsilon(\gamma) \) is the absorption coefficient at some wavelength \( \gamma \). \( b \) is the path length of the cell and \( c \) is the concentration. Absorbance is dimensionless and \( \varepsilon \) is the absorbance per unit concentration per unit path length. We can write this as

\[ [A] = b \left[ \varepsilon \right] c \]

where \([A]\) is the absorbance vector and \([\varepsilon]\) is the vector of absorption coefficients. Each element in the vectors corresponds to a particular wavelength of light. For a given absorption process, a molecule absorbs only one discrete amount of energy and thus absorbs radiation of only one wavelength. If all molecules of a compound were in exactly the same state then a plot of the absorption vector would have very narrow absorption lines. However, molecules have different vibrational and rotational states with each state at highly different energy levels. Thus the base state is variable and the amount of energy required for a transition to a higher energy state will be a function of base state. Thus, an ensemble of molecules absorbs radiations at slightly different wavelengths as the individual molecules move from their various base states and thus the result is a broad absorption band.

4.2 MAXIMUM ABSORBANCE VALUES

High absorbance values are suspected because they indicate that most of incident light has been absorbed by the sample and remaining transmitted light intensity is very weak. Figures 4.1-4.2 show the maximum absorbance at different concentration of dye in
Figure 4.1. Absorption spectra of Methylene blue at different concentration in aqueous solution.
Figure 4.2: Absorption spectra of methylene blue in 50% methanol at different concentration of dye
aqueous methanol and water. Absorbance increases as the concentration of dye increases as show in Table 10. Concentration did not result in increase in absorbance as shown in Figures 4.1-4.2 because the maximum measurable absorbance is approximately 2.5.

**Table - 10**

**Absorbance measurements at various concentration of methylene blue in 50% Methanol and Water. $\lambda_{\text{max}} = 661 \text{ nm}$**

<table>
<thead>
<tr>
<th>[Methylene blue] [mol dm$^{-3}$]</th>
<th>Absorbance 50% Methanol</th>
<th>Absorbance H$_2$O</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1 \times 10^{-6}$</td>
<td>0.082</td>
<td>0.084</td>
</tr>
<tr>
<td>$2 \times 10^{-6}$</td>
<td>0.175</td>
<td>0.231</td>
</tr>
<tr>
<td>$3 \times 10^{-6}$</td>
<td>0.410</td>
<td>0.439</td>
</tr>
<tr>
<td>$4 \times 10^{-6}$</td>
<td>0.576</td>
<td>0.547</td>
</tr>
<tr>
<td>$5 \times 10^{-6}$</td>
<td>0.686</td>
<td>0.676</td>
</tr>
<tr>
<td>$6 \times 10^{-6}$</td>
<td>1.068</td>
<td>0.806</td>
</tr>
<tr>
<td>$7 \times 10^{-6}$</td>
<td>1.341</td>
<td>1.392</td>
</tr>
<tr>
<td>$8 \times 10^{-6}$</td>
<td>1.520</td>
<td>1.592</td>
</tr>
<tr>
<td>$9 \times 10^{-6}$</td>
<td>1.527</td>
<td>1.286</td>
</tr>
<tr>
<td>$1 \times 10^{-5}$</td>
<td>1.718</td>
<td>1.392</td>
</tr>
<tr>
<td>$2 \times 10^{-5}$</td>
<td>1.912</td>
<td>1.714</td>
</tr>
<tr>
<td>$1 \times 10^{-4}$</td>
<td>2.490</td>
<td>2.490</td>
</tr>
</tbody>
</table>
The values of molar absorption coefficients at different concentrations of the dye and solvents are summarized in Table 11.

Table - 11

<table>
<thead>
<tr>
<th>Molar Absorption Coefficients of Methylene Blue at Different Concentrations and Different Solvents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td><strong>H₂O</strong></td>
</tr>
<tr>
<td>1 × 10⁻⁴</td>
</tr>
<tr>
<td>1 × 10⁻⁵</td>
</tr>
<tr>
<td>1 × 10⁻⁶</td>
</tr>
<tr>
<td><strong>50 % Aqueous Methanol</strong></td>
</tr>
<tr>
<td>1 × 10⁻⁴</td>
</tr>
<tr>
<td>1 × 10⁻⁵</td>
</tr>
<tr>
<td>1 × 10⁻⁶</td>
</tr>
<tr>
<td><strong>100 % Methanol</strong></td>
</tr>
<tr>
<td>1 × 10⁻⁴</td>
</tr>
<tr>
<td>1 × 10⁻⁵</td>
</tr>
<tr>
<td>1 × 10⁻⁶</td>
</tr>
</tbody>
</table>

The molar absorption coefficients of methylene blue in dye concentration of 1×10⁻⁴ mol dm⁻³ were 2.47×10⁻⁴, 2.49×10⁻⁴ and 2.49×10⁻⁴ in aqueous, 50 % aqueous methanol and pure methanol respectively, as summarized in Table 11.

The results tabulated in Table 12 show that in pure methanol, 50 % aqueous methanol and aqueous solution, two bands appear in spectrum for concentration 1×10⁻⁴ mol dm⁻³ at λₘₐₓ 683 and 650 nm, 667 nm and 648 nm and 681 nm and 647 nm respectively.
<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\lambda_{\text{max}}$ [nm]</th>
<th>$\varepsilon \times 10^6$ mol$^{-1}$ cm$^{-1}$ dm$^3$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$O</td>
<td>681</td>
<td>2.470</td>
<td></td>
</tr>
<tr>
<td></td>
<td>647</td>
<td>2.467</td>
<td></td>
</tr>
<tr>
<td>50 % Aqueous Methanol</td>
<td>667</td>
<td>2.490</td>
<td></td>
</tr>
<tr>
<td></td>
<td>648</td>
<td>2.490</td>
<td></td>
</tr>
<tr>
<td>100 % Methanol</td>
<td>683</td>
<td>2.490</td>
<td></td>
</tr>
<tr>
<td></td>
<td>650</td>
<td>2.460</td>
<td></td>
</tr>
</tbody>
</table>

The values of molar absorption coefficients are in agreement with the values of $8.16 \times 10^4$ mol$^{-1}$ cm$^{-1}$ dm$^3$ at $\lambda = 665$ nm, $2.25 \times 10^4$ mol$^{-1}$ cm$^{-1}$ dm$^3$ at $\lambda = 420$ nm and $8.8 \times 10^4$ mol$^{-1}$ cm$^{-1}$ as reported by Matsumoto [78], Sayser et al [61], Somer et al [93] and Parker and Hatchard [76] respectively. The spectra of the solution of methylene blue in absolute methanol, 50% aqueous methanol and aqueous solution at concentration $1 \times 10^6$ mol dm$^{-3}$ are shown in the Figure 4.3.
Figure: 4.3  Absorption spectra of methylene blue of concentration

\[ 1 \times 10^{-6} \text{mol dm}^{-3} \]

a) 50% aqueous methanol  b) 100% methanol  c) Water
4.3 DETERMINATION OF QUANTUM YIELD FOR THE PHOTOREDUCTION OF METHYLENE BLUE WITH REDUCING SUGARS

The kinetics of reduction of methylene blue with reducing sugars was carried out in deoxygenated system. Calculated volume of dye, acetate buffer, solvent, acid, base and reductant were pipette out into reaction cell. The quantum yield for the reaction of methylene blue with reducing sugars was determined by using following relation as follows.

Intensity of the incident beam : $I_0$ Einstein second$^{-1}$
Intensity of the beam at the back of the first window : $I_1$ Einstein second$^{-1}$
Intensity of the beam at the front of the second window : $I_2$ Einstein second$^{-1}$
Intensity of the beam at the back of the second window : $I_3$ Einstein second$^{-1}$
Light absorbed in the solution : $I_a$ Einstein second$^{-1}$
Light absorbed per unit volume : $[I_a]$ Einstein second$^{-1}$ liter$^{-1}$
Signal recorded by deflection on galvanometer when no cell in the beam : $D_0$
Signal recorded when cell with solvent (50% aqueous methanol) in the beam : $D_m$
Signal recorded when cell with solution in the beam : $D_n$
Fraction of light lost at each window : $\alpha$
Fraction of light transmitted through the window : $1 - \alpha$

The deflection is proportional to light intensity:

\[ I_0 \propto D_0 \]
\[ I_0 = \beta D_0 \]
Where $\beta$ is the proportionality factor for the relation of deflection proportional to the intensity of beam.

For solvent in the cell:  
$$I_3 = \beta D_m$$  \hspace{1cm} 4.3.2

For solution in the cell:  
$$I_3 = \beta D_n \quad \text{and} \quad I_s = \beta D_a$$  \hspace{1cm} 4.3.3

The intensity of the beam entering the solution:  
$$I_1 = I_o (1 - \alpha) \quad \text{and} \quad I_3 = I_1 (1 - \alpha)$$  \hspace{1cm} 4.3.4

$$I_s = I_1 - I_2$$  \hspace{1cm} 4.3.5

Put the value of $I_1$ and $I_2$ in equation 4.3.7

$$I_a = I_o (1 - \alpha) - \frac{I_a}{(1 - \alpha)}$$  \hspace{1cm} 4.3.6

In terms of deflection:

$$\beta D_n = \beta D_o (1 - \alpha) - \frac{\beta D_a}{(1 - \alpha)}$$  \hspace{1cm} 4.3.7

Dividing equation 4.3.8 by $\beta$:

$$D_a = D_n (1 - \alpha) - \frac{D_n}{(1 - \alpha)}$$  \hspace{1cm} 4.3.8

$$D_a = D_o (1 - \alpha)^2 - \frac{D_n}{(1 - \alpha)}$$  \hspace{1cm} 4.3.9

If the solvent transmits 100% light:

$$I_s = I_o (1 - \alpha)^2$$  \hspace{1cm} 4.3.10

In terms of deflection:

$$\beta D_m = \beta D_o (1 - \alpha)^2$$  \hspace{1cm} 4.3.11

Dividing by $\beta$:

$$D_m = D_o (1 - \alpha)^2$$  \hspace{1cm} 4.3.12

$$\frac{D_m}{D_o} = (1 - \alpha)^2$$  \hspace{1cm} 4.3.13

Put the value of $(1 - \alpha)^2$ in equation 4.3.10:
\[
\frac{D_o \times \frac{D_m}{D_o} - D_n}{(1 - \alpha)} = \frac{D_n - D_m}{(1 - \alpha)} \quad 4.3.16
\]

From equation 4.3.4:

\[I_a = \beta D_a\]

Put the value of \(D_a\):

\[I_a = \beta \frac{D_m - D_n}{(1 - \alpha)} \quad 4.3.17\]

Quantum yield \((\phi)\) is defined as number of dye molecules of reactants converted into product per unit Einstein of light absorbed.

\[
\phi = \frac{\text{No. of moles of reactant converted into product}}{\text{No. of Einstein of light absorbed}}
\]

With the light beam the concentration of the dye converted \textit{per unit volume of the beam in time } \(dt\) be the absorption of light \([I_a]_b\), \(dt\) is \(d[DH^+]_b\). The rate of formation of singly protonated form of dye is proportional to the light intensity absorbed:

\[
\frac{d[DH^+]_b}{dt} = \alpha [I_a]_b \quad 4.3.18
\]

Where, \(D\) is used for dye methylene blue and \(DH^+\) is the singly protonated form of methylene blue:

\[
\frac{d[DH^+]_b}{dt} = \phi [I_a]_b \quad 4.3.19
\]

Where \([DH^+]_b\) is the singly protonated form of dye (methylene blue), \([I_a]_b\) is the intensity of light absorbed and subscript 'b' stands for light beam.

As the volume of the beam in the solution is \(V_b\), then for the whole solution equation 4.3.19 becomes:

\[
\frac{d [DH^+]_b}{dt} \times \frac{V_b}{V} = \phi [I_a]_b \times \frac{V_b}{V} \quad 4.3.20
\]

\[
\frac{d [DH^+]_b}{V} = \phi [I_a]_b \frac{V_b}{V} = d [DH^+] \]

as
Equation 4.3.20 becomes:

\[
\frac{d [DH^+]_b}{dt} = \frac{\phi [I_a]_b V_b}{V}
\]

Since \([I_a]_b\) is the light absorbed in unit volume, the value \(V_b \ [I_a]_b\) is the total light absorption in the solution i.e., \(I_a\).

Put the value \(V_b \ [I_a]_b = I_a\) in equation 4.3.21:

\[
\frac{d [DH^+]}{dt} = \frac{\phi I_a}{V}
\]

According to equation 4.3.7:

\[I_a = I_1 - I_2\]

Applying Beer-Lambert law for equation 4.3.7, the value of,

\[I_2 = I_1 \times 10^{-\varepsilon [DH^+]L}\]

As \(I_2\) is the light intensity behind the solution which is equivalent to the transmittance of light after the solution where \(\varepsilon\) is the molar absorption coefficient and \(L\) is the length of the cell.

Therefore following expression is obtained by placing the value of \(I_2\):

\[I_a = I_1 \times I_1 10^{-\varepsilon [DH^+]L}\]

\[I_2 = I_1 \left(1 - 10^{-\varepsilon [DH^+]L}\right)\]

According to equation 4.3.5:

\[I_1 = I_o (1 - \alpha)\]

Put the value of \(I_1\) in equation 4.3.23:

\[I_a = I_o (1 - \alpha) \left(1 - 10^{-\varepsilon [DH^+]L}\right)\]

\[\frac{I_a}{I_o (1 - \alpha)} = \left[1 - 10^{-\varepsilon [DH^+]L}\right]

\[1 - 10^{-\varepsilon [DH^+]L} = 1 - \frac{I_a}{I_o (1 - \alpha)}\]

Taking natural logarithm:

\[-2.303 \varepsilon [DH^+]L = \ln \left(1 - \frac{I_a}{I_o (1 - \alpha)}\right)\]

\[\]
Substituting the value of \( \varepsilon^* = 2.303 \varepsilon \) in equation 4.3.26:

\[
- \varepsilon^* [DH^+] L = \ln \left[ 1 - \frac{I_a}{I_o (1 - \alpha)} \right] \quad \text{(4.3.27)}
\]

Differentiating the equation 3.1.77 with reference to \( I_o \):

\[
- \varepsilon^* d [DH^+] L = - \frac{d I_o}{I_o (1 - \alpha)} \left[ 1 - \frac{I_a}{I_o (1 - \alpha)} \right] \quad \text{(4.3.28)}
\]

\[
- \varepsilon^* d [DH^+] L = - \frac{d I_a}{I_o (1 - \alpha) - I_o (1 - \alpha) - I_a} \quad \text{(4.3.29)}
\]

Substituting the value of \( d[DH^+] \) from equation 4.3.22 in equation 4.3.29:

\[
- \varepsilon^* \left[ \frac{\phi I_a}{\nu} \cdot dt \right] L = - \frac{d I_a}{I_o (1 - \alpha) - I_o (1 - \alpha) - I_a} \quad \text{(4.3.30)}
\]

On integrating equation 4.3.30:

\[
\frac{\varepsilon^* \phi L}{\nu} \int dt = \frac{1}{I_o (1 - \alpha)} \int \left[ \frac{1}{I_a} + \frac{1}{I_o (1 - \alpha) - I_a} \right] dI_a
\]

\[
\frac{\varepsilon^* \phi L}{\nu} \int dt = \frac{1}{I_o (1 - \alpha)} \int \frac{d I_a}{I_a} + \int \frac{d I_a}{I_o (1 - \alpha) - I_a}
\]
\[ \frac{\varepsilon^* \phi}{V} L \ t + C = \frac{1}{I_o (1 - \alpha)} \left[ \ln I_s - \ln \left( \frac{I_o (1 - \alpha) - I_s}{I_s} \right) \right] \]

\[ \frac{\varepsilon^* \phi}{V} t + C = -\frac{1}{I_o (1 - \alpha)} \ln \left[ \frac{I_o (1 - \alpha) - I_s}{I_s} \right] \quad 4.3.31 \]

Where \( C \) is the integration constant.

Applying the initial condition when \( t = 0, I_s = I_{s0} \), equation 4.3.31 reduces to:

\[ C = -\frac{1}{I_o (1 - \alpha)} \ln \left[ \frac{I_o (1 - \alpha) - I_{s0}}{I_{s0}} \right] \quad 4.3.32 \]

Substituting the value of \( C \) in equation 4.3.31:

\[ \frac{\varepsilon^* \phi}{V} L \ t = -\frac{1}{I_o (1 - \alpha)} \ln \left[ \frac{I_o (1 - \alpha) - I_{s0}}{I_{s0}} \right] \quad 4.3.33 \]

\[ \frac{\varepsilon^* \phi}{V} \ t = -\frac{1}{I_o (1 - \alpha)} \ln \left( \frac{I_o (1 - \alpha) - I_{s0}}{I_{s0}} \right) \]

For any time \( t, I_s = I_{st} \), substitute the value in equation 4.3.33:

\[ \frac{\varepsilon^* \phi}{V} L \ t = -\frac{1}{I_o (1 - \alpha)} \ln \left( \frac{I_o (1 - \alpha) - I_{st}}{I_{st}} \right) \]

\[ \frac{\varepsilon^* \phi}{V} L \ t = -\frac{1}{I_o (1 - \alpha)} \ln \left[ \frac{I_o (1 - \alpha) - I_{st}}{I_{st}} \right] + \frac{1}{I_o (1 - \alpha)} \ln \left[ \frac{I_o (1 - \alpha) - I_{s0}}{I_{s0}} \right] \]

\[ \frac{\varepsilon^* \phi}{V} t = -\frac{1}{I_o (1 - \alpha)} \left[ \ln \left( \frac{I_o (1 - \alpha) - I_{st}}{I_{st}} \right) - \ln \left( \frac{I_o (1 - \alpha) - I_{s0}}{I_{s0}} \right) \right] \]

\[ \frac{\varepsilon^* \phi}{V} t I_o (1 - \alpha) = \ln \left[ \frac{I_o (1 - \alpha) - I_{st}}{I_{st}} \right] - \ln \left[ \frac{I_o (1 - \alpha) - I_{s0}}{I_{s0}} \right] \quad 4.3.35 \]
As for $t = 0$, $I_a = I_{ai}$ so $D_n = D_{ni}$

And at any time $t$: $I_a = I_{at}$ so $D_n = D_{nt}$

The equation 4.3.17 for initial condition and at any time $t'$ can be represented as follows respectively:

$$I_{ai} = \frac{\beta (D_m - D_{ni})}{(1 - \alpha)}$$

$$I_{at} = \frac{\beta (D_m - D_{nt})}{(1 - \alpha)}$$

Substitute the value of $I_{ai}$ and $I_{at}$ in equation 4.3.35

$$\varepsilon^* \varphi \frac{L L_0 (1 - \alpha) t}{V} =$$

$$= \left[ \begin{array}{c}
I_o (1 - \alpha) - \frac{\beta (D_m - D_{nt})}{(1 - \alpha)} \\
\frac{\beta (D_m - D_{nt})}{(1 - \alpha)} \\
\frac{\beta (D_m - D_{nt})}{(1 - \alpha)} \\
\frac{\beta (D_m - D_{ni})}{(1 - \alpha)}
\end{array} \right]$$

$$- \varepsilon^* \varphi \frac{L L_0 (1 - \alpha) t}{V} =$$

$$= \left[ \begin{array}{c}
I_o (1 - \alpha)^2 - \frac{\beta (D_m - D_{nt})}{(1 - \alpha)} \\
\frac{\beta (D_m - D_{nt})}{(1 - \alpha)} \\
\frac{\beta (D_m - D_{nt})}{(1 - \alpha)} \\
\frac{\beta (D_m - D_{ni})}{(1 - \alpha)}
\end{array} \right]$$
According to equation 4.3.1 and 4.3.15, substitute the value of \( I_o = \beta D_o \) and in equation 4.3.38, we get:

\[
(1 - \alpha)^2 = \frac{D_m}{D_n} \left\{ \ln \left[ \frac{\beta D_o \times \frac{D_m}{D_n} - 3(D_m - D_{nt})}{\beta (D_m - D_at)} \right] \right. - \\
- \left. \frac{\varepsilon^* \varphi L I_o (1 - \alpha) t}{V} = \ln \left[ \frac{\beta[D_m - (D_m - D_{at})]}{\beta (D_m - D_at)} \right] - \ln \left[ \frac{\beta[D_n - (D_m - D_{nt})]}{\beta (D_m - D_{ni})} \right] \right. \\
- \left. \frac{\varepsilon^* \varphi L I_o (1 - \alpha) t}{V} = \ln \left[ \frac{(D_m - D_{nt})}{(D_m - D_{nt})} \right] - \ln \left[ \frac{(D_m - D_{nt} + D_{at})}{(D_m - D_{nt})} \right] \\
- \left. \frac{\varepsilon^* \varphi L I_o (1 - \alpha) t}{V} 4.3.39 = \ln \left( \frac{D_{nt}}{D_m - D_{nt}} \right) - \ln \left( \frac{D_{ni}}{D_m - D_{ni}} \right) \right. \\
- \left. \frac{\varepsilon^* \varphi L I_o (1 - \alpha) t}{V} = - \ln \left( \frac{D_m - D_{nt}}{D_{ni}} \right) + \ln \left( \frac{D_m - D_{ni}}{D_{ni}} \right) \right. \\
- \left. \frac{\varepsilon^* \varphi L I_o (1 - \alpha) t}{V} 4.3.40 = - \ln \left( \frac{D_m - D_{nt}}{D_{nt}} \right) - \ln \left( \frac{D_{ni}}{D_{ni}} \right) \right. \\

\]

or

\[
\frac{\varepsilon^* \varphi L I_o (1 - \alpha) t}{V} = - \ln \left( \frac{D_m - D_{nt}}{D_{nt}} \right) - \ln \left( \frac{D_{ni}}{D_{ni}} \right) \\

\]

Substituting the value \( \varepsilon^* = 2.303 \varepsilon \)

\[
\frac{2303 \varepsilon \varphi L I_o (1 - \alpha) t}{V} = 2.303 \ln \left( \frac{D_m - D_{nt}}{D_{nt}} \right) - \\
2.303 \ln \left( \frac{D_m - D_{ni}}{D_{ni}} \right) \\

Dividing equation 4.3.40 by 2.303, we get:
\[
\frac{\varepsilon \varphi \cdot L \cdot I_0 \cdot (1 - \alpha)}{V} = \log \left( \frac{D_m - D_{nt}}{D_{nt}} \right) - \log \left( \frac{D_m - D_{ni}}{D_{ni}} \right) \quad \text{4.3.41}
\]

\[
\log \left( \frac{D_m - D_{nt}}{D_{nt}} \right) = \frac{\varepsilon \varphi \cdot L \cdot I_0 \cdot (1 - \alpha) \cdot t}{V} + \log \left( \frac{D_m - D_{ni}}{D_{ni}} \right) \quad \text{4.3.42}
\]

Equation 4.3.42 is a straight line equation as the plot of \( \log \left( \frac{D_m - D_{nt}}{D_{nt}} \right) \) against time 't'.

will be a straight line with the slope \( \frac{I_0 \cdot (1 - \alpha) \cdot \varepsilon \varphi \cdot L}{V} \)

and intercept \( \log \left( \frac{D_m - D_{nt}}{D_{nt}} \right) \). Representative plots are shown in Figures 4.4 - 4.8. The quantum yield (\( \phi \)) was calculated from the slope using equation 4.3.42 as follows:

\[
\text{Slope} = \frac{I_0 \cdot (1 - \alpha) \cdot \varepsilon \varphi \cdot L}{V}
\]

\[
\varphi = \frac{\text{Slope} \times V}{I_0 \cdot (1 - \alpha) \cdot \varepsilon \cdot L}
\]

where \( L \) is the length of path travelled 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\(^3\), \( I_0 \) is the initial intensity of the beam and \( (1 - \alpha) \) is the fraction of light transmitted through the window were measured during the experiment.
Figure 4.4: A plot of $\log \frac{D_m-D_n}{D_n}$ vs Time for monosacharides (Galactose) at two different acidities
4.4 THE INERT ATMOSPHERE FOR PHOTO REDUCTION OF
METHYLENE BLUE WITH REDUCING SUGARS AT TWO
DIFFERENT ACIDITIES H_o = 4.2 AND 7.7

The methylene blue (MB) is a stable dye but leuco methylene blue (MB) will immediately
be oxidized to MB in the presence of oxygen, therefore to investigate the photoreduction of
methylene blue, it is necessary to provide the oxygen free atmosphere by bubbling the
nitrogen through reaction mixture [140]. The inert atmosphere was created for 10 to 60
minutes and at every 10 minutes of time interval. The quantum yield for the photochemical
reaction of MB in presence of monosaccharides and disaccharides were determined and
tabulated in Tables 13 – 14. From tables it was observed that when nitrogen was bubbled
for forty minutes, maximum quantum yield was obtained. Thus for completely
deoxygenated system, it is necessary to pass the nitrogen for forty minutes. It is therefore
concluded that inert atmosphere plays a significant role in photoreduction of methylene
blue with reducing sugars, using acetate buffer solution. In present work nitrogen was
bubbled for forty minutes before each run for complete removal of oxygen. The reacting
solution was irradiated with a wavelength of 661 nm, obtained by using red Kodak filter
no. 15 as follows when:

1) No cell was placed in path of the beam in cell holder.
2) Cell containing solvent
3) Cell containing deoxygenated reaction mixture.
4) No. of experiment carried out for determination of quantum yield of each set were
   three.
Figure 4.5: A plot of $\log D_m - D_m/D_{in}$ versus time for monosaccharides (D-mannose) at two different acidities.
Figure 4.6: A plot of log $D_m - D_n / D_n$ versus time for monosacharides (D- (+) Ribose) at two different acidities.
Figure 4.7: A plot of $\log D_m - D_{h1}/D_{n1}$ versus time for diastereides (Maltose) at two different acidities.
Figure 4.8: A plot of log $D_m - D_{m+} / D_m$ versus time for diastereoisomers (Lactose) at two different acidities.
4.4 THE INERT ATMOSPHERE FOR PHOTO REDUCTION OF METHYLENE BLUE WITH REDUCING SUGARS AT TWO DIFFERENT ACIDITIES $H_0 = 4.2$ AND 7.7

The methylene blue (MB) is a stable dye but leuco methylene blue (MB) will immediately be oxidized to MB in the presence of oxygen, therefore to investigate the photoreduction of methylene blue, it is necessary to provide the oxygen free atmosphere by bubbling the nitrogen through reaction mixture [140]. The inert atmosphere was created for 10 to 60 minutes and at every 10 minutes of time interval. The quantum yield for the photochemical reaction of MB in presence of monosaccharides and disaccharides were determined and tabulated in Tables 13 – 14. From tables it was observed that when nitrogen was bubbled for forty minutes, maximum quantum yield was obtained. Thus for completely deoxygenated system, it is necessary to pass the nitrogen for forty minutes. It is therefore concluded that inert atmosphere plays a significant role in photoreduction of methylene blue with reducing sugars, using acetate buffer solution. In present work nitrogen was bubbled for forty minutes before each run for complete removal of oxygen. The reacting solution was irradiated with a wavelength of 661 nm, obtained by using red Kodak filter no. 15 as follows when:

1) No cell was placed in path of the beam in cell holder.
2) Cell containing solvent
3) Cell containing deoxygenated reaction mixture.
4) No. of experiment carried out for determination of quantum yield of each set were three.
Table - 13
EFFECT OF NITROGEN ON QUANTUM YIELD FOR PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE WITH MONOSACCHARIDES

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Buffer</th>
<th>Temp.</th>
<th>(10^6 \cdot [\text{MB}]) mol.dm(^{-3})</th>
<th>(10^3 \cdot [\text{RCHO}]) mol.dm(^{-3})</th>
<th>Time for nitrogen bubbling (min)</th>
<th>(\phi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% Aqueous methanol</td>
<td>Acetate</td>
<td>25(^\circ)C</td>
<td>1.0</td>
<td>0.8</td>
<td>10</td>
<td>0.016 ± 0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
<td>0.8</td>
<td>20</td>
<td>0.085 ± 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
<td>0.8</td>
<td>30</td>
<td>0.127 ± 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
<td>0.8</td>
<td>40</td>
<td>0.151 ± 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
<td>0.8</td>
<td>50</td>
<td>0.149 ± 0.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.0</td>
<td>0.8</td>
<td>60</td>
<td>0.151 ± 0.0001</td>
</tr>
<tr>
<td>[(H_0)] = 4.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[(H_0)] = 7.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

|                |          |       | 1.0                                         | 0.8                                      | 10                              | 0.061 ± 0.002   |
|                |          |       | 1.0                                         | 0.8                                      | 20                              | 0.062 ± 0.002   |
|                |          |       | 1.0                                         | 0.8                                      | 30                              | 0.072 ± 0.004   |
|                |          |       | 1.0                                         | 0.8                                      | 40                              | 0.099 ± 0.008   |
|                |          |       | 1.0                                         | 0.8                                      | 50                              | 0.099 ± 0.001   |
|                |          |       | 1.0                                         | 0.8                                      | 60                              | 0.099 ± 0.009   |
Table - 14

EFFECT OF NITROGEN ON QUANTUM YIELD FOR PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE WITH DISACCHARIDES

Solvent = 50% Aqueous methanol
Buffer = Acetate  Temp. = 25°C

<table>
<thead>
<tr>
<th>$10^6 \cdot [\text{MB}]$</th>
<th>$10^3 \cdot [\text{RCHO}]$</th>
<th>Time for nitrogen bubbling (min)</th>
<th>$\phi$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mol.dm$^{-3}$</td>
<td>mol.dm$^{-3}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0.8</td>
<td>10</td>
<td>0.015 ± 0.001</td>
</tr>
<tr>
<td>1.0</td>
<td>0.8</td>
<td>20</td>
<td>0.083 ± 0.003</td>
</tr>
<tr>
<td>1.0</td>
<td>0.8</td>
<td>30</td>
<td>0.131 ± 0.001</td>
</tr>
<tr>
<td>1.0</td>
<td>0.8</td>
<td>40</td>
<td>0.15 ± 0.0012</td>
</tr>
<tr>
<td>1.0</td>
<td>0.8</td>
<td>50</td>
<td>0.151 ± 0.001</td>
</tr>
<tr>
<td>1.0</td>
<td>0.8</td>
<td>60</td>
<td>0.151 ± 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$[\text{H}_4]$ = 4.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0.8</td>
<td>10</td>
<td>0.055 ± 0.001</td>
</tr>
<tr>
<td>1.0</td>
<td>0.8</td>
<td>20</td>
<td>0.067 ± 0.002</td>
</tr>
<tr>
<td>1.0</td>
<td>0.8</td>
<td>30</td>
<td>0.082 ± 0.003</td>
</tr>
<tr>
<td>1.0</td>
<td>0.8</td>
<td>40</td>
<td>0.093 ± 0.001</td>
</tr>
<tr>
<td>1.0</td>
<td>0.8</td>
<td>50</td>
<td>0.091 ± 0.001</td>
</tr>
<tr>
<td>1.0</td>
<td>0.8</td>
<td>60</td>
<td>0.092 ± 0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$[\text{H}_4]$ = 7.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.5 VARIATION OF ACIDITY ON QUANTUM YIELD OF METHYLENE BLUE IN ABSENCE OF REDUCTANT

Influence of acidity on quantum yield (Φ) of photochemical reduction of methylene blue in presence of acetate buffer solution at concentration of $1 \times 10^{-5}$ mol dm$^{-3}$ in 50% aqueous methanol was studied at different acidities ($H_0$) as shown in Table 15. It was observed that dye reduced to leuco dye by absorption of photon of light of 661 nm in absence of reductant and oxygen, upon exposure to light and on entering the air, the dye oxidized to its blue color in absence of reductant but quantum yield was very low as tabulated in Table 15.

Very low values of quantum yield indicate that dye reduced in absence of reducing agent with solvent which is also proved by analysis of reaction mixture where very less quantity of leuco dye with solvent is obtained. From the values summarized in the Table 15, it was observed that quantum yield was twice in low acidity region $H_0 = 4.2$ as compared to high acidity region $H_0 = 7.7$ in the absence of reductant.
Table - 15

INFLUENCE OF ACIDITY (Hₐ) ON QUANTUM YIELD OF PHOTOREDUCTION OF METHYLENE BLUE IN ABSENCE OF REDUCING SUGARS

\[ [MB] = \cdot \times 10^{-6} \text{ mol dm}^{-3} \]

Buffer = Acetate

Temp. = 25°C

Solvent = 50% aqueous methanol

<table>
<thead>
<tr>
<th>Acidity [Hₐ]</th>
<th>Quantum yield $10^{-3} \cdot \phi$</th>
<th>$\phi_1 - \phi_2$</th>
<th>$\frac{1}{\phi}$</th>
<th>$10^6 \cdot \frac{1}{h_0}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2</td>
<td>8.612 ± 0.002</td>
<td>-</td>
<td>116.1170</td>
<td>0.01584</td>
</tr>
<tr>
<td>4.7</td>
<td>8.311 ± 0.001</td>
<td>0.0887</td>
<td>120.322</td>
<td>0.05018</td>
</tr>
<tr>
<td>5.3</td>
<td>7.832 ± 0.002</td>
<td>0.2676</td>
<td>127.681</td>
<td>0.199526</td>
</tr>
<tr>
<td>6.6</td>
<td>7.235 ± 0.001</td>
<td>0.5943</td>
<td>138.217</td>
<td>3.981071</td>
</tr>
<tr>
<td>6.9</td>
<td>7.015 ± 0.001</td>
<td>0.7615</td>
<td>142.551</td>
<td>7.9432</td>
</tr>
<tr>
<td>7.0</td>
<td>6.812 ± 0.001</td>
<td>0.9503</td>
<td>146.799</td>
<td>10.000</td>
</tr>
<tr>
<td>7.3</td>
<td>6.413 ± 0.001</td>
<td>1.470</td>
<td>155.933</td>
<td>19.9526</td>
</tr>
<tr>
<td>7.4</td>
<td>5.516 ± 0.001</td>
<td>5.1772</td>
<td>181.290</td>
<td>25.1188</td>
</tr>
<tr>
<td>7.6</td>
<td>5.210 ± 0.001</td>
<td>11.650</td>
<td>191.385</td>
<td>39.8107</td>
</tr>
<tr>
<td>7.7</td>
<td>4.918 ± 0.002</td>
<td>-</td>
<td>203.334</td>
<td>50.1187</td>
</tr>
</tbody>
</table>

Confidence Interval = 0.91529
4.6 THE INFLUENCE OF ACIDITY ON THE QUANTUM YIELD OF PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE WITH REDUCING SUGARS

The dependence of quantum yield with the variation in acidity from low $H_0 = 4.2$ to higher $H_0 = 7.7$ was observed in 50% aqueous methanol with reducing sugars like, Galactose, D. Mannose, D-(+)-Ribose, Maltose and Lactose.

The quantum yields were determined at two different concentrations of reductants i.e. $8.0 \times 10^{-4}$ and $6.0 \times 10^{-2}$ mol dm$^{-3}$ in presence of acetate buffer in 50% aqueous methanol. The results are tabulated in Tables 16 – 20.

It was shown in the tables that a significant decrease in the quantum yield was observed with the increase in the value of $H_0$. The temperature was maintained constant at $25^\circ C \pm 0.1^\circ C$. The plots of quantum yield ($\phi$) versus acidities ($H_0$) for photoreduction of methylene blue are shown in the Figures 4.9 – 4.13. The plots of reciprocal of quantum yield $(1/\phi)$ against $1/H_0$ are straight lines as shown in the Figures 4.14 – 4.18. The values of slopes and intercepts give the value of quantum yield at infinite acidity. Taking $\phi_1$ and $\phi_2$ as the extreme values of quantum yield at high and low acidities, the plot of $\phi_1 - \phi / \phi - \phi_2$ versus $1/H_0$ were straight lines passing through the origin. The corresponding plots of $\phi_1 - \phi / \phi - \phi_2$ against $1/H_0$ are shown in Figures 4.19 – 4.23 for Galactose, D-Mannose, D-(+)-Ribose, Maltose and Lactose respectively.
Table - 16

INFLUENCE OF ACIDITY ON QUANTUM YIELD OF PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE WITH GALACTOSE

\[ [\text{MB}] = 1 \times 10^{-6} \text{ mol dm}^{-3} \quad \text{RCHO} = \text{Galactose} \]

Buffer = Acetate

Solvent = 50% aqueous methanol

\[ \text{Temp.} = 25^\circ \text{C} \]

<table>
<thead>
<tr>
<th>Acidity [H_0]</th>
<th>Quantum yield (\phi)</th>
<th>(\phi_1 \cdot \phi)</th>
<th>(\phi - \phi_2)</th>
<th>(1 / \phi)</th>
<th>(10^{-6} \cdot 1 / h_0)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(4.2)</td>
<td>0.151 ± 0.001</td>
<td></td>
<td></td>
<td></td>
<td>6.6225</td>
</tr>
<tr>
<td>(4.7)</td>
<td>0.149 ± 0.001</td>
<td>0.0385</td>
<td></td>
<td>6.706</td>
<td>0.0501</td>
</tr>
<tr>
<td>(5.3)</td>
<td>0.134 ± 0.002</td>
<td>0.4880</td>
<td></td>
<td>7.4515</td>
<td>0.1995</td>
</tr>
<tr>
<td>(6.6)</td>
<td>0.122 ± 0.001</td>
<td>1.3060</td>
<td></td>
<td>8.1967</td>
<td>3.9810</td>
</tr>
<tr>
<td>(6.9)</td>
<td>0.111 ± 0.008</td>
<td>4.6660</td>
<td></td>
<td>8.9445</td>
<td>7.9432</td>
</tr>
<tr>
<td>(7.0)</td>
<td>0.104 ± 0.005</td>
<td>9.8930</td>
<td></td>
<td>9.5693</td>
<td>10.0000</td>
</tr>
<tr>
<td>(7.3)</td>
<td>0.103 ± 0.002</td>
<td>14.0580</td>
<td></td>
<td>9.6899</td>
<td>19.9520</td>
</tr>
<tr>
<td>(7.4)</td>
<td>0.101 ± 0.001</td>
<td>23.3800</td>
<td></td>
<td>9.8135</td>
<td>25.1180</td>
</tr>
<tr>
<td>(7.6)</td>
<td>0.100 ± 0.001</td>
<td>45.5400</td>
<td></td>
<td>9.9108</td>
<td>39.8100</td>
</tr>
<tr>
<td>(7.7)</td>
<td>0.099 ± 0.008</td>
<td></td>
<td></td>
<td>10.0200</td>
<td>50.1180</td>
</tr>
</tbody>
</table>

\[ \text{[RCHO]} = 8 \times 10^{-4} \text{ mol dm}^{-3} \]

Confidence Interval = 0.01462

\[ \text{[RCHO]} = 6 \times 10^{-3} \text{ mol dm}^{-3} \]

Confidence Interval = 0.05767
Table - 17

INFLUENCE OF ACIDITY ON QUANTUM YIELD OF PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE WITH D-MANNOSE

\[
\begin{align*}
[\text{MB}] &= 1 \times 10^{-6} \text{ mol dm}^{-3} & \text{RCHO} &= \text{D-Mannose} \\
\text{Buffer} &= \text{Acetate} & \text{Temp.} &= 25^\circ\text{C} \\
\text{Solvent} &= 50 \% \text{aqueous methanol} \\
\end{align*}
\]

<table>
<thead>
<tr>
<th>Acidity [Hₐ]</th>
<th>Quantum yield $\phi$</th>
<th>$\frac{\phi_1 - \phi}{\phi_1 - \phi_2}$</th>
<th>$\frac{1}{\phi}$</th>
<th>$10^{-6} \cdot \frac{1}{h_0}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>[RCHO] = $8 \times 10^{-4}$ mol dm$^{-3}$</td>
<td>Confidence Interval = 0.01722</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td>0.179 ± 0.002</td>
<td>—</td>
<td>5.5648</td>
<td>0.0158</td>
</tr>
<tr>
<td>4.7</td>
<td>0.159 ± 0.009</td>
<td>0.3639</td>
<td>6.2539</td>
<td>0.0501</td>
</tr>
<tr>
<td>5.3</td>
<td>0.146 ± 0.005</td>
<td>0.8099</td>
<td>6.8259</td>
<td>0.1995</td>
</tr>
<tr>
<td>6.6</td>
<td>0.130 ± 0.001</td>
<td>2.0040</td>
<td>7.6804</td>
<td>3.9810</td>
</tr>
<tr>
<td>6.9</td>
<td>0.129 ± 0.005</td>
<td>2.0916</td>
<td>7.7220</td>
<td>7.9432</td>
</tr>
<tr>
<td>7.0</td>
<td>0.120 ± 0.001</td>
<td>3.8181</td>
<td>8.2712</td>
<td>10.0000</td>
</tr>
<tr>
<td>7.3</td>
<td>0.116 ± 0.001</td>
<td>5.7454</td>
<td>9.0090</td>
<td>19.9520</td>
</tr>
<tr>
<td>7.4</td>
<td>0.111 ± 0.001</td>
<td>12.4900</td>
<td>8.5836</td>
<td>25.1180</td>
</tr>
<tr>
<td>7.6</td>
<td>0.109 ± 0.002</td>
<td>19.0540</td>
<td>9.1575</td>
<td>39.8100</td>
</tr>
<tr>
<td>7.7</td>
<td>0.105 ± 0.005</td>
<td>—</td>
<td>9.4786</td>
<td>50.1180</td>
</tr>
</tbody>
</table>

| [RCHO] = $6 \times 10^{-3}$ mol dm$^{-3}$ | Confidence Interval = 0.05717 |
| 4.2 | 0.499 ± 0.001 | — | 2.003 | 0.0158 |
| 4.7 | 0.466 ± 0.003 | 0.1734 | 2.1454 | 0.0501 |
| 5.3 | 0.388 ± 0.005 | 0.9813 | 2.5740 | 0.1995 |
| 6.6 | 0.333 ± 0.001 | 2.9036 | 3.0030 | 3.9810 |
| 6.9 | 0.323 ± 0.002 | 3.6520 | 3.0883 | 7.9432 |
| 7.0 | 0.317 ± 0.001 | 4.3040 | 3.1456 | 10.0000 |
| 7.3 | 0.291 ± 0.001 | 13.3140 | 3.4317 | 19.9520 |
| 7.4 | 0.284 ± 0.003 | 23.5384 | 3.5100 | 25.1180 |
| 7.6 | 0.280 ± 0.001 | 50.9302 | 3.5701 | 39.8100 |
| 7.7 | 0.275 ± 0.002 | — | 3.6258 | 50.1180 |
Table - 18

INFLUENCE OF ACIDITY ON QUANTUM YIELD OF PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE WITH D-(+)-RIBOSE

[MB] = 1 × 10^{-6} \text{ mol dm}^{-3} \quad \text{RCHO} = \text{D-(+)-Ribose}

Buffer = Acetate \quad \text{Temp.} = 25^\circ\text{C}

Solvent = 50 \text% aqueous methanol

<table>
<thead>
<tr>
<th>Acidity [H\text{\textsubscript{a}}]</th>
<th>Quantum yield $\phi$</th>
<th>$\phi_1 - \phi$</th>
<th>$\phi_1 - \phi_2$</th>
<th>$\frac{1}{\phi}$</th>
<th>$10^{-6} \cdot \frac{1}{h_0}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>[RCHO] = 8 × 10^{-4} \text{ mol dm}^{-3}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td>0.162 ± 0.001</td>
<td>—</td>
<td>—</td>
<td>6.1387</td>
<td>0.0158</td>
</tr>
<tr>
<td>4.7</td>
<td>0.145 ± 0.007</td>
<td>0.4056</td>
<td>6.8343</td>
<td>0.0501</td>
<td></td>
</tr>
<tr>
<td>5.3</td>
<td>0.126 ± 0.007</td>
<td>1.5470</td>
<td>7.8926</td>
<td>0.1995</td>
<td></td>
</tr>
<tr>
<td>6.6</td>
<td>0.120 ± 0.003</td>
<td>2.5050</td>
<td>8.3125</td>
<td>3.9810</td>
<td></td>
</tr>
<tr>
<td>6.9</td>
<td>0.115 ± 0.001</td>
<td>3.9666</td>
<td>8.6730</td>
<td>7.9432</td>
<td></td>
</tr>
<tr>
<td>7.0</td>
<td>0.111 ± 0.001</td>
<td>6.5443</td>
<td>8.9920</td>
<td>10.0000</td>
<td></td>
</tr>
<tr>
<td>7.3</td>
<td>0.110 ± 0.001</td>
<td>7.8955</td>
<td>9.0909</td>
<td>19.9520</td>
<td></td>
</tr>
<tr>
<td>7.4</td>
<td>0.109 ± 0.003</td>
<td>8.9330</td>
<td>9.1491</td>
<td>25.1180</td>
<td></td>
</tr>
<tr>
<td>7.6</td>
<td>0.105 ± 0.001</td>
<td>24.9130</td>
<td>9.4696</td>
<td>39.8100</td>
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<tr>
<td>7.7</td>
<td>0.103 ± 0.003</td>
<td>—</td>
<td>9.6805</td>
<td>50.1180</td>
<td></td>
</tr>
</tbody>
</table>

Confidence interval = 0.01362

| 4.2       | 0.509 ± 0.002   | —              | 1.9630        | 0.0158         |
| 4.7       | 0.475 ± 0.001   | 0.1655        | 2.1043        | 0.0501        |
| 5.3       | 0.421 ± 0.001   | 0.5833        | 2.3750        | 0.1995        |
| 6.6       | 0.375 ± 0.001   | 1.2536        | 2.6602        | 3.9810        |
| 6.9       | 0.342 ± 0.002   | 2.2749        | 2.9163        | 7.9432        |
| 7.0       | 0.314 ± 0.001   | 4.4040        | 3.1836        | 10.0000       |
| 7.3       | 0.293 ± 0.001   | 9.2307        | 3.5587        | 19.9520       |
| 7.4       | 0.281 ± 0.004   | 20.3750       | 3.7439        | 25.1180       |
| 7.6       | 0.267 ± 0.002   | 38.4285       | 3.7100        | 39.8100       |
| 7.7       | 0.260 ± 0.003   | —             | 3.8343        | 50.1180       |

Confidence interval = 0.06344
<table>
<thead>
<tr>
<th>Acidity [H₂]</th>
<th>Quantum yield Φ</th>
<th>( \frac{Φ_1 - Φ}{Φ_1 - Φ_2} )</th>
<th>( \frac{1}{Φ} )</th>
<th>( 10^{-6} \cdot \frac{1}{n_0} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>[RCHO] = 8 \times 10^{-4} mol dm^{-3}</td>
<td>Confidence Interval = 0.01541</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td>0.151 ± 0.002</td>
<td>—</td>
<td>6.6137</td>
<td>0.0158</td>
</tr>
<tr>
<td>4.7</td>
<td>0.140 ± 0.001</td>
<td>0.2246</td>
<td>7.1377</td>
<td>0.0501</td>
</tr>
<tr>
<td>5.3</td>
<td>0.131 ± 0.003</td>
<td>0.4901</td>
<td>7.6161</td>
<td>0.195</td>
</tr>
<tr>
<td>6.6</td>
<td>0.126 ± 0.001</td>
<td>0.7090</td>
<td>7.9302</td>
<td>3.9810</td>
</tr>
<tr>
<td>6.9</td>
<td>0.116 ± 0.002</td>
<td>1.3725</td>
<td>8.6058</td>
<td>7.9432</td>
</tr>
<tr>
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<td>2.8291</td>
<td>9.3896</td>
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</tr>
<tr>
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<td>0.098 ± 0.003</td>
<td>5.9605</td>
<td>10.1729</td>
<td>19.9520</td>
</tr>
<tr>
<td>7.4</td>
<td>0.095 ± 0.006</td>
<td>10.6340</td>
<td>10.4275</td>
<td>25.1180</td>
</tr>
<tr>
<td>7.6</td>
<td>0.093 ± 0.002</td>
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<td>39.8100</td>
</tr>
<tr>
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<td>0.090 ± 0.003</td>
<td>—</td>
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<td></td>
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<td>0.508 ± 0.001</td>
<td>—</td>
<td>1.9673</td>
<td>0.0158</td>
</tr>
<tr>
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<td>0.0501</td>
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<td>2.1968</td>
<td>0.195</td>
</tr>
<tr>
<td>6.6</td>
<td>0.424 ± 0.001</td>
<td>0.5115</td>
<td>2.3579</td>
<td>3.9810</td>
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<tr>
<td>6.9</td>
<td>0.397 ± 0.002</td>
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<td>2.5109</td>
<td>7.9432</td>
</tr>
<tr>
<td>7.0</td>
<td>0.353 ± 0.001</td>
<td>1.6461</td>
<td>2.8296</td>
<td>10.0000</td>
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<tr>
<td>7.3</td>
<td>0.318 ± 0.002</td>
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<td>3.1436</td>
<td>19.9520</td>
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<td>7.4</td>
<td>0.289 ± 0.001</td>
<td>7.3557</td>
<td>3.4590</td>
<td>25.1180</td>
</tr>
<tr>
<td>7.6</td>
<td>0.265 ± 0.002</td>
<td>42.6842</td>
<td>3.7735</td>
<td>39.8100</td>
</tr>
<tr>
<td>7.7</td>
<td>0.259 ± 0.002</td>
<td>—</td>
<td>3.8565</td>
<td>50.1180</td>
</tr>
</tbody>
</table>
Table - 20

INFLUENCE OF ACIDITY ON QUANTUM YIELD OF PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE WITH LACTOSE

\[ [\text{MB}] = 1 \times 10^{-6} \text{ mol dm}^{-3} \quad \text{RCHO} = \text{Lactose} \]

Buffer = Acetate  
Temp. = 25°C  
Solvent = 50 % aqueous methanol

<table>
<thead>
<tr>
<th>Acidity [ [H_3] ]</th>
<th>Quantum yield [ \phi ]</th>
<th>[ \phi_1 - \phi ]</th>
<th>[ \phi - \phi_2 ]</th>
<th>[ \frac{1}{\phi} ]</th>
<th>[ 10^{-6}. \frac{1}{h_0} ]</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ [\text{RCHO}] = 8 \times 10^{-4} \text{ mol dm}^{-3} ]</td>
<td>[ 4.2 ]</td>
<td>0.14 ± 0.001</td>
<td>—</td>
<td>—</td>
<td>6.8960</td>
</tr>
<tr>
<td></td>
<td>[ 4.7 ]</td>
<td>0.137 ± 0.003</td>
<td>0.1609</td>
<td>7.2510</td>
<td>0.0501</td>
</tr>
<tr>
<td></td>
<td>[ 5.3 ]</td>
<td>0.132 ± 0.002</td>
<td>0.3161</td>
<td>7.5350</td>
<td>0.1995</td>
</tr>
<tr>
<td></td>
<td>[ 6.6 ]</td>
<td>0.130 ± 0.002</td>
<td>0.4065</td>
<td>7.6800</td>
<td>3.9810</td>
</tr>
<tr>
<td></td>
<td>[ 6.9 ]</td>
<td>0.126 ± 0.001</td>
<td>0.5705</td>
<td>7.9110</td>
<td>7.9432</td>
</tr>
<tr>
<td></td>
<td>[ 7.0 ]</td>
<td>0.123 ± 0.002</td>
<td>0.7297</td>
<td>8.1030</td>
<td>10.0000</td>
</tr>
<tr>
<td></td>
<td>[ 7.3 ]</td>
<td>0.117 ± 0.003</td>
<td>1.1880</td>
<td>8.5320</td>
<td>19.9520</td>
</tr>
<tr>
<td></td>
<td>[ 7.4 ]</td>
<td>0.104 ± 0.003</td>
<td>3.9708</td>
<td>9.6060</td>
<td>25.1180</td>
</tr>
<tr>
<td></td>
<td>[ 7.6 ]</td>
<td>0.098 ± 0.004</td>
<td>10.3777</td>
<td>10.1720</td>
<td>39.8100</td>
</tr>
<tr>
<td></td>
<td>[ 7.7 ]</td>
<td>0.093 ± 0.001</td>
<td>—</td>
<td>—</td>
<td>10.6600</td>
</tr>
</tbody>
</table>

| \[ [\text{RCHO}] = 6 \times 10^{-3} \text{ mol dm}^{-3} \] | \[ 4.2 \] | 0.516 ± 0.002 | — | — | 1.937 | 0.0158 |
| | \[ 4.7 \] | 0.505 ± 0.001 | 0.0404 | 1.9782 | 0.0501 |
| | \[ 5.3 \] | 0.442 ± 0.003 | 0.3748 | 2.260 | 0.1995 |
| | \[ 26.6 \] | 0.393 ± 0.001 | 0.8325 | 2.543 | 3.9810 |
| | \[ 6.9 \] | 0.379 ± 0.002 | 1.0262 | 2.637 | 7.9432 |
| | \[ 7.0 \] | 0.353 ± 0.003 | 1.4981 | 2.825 | 10.0000 |
| | \[ 7.3 \] | 0.321 ± 0.004 | 2.5565 | 3.108 | 19.9520 |
| | \[ 7.4 \] | 0.294 ± 0.002 | 4.5276 | 3.394 | 25.1180 |
| | \[ 7.6 \] | 0.265 ± 0.001 | 12.7208 | 3.767 | 39.8100 |
| | \[ 7.7 \] | 0.248 ± 0.002 | — | — | 4.070 | 50.1180 |
Figure 4.9: A plot of $\phi$ vs $[H_0]$ for the photoreduction of methylene blue with galactose
Figure 4.10: A plot of $\phi$ verses $[H_0]$ for the photoreduction of methylene blue with D-Mannose
Figure 4.15: A plot of $1/\phi$ verses $1/h_0$ for the photoreduction of methylene blue with D-Mannose
Figure 4.16: A plot of $1/\phi$ vs $1/h_0$ for the photoreduction of methylene blue with D-(+)-Ribose
Figure 4.17: A plot of $1/\phi$ vs $1/h_o$ for the photoreduction of methylene blue with Maltose
Figure 4.18: A plot of $1/\phi$ vs $1/\h_0$ for the photoreduction of methylene blue with Lactose
Figure 4.19: A plot of $\phi_1 / \phi_2$ vs $1/h_0 \times 10^4$ for the photoreduction of methylene blue with Galactose
Figure 4.20: A plot of $\phi_1 - \phi / \phi_2$ vs $1/\nu_0 \times 10^{-6}$ for the photoreduction of methylene blue with D-Mannose
Figure 4.21: A plot of $\phi_1/\phi - \phi_2$ vs $1/h_0 \times 10^{-6}$ for the photoreduction of methylene blue with D-(-)-Ribose
Figure 4.22: A plot of $\phi_1 - \phi_2 / \phi_2$ vs $1/h_0 \times 10^{-6}$ for the photoreduction of methylene blue with Maltose
Figure 4.23: A plot of $\phi_1 - \phi / \phi - \phi_2$ vs $1/h_o \times 10^6$ for the photoreduction of methylene blue with Lactose
4.7 INFLUENCE OF CONCENTRATION OF REDUCING SUGARS ON THE QUANTUM YIELD OF PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE

The influence of concentration of reducing sugars such as Galactose, D-Mannose, D-(+)-Ribose, Maltose and Lactose on quantum yields of the photochemical reduction of methylene blue in 50% aqueous methanol was investigated. It was observed that for concentration of $1 \times 10^{-6}$ mol dm$^{-3}$ of methylene blue and reductant concentration from $8 \times 10^{-4}$ to $6 \times 10^{-3}$ mol dm$^{-3}$ at two acidities 4.2 and 7.7, quantum yields of photoreduction of methylene showed linear increase in quantum yield for reductants concentration i.e. quantum yield increases with the increase in the concentration of reducing sugars.

The results are for reducing sugars Galactose, D-Mannose, D-(+)-Ribose, Maltose and Lactose tabulated in Tables 21 to 25 respectively. The representative plots of $1/\phi$ versus $1/[RCHO]$ are shown in Figures 4.24 – 4.28 and the values of quantum yields at infinite concentration of reductants are calculated from the graphs and are shown in Table 26.
### Table 21

**INFLUENCE OF CONCENTRATION OF REDUCTANT GALACTOSE ON QUANTUM YIELD OF METHYLENE BLUE**

- **RCHO** = Galactose
- **Buffer** = Acetate
- **[MB]** = $1 \times 10^{-6}$ mol dm$^{-3}$
- **Temp.** = 25°C
- **Solvent** = 50% aqueous methanol

<table>
<thead>
<tr>
<th>$10^3 \cdot [\text{RCHO}]$ (mol dm$^{-3}$)</th>
<th>Quantum yield $\phi$</th>
<th>$10^{-3} \cdot \frac{1}{[\text{RCHO}]}$ (mol$^{-1}$ dm$^{-3}$)</th>
<th>$\frac{1}{\phi}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>$[H_0] = 4.2$</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>0.151 ± 0.001</td>
<td>1.250</td>
<td>6.6225</td>
</tr>
<tr>
<td>1.6</td>
<td>0.173 ± 0.001</td>
<td>0.625</td>
<td>5.7603</td>
</tr>
<tr>
<td>2.0</td>
<td>0.201 ± 0.001</td>
<td>0.500</td>
<td>4.9627</td>
</tr>
<tr>
<td>3.0</td>
<td>0.251 ± 0.002</td>
<td>0.333</td>
<td>3.9793</td>
</tr>
<tr>
<td>4.0</td>
<td>0.311 ± 0.001</td>
<td>0.250</td>
<td>3.2102</td>
</tr>
<tr>
<td>5.0</td>
<td>0.372 ± 0.001</td>
<td>0.200</td>
<td>2.6838</td>
</tr>
<tr>
<td>6.0</td>
<td>0.496 ± 0.001</td>
<td>0.166</td>
<td>2.0128</td>
</tr>
<tr>
<td><strong>$[H_0] = 7.7$</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>0.099 ± 0.008</td>
<td>1.250</td>
<td>10.0200</td>
</tr>
<tr>
<td>1.6</td>
<td>0.119 ± 0.001</td>
<td>0.625</td>
<td>8.4033</td>
</tr>
<tr>
<td>2.0</td>
<td>0.129 ± 0.008</td>
<td>0.500</td>
<td>7.7041</td>
</tr>
<tr>
<td>3.0</td>
<td>0.147 ± 0.003</td>
<td>0.333</td>
<td>6.7613</td>
</tr>
<tr>
<td>4.0</td>
<td>0.181 ± 0.001</td>
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</tr>
<tr>
<td>5.0</td>
<td>0.221 ± 0.001</td>
<td>0.200</td>
<td>4.5066</td>
</tr>
<tr>
<td>6.0</td>
<td>0.277 ± 0.003</td>
<td>0.166</td>
<td>4.3941</td>
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</table>

Confidence Interval = 0.11405

Confidence Interval = 0.05847
### Table 22

**INFLUENCE OF CONCENTRATION OF REDUCTANT D-MANNOSE ON QUANTUM YIELD OF METHYLENE BLUE**

<table>
<thead>
<tr>
<th>$10^3 \cdot [\text{RCHO}]$ (mol dm$^{-3}$)</th>
<th>Quantum yield $\phi$</th>
<th>$10^3 \cdot \frac{1}{[\text{RCHO}]}$ (mol$^{-1}$ dm$^3$)</th>
<th>$\frac{1}{\phi}$</th>
</tr>
</thead>
<tbody>
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<td></td>
</tr>
<tr>
<td>0.8</td>
<td>0.179 ± 0.002</td>
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<td>6.8965</td>
</tr>
<tr>
<td>1.6</td>
<td>0.192 ± 0.005</td>
<td>0.625</td>
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</tr>
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<td>2.0</td>
<td>0.224 ± 0.002</td>
<td>0.500</td>
<td>4.6926</td>
</tr>
<tr>
<td>3.0</td>
<td>0.276 ± 0.001</td>
<td>0.333</td>
<td>4.0070</td>
</tr>
<tr>
<td>4.0</td>
<td>0.326 ± 0.001</td>
<td>0.250</td>
<td>3.3921</td>
</tr>
<tr>
<td>5.0</td>
<td>0.399 ± 0.002</td>
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<tr>
<td>6.0</td>
<td>0.499 ± 0.001</td>
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<td>1.9379</td>
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<td>0.105 ± 0.005</td>
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</tr>
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<td>0.275 ± 0.002</td>
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Table - 23

**INFLUENCE OF CONCENTRATION OF REDUCTANT D-(+)-RIBOSE ON QUANTUM YIELD OF METHYLENE BLUE**

<table>
<thead>
<tr>
<th>RCHO</th>
<th>Buffer</th>
<th>[MB]</th>
<th>Temp.</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>= D-(+)-Ribose</td>
<td>= Acetate</td>
<td>= $1 \times 10^{-6}$ mol dm$^{-3}$</td>
<td>= 25°C</td>
<td>= 50% aqueous methanol</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$10^3 \cdot [\text{RCHO}]$ (mol dm$^{-3}$)</th>
<th>Quantum yield $\phi$</th>
<th>$10^3 \cdot \frac{1}{[\text{RCHO}]}$ (mol$^{-1}$ dm$^{-3}$)</th>
<th>$\frac{1}{\phi}$</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Confidence Interval = 0.11354</td>
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<td>0.8</td>
<td>0.162 ± 0.001</td>
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<td>6.1387</td>
</tr>
<tr>
<td>1.6</td>
<td>0.191 ± 0.001</td>
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<td>0.234 ± 0.001</td>
<td>0.500</td>
<td>4.2716</td>
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<td>0.336 ± 0.005</td>
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<tr>
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</tr>
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<td>1.6</td>
<td>0.128 ± 0.001</td>
<td>0.625</td>
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<td>2.0</td>
<td>0.151 ± 0.001</td>
<td>0.500</td>
<td>6.5832</td>
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<tr>
<td>3.0</td>
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<tr>
<td>4.0</td>
<td>0.213 ± 0.001</td>
<td>0.250</td>
<td>4.6860</td>
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<td>0.245 ± 0.001</td>
<td>0.200</td>
<td>4.0783</td>
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<tr>
<td>6.0</td>
<td>0.269 ± 0.003</td>
<td>0.166</td>
<td>3.7064</td>
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</table>
**Table - 24**

**EFFECT OF CONCENTRATION OF REDUCTANT MALTOSE ON QUANTUM YIELD OF METHYLENE BLUE**

<table>
<thead>
<tr>
<th>RCHO</th>
<th>Maltose</th>
<th>Buffer</th>
<th>Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>[MB]</td>
<td>$1 \times 10^{-6}$ mol dm$^{-3}$</td>
<td>Temp.</td>
<td>$25^\circ$C</td>
</tr>
<tr>
<td>Solvent</td>
<td>50% aqueous methanol</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$10^3 \cdot [\text{RCHO}]$ (mol dm$^{-3}$)</th>
<th>Quantum yield $\phi$</th>
<th>$10^{-3} \cdot \frac{1}{[\text{RCHO}]}$ (mol$^{-1}$ dm$^{-3}$)</th>
<th>$\frac{1}{\phi}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[H_0] = 4.2$</td>
<td></td>
<td>Confidence Interval = 0.115407</td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>0.151 $\pm$ 0.002</td>
<td>1.250</td>
<td>6.6137</td>
</tr>
<tr>
<td>1.6</td>
<td>0.189 $\pm$ 0.001</td>
<td>0.625</td>
<td>5.2882</td>
</tr>
<tr>
<td>2.0</td>
<td>0.213 $\pm$ 0.002</td>
<td>0.500</td>
<td>4.6838</td>
</tr>
<tr>
<td>3.0</td>
<td>0.242 $\pm$ 0.001</td>
<td>0.333</td>
<td>4.1322</td>
</tr>
<tr>
<td>4.0</td>
<td>0.302 $\pm$ 0.004</td>
<td>0.250</td>
<td>3.3057</td>
</tr>
<tr>
<td>5.0</td>
<td>0.381 $\pm$ 0.003</td>
<td>0.200</td>
<td>2.6226</td>
</tr>
<tr>
<td>6.0</td>
<td>0.508 $\pm$ 0.001</td>
<td>0.166</td>
<td>1.9673</td>
</tr>
<tr>
<td>$[H_0] = 7.7$</td>
<td></td>
<td>Confidence Interval = 0.5835</td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>0.090 $\pm$ 0.003</td>
<td>1.250</td>
<td>11.025</td>
</tr>
<tr>
<td>1.6</td>
<td>0.110 $\pm$ 0.001</td>
<td>0.625</td>
<td>9.0909</td>
</tr>
<tr>
<td>2.0</td>
<td>0.121 $\pm$ 0.001</td>
<td>0.500</td>
<td>8.2644</td>
</tr>
<tr>
<td>3.0</td>
<td>0.134 $\pm$ 0.001</td>
<td>0.333</td>
<td>7.4404</td>
</tr>
<tr>
<td>4.0</td>
<td>0.172 $\pm$ 0.004</td>
<td>0.250</td>
<td>5.7836</td>
</tr>
<tr>
<td>5.0</td>
<td>0.226 $\pm$ 0.001</td>
<td>0.200</td>
<td>4.4072</td>
</tr>
<tr>
<td>6.0</td>
<td>0.259 $\pm$ 0.002</td>
<td>0.166</td>
<td>3.8565</td>
</tr>
</tbody>
</table>
**Table - 25**

**EFFECT OF CONCENTRATION OF REDUCTANT LACTOSE ON QUANTUM YIELD OF METHYLENE BLUE**

<table>
<thead>
<tr>
<th>RCHO = Lactose</th>
<th>Buffer = Acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>[MB] = 1 × 10^{-3} mol dm^{-3}</td>
<td>Temp. = 25°C</td>
</tr>
<tr>
<td>Solvent = 50% aqueous methanol</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(10^3 \frac{[\text{RCHO}]}{\text{(mol dm}^{-3})})</th>
<th>Quantum yield (\phi)</th>
<th>(10^3 \cdot \frac{1}{[\text{RCHO}]}) (mol(^{-1}) dm(^3))</th>
<th>(\frac{1}{\phi})</th>
</tr>
</thead>
<tbody>
<tr>
<td>([H_0] = 4.2)</td>
<td>Confidence Interval = 0.012295</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>0.145 ± 0.001</td>
<td>1.250</td>
<td>6.8965</td>
</tr>
<tr>
<td>1.6</td>
<td>0.172 ± 0.001</td>
<td>0.625</td>
<td>5.8139</td>
</tr>
<tr>
<td>2.0</td>
<td>0.213 ± 0.001</td>
<td>0.500</td>
<td>4.6926</td>
</tr>
<tr>
<td>3.0</td>
<td>0.245 ± 0.002</td>
<td>0.333</td>
<td>4.0070</td>
</tr>
<tr>
<td>4.0</td>
<td>0.294 ± 0.001</td>
<td>0.250</td>
<td>3.3921</td>
</tr>
<tr>
<td>5.0</td>
<td>0.401 ± 0.002</td>
<td>0.200</td>
<td>2.4919</td>
</tr>
<tr>
<td>6.0</td>
<td>0.516 ± 0.002</td>
<td>0.166</td>
<td>1.9379</td>
</tr>
<tr>
<td>([H_0] \approx 7.7)</td>
<td>Confidence Interval = 0.05006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>0.093 ± 0.002</td>
<td>1.250</td>
<td>10.660</td>
</tr>
<tr>
<td>1.6</td>
<td>0.114 ± 0.001</td>
<td>0.625</td>
<td>8.7260</td>
</tr>
<tr>
<td>2.0</td>
<td>0.137 ± 0.001</td>
<td>0.500</td>
<td>7.2674</td>
</tr>
<tr>
<td>3.0</td>
<td>0.152 ± 0.001</td>
<td>0.333</td>
<td>6.5445</td>
</tr>
<tr>
<td>4.0</td>
<td>0.172 ± 0.002</td>
<td>0.250</td>
<td>5.8139</td>
</tr>
<tr>
<td>5.0</td>
<td>0.215 ± 0.001</td>
<td>0.200</td>
<td>4.6511</td>
</tr>
<tr>
<td>6.0</td>
<td>0.245 ± 0.002</td>
<td>0.166</td>
<td>4.0700</td>
</tr>
</tbody>
</table>
Table - 36
QUANTUM YIELD OF PHOTOREDUCTION OF METHYLENE BLUE AT INFINTE CONCENTRATION OF REDUCTANT

\([MB] = 1 \times 10^{-6} \text{ mol dm}^{-3}\) \hspace{1cm} Buffer = Acetate
Solvent = 50 % aqueous methanol \hspace{1cm} Temp. = 25°C

<table>
<thead>
<tr>
<th>Reductant</th>
<th>Quantum yield</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(H_o = 4.2)</td>
<td>(H_o = 7.7)</td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>0.440 ± 0.001</td>
<td>0.230 ± 0.001</td>
<td></td>
</tr>
<tr>
<td>D-mannose</td>
<td>0.465 ± 0.001</td>
<td>0.261 ± 0.001</td>
<td></td>
</tr>
<tr>
<td>D-(+)-Ribose</td>
<td>0.481 ± 0.001</td>
<td>0.293 ± 0.001</td>
<td></td>
</tr>
<tr>
<td>Maltose</td>
<td>0.447 ± 0.001</td>
<td>0.239 ± 0.001</td>
<td></td>
</tr>
<tr>
<td>Lactose</td>
<td>0.468 ± 0.001</td>
<td>0.244 ± 0.001</td>
<td></td>
</tr>
</tbody>
</table>
Figure 4.24: A plot of $1/\phi$ vs. $1/[\text{RCHO}]$ for the photoreduction of methylene blue with

Galactose
Figure 4.25: A plot of $1/\phi$ vs $1/[\text{RCH}:\cdot]$ for the photoreduction of methylene blue with D-Mannose
Figure 4.26: A plot of $1/\phi$ vs $1/[\text{RCHO}]$ for the photoreduction of methylene blue with D-(+)-Ribose
Figure 4.27: A plot of $1/\phi$ vs $1/[RCHO]$ for the photoreduction of methylene blue with Maltose
Figure 4.28: A plot of $1/\phi$ vs $1/[\text{RCHO}]$ for the photoreduction of methylene blue with Lactose.
4.8 INFLUENCE OF CONCENTRATION OF METHYLENE BLUE ON THE QUANTUM YIELD OF PHOTOCHEMICAL REDUCTION

The effect of methylene blue concentration on the quantum yield was studied at different concentration of methylene blue ranging from $1 \times 10^{-6}$ mol dm$^{-3}$ to $4 \times 10^{-6}$ mol dm$^{-3}$ in 50% aqueous methanol as shown in Tables 27 to 31.

The acidity of the reacting solution was kept constant at 4.2 and temperature maintained at 25°C ± 0.1°C. The effect of change in concentration of methylene blue was studied with different reducing sugars like galactose, mannose, ribose, maltose and lactose and it is revealed that there is no significant change in quantum yield of methylene blue observed with different reductants of same concentration as shown in Tables 27 – 31.

These results indicated that quantum yield of photochemical reduction of methylene blue is independent upon the concentration of the dye. The results are also supported by earlier workers [78,141, 215]. The representative plot of $\phi$ versus [MB] is shown in the Figure 4.29.
Table - 27
EFFECT OF CONCENTRATION OF METHYLENE BLUE ON QUANTUM YIELD OF PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE WITH GALACTOSE

\[
\begin{align*}
\text{RCHO} & = \text{Galactose} & \text{Buffer} & = \text{Acetate} \\
[H_2] & = 4.2 & \text{Solvent} & = 50\% \text{ aqueous methanol} \\
\text{Temp.} & = 25 \pm 0.1 ^\circ\text{C}
\end{align*}
\]

<table>
<thead>
<tr>
<th>(10^6 \cdot [\text{MB}] \ (\text{mol dm}^{-3}))</th>
<th>(10^4 \cdot [\text{RCHO}] \ (\text{mol dm}^{-3}))</th>
<th>Quantum yield (\phi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1.00)</td>
<td>8.0</td>
<td>0.151 (\pm) 0.001</td>
</tr>
<tr>
<td>(1.5)</td>
<td>8.0</td>
<td>0.155 (\pm) 0.001</td>
</tr>
<tr>
<td>(2.0)</td>
<td>8.0</td>
<td>0.155 (\pm) 0.001</td>
</tr>
<tr>
<td>(2.5)</td>
<td>8.0</td>
<td>0.158 (\pm) 0.001</td>
</tr>
<tr>
<td>(3.0)</td>
<td>8.0</td>
<td>0.149 (\pm) 0.001</td>
</tr>
<tr>
<td>(3.5)</td>
<td>8.0</td>
<td>0.155 (\pm) 0.001</td>
</tr>
<tr>
<td>(4.0)</td>
<td>8.0</td>
<td>0.156 (\pm) 0.001</td>
</tr>
</tbody>
</table>

Confidence Interval = 0.00284
Table 28

EFFECT OF CONCENTRATION OF METHYLENE BLUE ON QUANTUM YIELD OF PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE WITH D-MANNOSE

\[ \text{RCHO} = \text{D-Mannose} \quad \text{BUFFER} = \text{Acetate} \]

\[ [H_+ ] = 4.2 \quad \text{Solvent} = 50\% \text{ aqueous methanol} \]

\[ \text{Temp.} = 25 \pm 0.1 \degree C \]

<table>
<thead>
<tr>
<th>(10^6 \cdot [\text{MB}] ) (mol dm(^{-3}))</th>
<th>(10^6 \cdot [\text{RCHO}] ) (mol dm(^{-3}))</th>
<th>Quantum yield ( \phi )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>8.0</td>
<td>0.179 ± 0.0002</td>
</tr>
<tr>
<td>1.5</td>
<td>8.0</td>
<td>0.175 ± 0.001</td>
</tr>
<tr>
<td>2.0</td>
<td>8.0</td>
<td>0.175 ± 0.002</td>
</tr>
<tr>
<td>2.5</td>
<td>8.0</td>
<td>0.175 ± 0.003</td>
</tr>
<tr>
<td>3.0</td>
<td>8.0</td>
<td>0.175 ± 0.001</td>
</tr>
<tr>
<td>3.5</td>
<td>8.0</td>
<td>0.179 ± 0.002</td>
</tr>
<tr>
<td>4.0</td>
<td>8.0</td>
<td>0.179 ± 0.002</td>
</tr>
</tbody>
</table>

Confidence Interval = 0.00197
Table - 29
EFFECT OF CONCENTRATION OF METHYLENE BLUE ON QUANTUM YIELD OF PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE WITH D-(+)-RIBOSE

\[
\begin{align*}
\text{RCHO} & = \text{D-(+)-Ribose} & \text{BUFFER} & = \text{Acetate} \\
[\text{H}_3] & = 4.2 & \text{Solvent} & = 50\% \text{ aqueous methanol}
\end{align*}
\]

<table>
<thead>
<tr>
<th>10^6 \cdot [MB] mol dm^{-3}</th>
<th>10^4 \cdot [RCHO] mol dm^{-3}</th>
<th>Quantum yield ( \phi )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>8.0</td>
<td>0.162 ± 0.001</td>
</tr>
<tr>
<td>1.5</td>
<td>8.0</td>
<td>0.163 ± 0.001</td>
</tr>
<tr>
<td>2.0</td>
<td>8.0</td>
<td>0.163 ± 0.002</td>
</tr>
<tr>
<td>2.5</td>
<td>8.0</td>
<td>0.162 ± 0.001</td>
</tr>
<tr>
<td>3.0</td>
<td>8.0</td>
<td>0.162 ± 0.001</td>
</tr>
<tr>
<td>3.5</td>
<td>8.0</td>
<td>0.162 ± 0.001</td>
</tr>
<tr>
<td>4.0</td>
<td>8.0</td>
<td>0.162 ± 0.001</td>
</tr>
</tbody>
</table>

Confidence Interval = 0.00045
Table - 39

EFFECT OF CONCENTRATION OF METHYLENE BLUE ON QUANTUM YIELD OF PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE WITH MALTOSE

<table>
<thead>
<tr>
<th>RCHO = Maltose</th>
<th>Buffer = Acetate</th>
<th>Solvent = 50% aqueous methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Hₐ] = 4.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>10⁶ [MB] mol dm⁻³</th>
<th>10⁴ [RCHO] mol dm⁻³</th>
<th>Quantum yield ( \phi )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Confidence Interval = 0.000903</td>
</tr>
<tr>
<td>1.00</td>
<td>8.0</td>
<td>0.151 ± 0.002</td>
</tr>
<tr>
<td>1.5</td>
<td>8.0</td>
<td>0.151 ± 0.002</td>
</tr>
<tr>
<td>2.0</td>
<td>8.0</td>
<td>0.151 ± 0.002</td>
</tr>
<tr>
<td>2.5</td>
<td>8.0</td>
<td>0.149 ± 0.001</td>
</tr>
<tr>
<td>3.0</td>
<td>8.0</td>
<td>0.151 ± 0.001</td>
</tr>
<tr>
<td>3.5</td>
<td>8.0</td>
<td>0.149 ± 0.001</td>
</tr>
<tr>
<td>4.0</td>
<td>8.0</td>
<td>0.151 ± 0.001</td>
</tr>
</tbody>
</table>
Table 31

EFFECT OF CONCENTRATION OF METHYLENE BLUE ON QUANTUM YIELD OF PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE WITH LACTOSE

<table>
<thead>
<tr>
<th>RCHO = Lactose</th>
<th>Buffer = Acetate</th>
<th>Solvent = 50% aqueous methanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>[H₃] = 4.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(10^6 \cdot [MB] \text{ mol dm}^{-3})</th>
<th>(10^4 \cdot [\text{RCHO}] \text{ mol dm}^{-3})</th>
<th>Quantum yield (\phi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>8.0</td>
<td>0.145 ± 0.001</td>
</tr>
<tr>
<td>1.5</td>
<td>8.0</td>
<td>0.139 ± 0.001</td>
</tr>
<tr>
<td>2.0</td>
<td>8.0</td>
<td>0.139 ± 0.002</td>
</tr>
<tr>
<td>2.5</td>
<td>8.0</td>
<td>0.139 ± 0.003</td>
</tr>
<tr>
<td>3.0</td>
<td>8.0</td>
<td>0.139 ± 0.002</td>
</tr>
<tr>
<td>3.5</td>
<td>8.0</td>
<td>0.139 ± 0.001</td>
</tr>
<tr>
<td>4.0</td>
<td>8.0</td>
<td>0.139 ± 0.001</td>
</tr>
</tbody>
</table>

Confidence Interval = 0.00209
Figure 4.29: A plot of $\phi$ Vs [MB] for photochemical reduction of methylene with Galactose as reducing sugar (Galactose) at $H_0 = 4.2$
4.9 INFLUENCE OF TEMPERATURE ON QUANTUM YIELD OF PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE WITH REDUCING SUGARS

The variation of temperature on quantum yield of photochemical reduction of methylene blue with reducing sugars such as Galactose, D-Mannose, D-(+)-Ribose, Maltose and Lactose was studied at two different acidities 4.2 and 7.7 and concentration of reductant was $8.0 \times 10^{-4}$ mol dm$^{-3}$ whereas the dye concentration was $1 \times 10^{-6}$ mol dm$^{-3}$ in deoxygenated system with acetate buffer. The temperatures were varied from 20°C to 45°C with interval of 5°C. The results are tabulated in Tables 32 – 36 respectively.

The temperature coefficients for every five degree rise in temperature were calculated at two acidities 4.2 and 7.7. It was observed that average temperature coefficient at two acidities 4.2 and 7.7 were 1.1508 and 1.1841. It was also found that quantum yield of photochemical reduction of methylene blue increases with the increase in temperature as given in Tables 32 – 36 respectively.

The values of quantum yield tabulated in tables 32 to 36 at temperatures from 20°C to 45°C were used to evaluate the activation parameters like $E_a$ (energy of activation), $\Delta H^\dagger$ (change in enthalpy of activation), $\Delta S^\dagger$ (change in entropy of activation) and $\Delta G^\dagger$ (change in free energy of activation) as a function of concentration of reductants and acidity of the reaction mixture. The plots of logarithm of quantum yield ($\log \phi$) versus reciprocal of absolute temperature ($1/T$) were drawn in the Figures 4.30 – 4.34 for reducing sugars such as Galactose, D-Mannose, D-(+)-Ribose, Maltose and Lactose. From the Arrhenius relation, the values of energy of activation $E_a$ were calculated. The plot of quantum yield ($\phi$) versus temperature is a straight line showing that quantum yield increases with the increase in temperature as shown in Figure 4.35.
Table - 32

INFLUENCE OF TEMPERATURE ON QUANTUM YIELD OF PHOTOCHEMICAL REACTION OF METHYLENE BLUE WITH GALACTOSE

\[ [\text{MB}] = 1 \times 10^{-6} \text{ mol dm}^{-3} \]
\[ [\text{RCHO}] = 8 \times 10^{-4} \text{ mol dm}^{-3} \]
RCHO = Galactose
Buffer = Acetate
Solvent = 50% aqueous methanol

<table>
<thead>
<tr>
<th>Acidity [H+]</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
<th>45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confidence Interval = 0.05184</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.2 ( \phi )</td>
<td>0.130</td>
<td>0.151</td>
<td>0.195</td>
<td>0.217</td>
<td>0.232</td>
<td>0.260</td>
</tr>
<tr>
<td>( \pm 0.001 \pm 0.001 \pm 0.002 \pm 0.002 \pm 0.001 \pm 0.003 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confidence Interval = 0.028800</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.7 ( \phi )</td>
<td>0.065</td>
<td>0.099</td>
<td>0.108</td>
<td>0.118</td>
<td>0.130</td>
<td>0.144</td>
</tr>
<tr>
<td>( \pm 0.002 \pm 0.008 \pm 0.001 \pm 0.004 \pm 0.002 \pm 0.002 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TEMPERATURE COEFFICIENTS

<table>
<thead>
<tr>
<th>( \phi_{25}/\phi_{20} )</th>
<th>( \phi_{30}/\phi_{25} )</th>
<th>( \phi_{35}/\phi_{30} )</th>
<th>( \phi_{40}/\phi_{35} )</th>
<th>( \phi_{45}/\phi_{40} )</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2</td>
<td>1.161</td>
<td>1.291</td>
<td>1.111</td>
<td>1.071</td>
<td>1.120</td>
</tr>
<tr>
<td>7.7</td>
<td>1.533</td>
<td>1.087</td>
<td>1.090</td>
<td>1.099</td>
<td>1.111</td>
</tr>
</tbody>
</table>
Table 33

INFLUENCE OF TEMPERATURE ON QUANTUM YIELD OF PHOTOCHEMICAL REACTION OF METHYLENE BLUE WITH D-MANNOSE

\[ [\text{MB}] = 1 \times 10^{-4} \text{ mol dm}^{-3} \quad [\text{RCHO}] = 8 \times 10^{-4} \text{ mol dm}^{-3} \]

RCHO = D-Mannose

Buffer = Acetate

<table>
<thead>
<tr>
<th>Acidity [Hₐ]</th>
<th>TEMPERATURE °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td>4.2 ϕ</td>
<td>0.144 ± 0.001</td>
</tr>
<tr>
<td>7.7 ϕ</td>
<td>0.086 ± 0.001</td>
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Confidence Interval = 0.05516

Confidence Interval = 0.04287

TEMPERATURE COEFFICIENTS

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<tr>
<th></th>
<th>(\phi_{25}/\phi_{20})</th>
<th>(\phi_{30}/\phi_{25})</th>
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<td>4.2</td>
<td>1.241</td>
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<td>1.060</td>
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<td>1.1504</td>
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<td>7.7</td>
<td>1.226</td>
<td>1.323</td>
<td>1.0766</td>
<td>1.155</td>
<td>1.125</td>
<td>1.1811</td>
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</table>
Table 34

INFLUENCE OF TEMPERATURE ON QUANTUM YIELD OF
PHOTOCHEMICAL REACTION OF METHYLENE BLUE WITH
D-(+)-RIBOSE

\[[\text{MB}] = 1 \times 10^{-6} \text{ mol dm}^{-3}\]
\[[\text{RCHO}] = 8 \times 10^{-4} \text{ mol dm}^{-3}\]
RCHO = D-(+) Ribose
Buffer = Acetate
Solvent = 50% aqueous methanol

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<th>Acidity [H+]</th>
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<td>Confidence Interval = 0.06151</td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>4.2 φ</td>
<td>0.140 ± 0.001</td>
<td>0.162 ± 0.001</td>
<td>0.192 ± 0.002</td>
<td>0.241 ± 0.002</td>
<td>0.269 ± 0.001</td>
<td>0.283 ± 0.002</td>
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<td>Confidence Interval = 0.28693</td>
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<tr>
<td>7.7 φ</td>
<td>0.812 ± 0.001</td>
<td>0.103 ± 0.003</td>
<td>0.128 ± 0.001</td>
<td>0.149 ± 0.002</td>
<td>0.165 ± 0.003</td>
<td>0.184 ± 0.001</td>
</tr>
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</table>

<table>
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<tr>
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<tbody>
<tr>
<td></td>
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<td>(\phi_{35}/\phi_{30})</td>
<td>(\phi_{40}/\phi_{35})</td>
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<td>1.1630</td>
<td>1.103</td>
<td>1.116</td>
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</table>
Table - 35
INFLUENCE OF TEMPERATURE ON QUANTUM YIELD OF PHOTOCHEMICAL REACTION OF METHYLENE BLUE WITH MALTOSE

\[ [MB] = 1 \times 10^{-6} \text{ mol dm}^{-3} \quad [\text{RCHO}] = 8 \times 10^{-4} \text{ mol dm}^{-3} \]

RCHO = Maltose
Buffer = Acetate

Solvent = 50% aqueous methanol

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<thead>
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<th>Acidity [H+]</th>
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</thead>
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</table>

Confidence Interval = 0.05806

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<tbody>
<tr>
<td>[\phi] \pm 0.001</td>
<td>\pm 0.002</td>
<td>\pm 0.003</td>
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Confidence Interval = 0.004124

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<tr>
<td>[\phi] \pm 0.002</td>
<td>\pm 0.003</td>
<td>\pm 0.001</td>
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TEMPERATURE COEFFICIENTS

<table>
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<th>[\phi_{35}/\phi_{30}]</th>
<th>[\phi_{40}/\phi_{35}]</th>
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<td>1.327</td>
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<tr>
<td>Acidity [H+]</td>
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<td>25</td>
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<td>35</td>
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<td>-----------------</td>
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<tr>
<td>Confidence Interval = 0.06777</td>
<td>4.2</td>
<td>0.130 ± 0.001</td>
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<td>0.162 ± 0.003</td>
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<td>Confidence Interval = 0.04658</td>
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<td>0.078 ± 0.001</td>
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<td>0.125 ± 0.002</td>
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<td>TEMPERATURE COEFFICIENTS</td>
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<td>φ_{30}/φ_{25}</td>
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<td>1.200</td>
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<td>1.339</td>
<td>1.321</td>
<td>1.142</td>
<td>1.166</td>
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</table>
Figure 4.30: A plot of $\log \phi$ versus $1/T$ for Galactose at $H_o = 4.2$ and 7.7
Figure 4.31: A plot of log $\phi$ versus $1/T$ for D-Mannose at $H_0 = 4.2$ and 7.7
Figure 4.32: A plot of $\log \phi$ versus $1/T$ for D-(+)-Ribose at $H_o = 4.2$ and 7.7
Figure 4.33: A plot of log $\phi$ versus $1/T$ for Maltose at $H_o = 4.2$ and 7.7
Figure 4.34: A plot of $\log \phi$ versus $1/T$ for Lactose at $H_0 = 4.2$ and 7.7
Figure 4.35: Plot of $\phi$ vs temperature for photoreduction of methylene blue with galactose at two different acidities.
4.10 ANALYSIS OF IRRADIATED REACTION MIXTURE OF METHYLENE BLUE WITH REDUCING SUGARS

An attempt has been made to analyze the photochemical product after passing monochromatic radiation of 661 nm by column chromatographic technique by taking TLC (Thin layer chromatography) of the irradiated reaction mixture when galactose as a reductant in the reaction mixture. It was observed that two spots were appeared on TLC plates which were shown optical activity under U.V. lamp. When ceric sulphate was sprayed on the TLC plate, it was found that unreacted dye (blue spot) was in the lower region and product spot was present in the upper region of the plate, as the concentration of irradiated mixture was very low as $1 \times 10^{-6} \text{ mol dm}^{-3}$ and $8 \times 10^{-4} \text{ mol dm}^{-3}$. The isolation of photochemical product in some quantity was very difficult and which cannot be subjected for further mass, I.R techniques and it was very difficult to take help for mechanism of the reaction by analyzing the mixture. Therefore the concentration of the dye was increased to $1 \times 10^{-4} \text{ mol dm}^{-3}$ and reaction mixture was prepared by the same method as it was used for the photochemical reaction. When TLC of this reaction mixture was taken it was the same as for $1 \times 10^{-6} \text{ mol dm}^{-3}$ of the dye.

The R.F (relative flow) value of $1 \times 10^{-6} \text{ mol dm}^{-3}$ and $1 \times 10^{-4} \text{ mol dm}^{-3}$ of the two product spots were same. The TLC of the reaction mixture was also taken in butanol acetic acid water (BWA) solvent system where it was observed that product spot was spreaded into two or more spots, and then reaction mixture was loaded on the rotary evaporator to evaporate the 50% aqueous methanol. After evaporation, evaporated mixture was loaded on the pencil column to purify the compound. The column was filled with mesh silica gel of size (230 – 400). The column was then run with hexane and ethylacetate and fractions were
collected in the vials 1 – 35 when it was observed that no more fractions were collected with ethylacetate then column was run with 1 % methanol. Similarly the polarity of the column was increased by 2 – 4% of methanol and till colorless compound leuco methylene blue was separated in vials 22 – 32, then T.I.C of the all vials were taken. It was observed during TLC that leuco dye was separated in vials 22 – 32. After evaporation of the solvent, the solid leuco dye was subjected to Mass, I.R and other techniques for kinetics and establishing mechanism. Spectrum was used for justification of the proposed mechanism and many side reactions were reported.
CHAPTER 5

DISCUSSION
5. DISCUSSION

5.1 THE PHOTOBLEACHING OF METHYLENE BLUE

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. The present work was performed to study the photoreduction of methylene blue by monochromatic radiations at wavelength of 661 nm with reducing sugars like monosaccharides and disaccharides. This is strikingly visible example of the conversion of light to chemical energy. The reaction is reversible and the rates of both forward and reverse reaction are easily measurable.

The reaction was observed under different parameters like effect of concentration of dye, concentration of reductants i.e. reducing sugars such as Galactose, D-Mannose, D-(+)-Ribose, Mallose and Lactose acidity (H⁺) of the reaction mixture and temperature. It is revealed that quantum yield (φ) depends upon acidity of reaction mixture, concentration of reductant and temperature of reaction. It was also found that reduction process is independent upon the concentration of dye. After passing radiation from reaction mixture in presence of reducing agent, mixture was subjected for further analysis by TLC and column chromatographic techniques.

Absorption of methylene blue solution at wavelength of 661 nm results in photo bleaching of the dye molecule, which means 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 [190] 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.

\[
\text{HOCH}_2 \text{(CHOH)}_4 \text{CHO} + 3\text{OH}^- \rightarrow \text{HOCH}_2 \text{(CHOH)}_4 \text{CO}_2 + 2\text{H}_2\text{O} + 2e^-
\]

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 ring structure in triplet excited state of the molecule. A possible explanation of redox reaction on the basis of analysis of reaction mixture involve is given below. Methylene blue is an organic dye that is reduced to colorless compound leuco methylene blue by a reducing sugar when light is passed, upon exposure to the air; the colorless solution is reoxidized by oxygen above the liquid into the blue dye, i.e. methylene blue.

### 5.2 VISIBLE ABSORPTION SPECTRA OF METHYLENE BLUE WITH DIFFERENT REDUCTANTS AND AT DIFFERENT ACIDITIES

The visible spectra of reaction mixture of methylene blue at concentration \( 1 \times 10^{-6} \text{ mol dm}^{-3} \) and reductants (monosaccharides and disaccharides) at concentration \( 8 \times 10^{-4} \text{ mol dm}^{-3} \) in 50 % 1:1 aqueous methanol at two different acidities i.e 4.2 and 7.7 were taken by shimadzo spectrophotometer. The spectra of methylene blue were observed in absence of reductants and values of \( e \) are given in Table 37. \( \lambda_{\text{max}} \) at \( H_0 = 4.2 \) in absence of reductant was 659 and 660 at \( H_0 = 7.7 \). Spectra showed the high values of absorbance at \( H_0 = 4.2 \) and \( H_0 = 7.7 \) in absence of reductant. The spectra were taken before irradiation and after
irradiation. Those spectra are given in the Figures 5.1-5.3. It was observed that before irradiation, methylene blue at $H_0 = 4.2$ shows a sharp peak at $\lambda_{\text{max}}$ 661 nm and 659 nm for monosaccharides which proves the brightness of color of methylene blue. However at $H_0 = 7.7$ (higher pH range) a broad spectrum was observed with $\lambda_{\text{max}}$ 662 nm and 664 nm for monosaccharides and 664 nm and 661 nm for disaccharides respectively. Such a change in absorbance measurement has been supported by the literature [140, 219 - 225] regarding the dimeric form of methylene blue.

After irradiation and passing nitrogen from the reaction mixture it was found that change in buffer composition to maintain acidities at 4.2 and 7.7 are highly dominating that no significant shift in $\lambda_{\text{max}}$ were observed after passing monochromatic radiation from the reaction mixture of methylene blue. But it shows the decrease in absorbance in presence of monosaccharides and disaccharides mainly at lower acidity region which confirms the photoreduction of methylene blue with reducing sugars. More or less same spectra were observed for all monosaccharides and disaccharides which confirmed that reduction reaction was taken place in between the aldehydic group of reducing sugars and the dye.

$$
RCHO + MB^+ \xrightarrow{\text{OH}^-} \xrightarrow{H^+/e^-} MBH + RCOOH \quad (\text{aldonic acid})
$$

The values of absorbance before irradiation in presence of reductants was at $H_0 = 4.2$ were 0.061, 0.089, 0.144 and 0.120 for Galactose, D-Mannose, Maltose and Lactose respectively and after irradiation the values decrease to 0.021 and 0.017 for monosaccharides and disaccharides respectively showing the bleaching of the dye in presence of reductant as shown in the Figures 5.2 - 5.3 The values of absorbance with respect to $H_0$ were tabulated in Tables 37-39 and spectra are shown in Figures 5.1 - 5.3. Results represent that value of absorbance are high in $H_0$ range 7.7
Table 37
MOLAR ABSORPTIVITY COEFFICIENT OF $1 \times 10^{-6}$ mol dm$^{-3}$ METHYLENE BLUE IN 50% AQUEOUS METHANOL AT TWO DIFFERENT $H_o$.

<table>
<thead>
<tr>
<th>$H_o$</th>
<th>$\lambda_{\text{max}}$ [nm]</th>
<th>$\varepsilon \times 10^4$ mol$^{-1}$ dm$^3$ cm$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2</td>
<td>659</td>
<td>0.47</td>
</tr>
<tr>
<td>7.7</td>
<td>660</td>
<td>1.06</td>
</tr>
</tbody>
</table>

Figure 5.1: Absorption spectrum of methylene blue in absence of reductant at two different acidities $H_o = 4.2$ and 7.7
Table - 38

VISIBLE SPECTRUM ANALYSIS OF THE STUDY OF PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE WITH MONOSACCHARIDES AND DIASACCHARIDES BEFORE IRRADIATION

\[
\begin{align*}
[\text{MB}] &= 1 \times 10^{-4} \text{ mol dm}^{-3} \\
[\text{RCHO}] &= 8 \times 10^{-4} \text{ mol dm}^{-3} \\
\text{Solvent} &= 50\% \text{ aqueous methanol} \\
\text{Buffer} &= \text{Acetate}
\end{align*}
\]

<table>
<thead>
<tr>
<th>Reaction Mixture before irradiation</th>
<th>Acidities ([\text{H}_a])</th>
<th>(\lambda_{\text{max}} ) (nm)</th>
<th>Absorbance</th>
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</thead>
<tbody>
<tr>
<td>Galactose</td>
<td>4.2</td>
<td>661</td>
<td>0.061</td>
</tr>
<tr>
<td></td>
<td>7.7</td>
<td>662</td>
<td>0.149</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>4.2</td>
<td>559</td>
<td>0.089</td>
</tr>
<tr>
<td></td>
<td>7.7</td>
<td>664</td>
<td>0.153</td>
</tr>
<tr>
<td>Maltose</td>
<td>4.2</td>
<td>661</td>
<td>0.144</td>
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<td></td>
<td>7.7</td>
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<td>Lactose</td>
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<tr>
<td></td>
<td>7.7</td>
<td>664</td>
<td>0.143</td>
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</tbody>
</table>
Visible Spectrum Analysis of the Study of Photochemical Reduction of Methylene Blue with Monosaccharides and Diasaccharides After Irradiation

\[ [MB] = 1 \times 10^{-5} \text{ mol dm}^{-3} \quad [RCHO] = 8 \times 10^{-4} \text{ mol dm}^{-3} \]

Solvent = 50% aqueous methanol
Buffer = Acetate

<table>
<thead>
<tr>
<th>Reaction Mixture after irradiation</th>
<th>Acidities [H₂]</th>
<th>( \lambda_{\max} ) (nm)</th>
<th>Absorbance</th>
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<tr>
<td>monosaccharides</td>
<td>4.2</td>
<td>663</td>
<td>0.021</td>
</tr>
<tr>
<td></td>
<td>7.7</td>
<td>664</td>
<td>0.024</td>
</tr>
<tr>
<td>Diasaccharides</td>
<td>4.2</td>
<td>665</td>
<td>0.017</td>
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<tr>
<td></td>
<td>7.7</td>
<td>664</td>
<td>0.023</td>
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</table>
Figure 5.2: Spectral change of photobleaching of methylene blue with monosaccharides before irradiation and after irradiation at acidity 4.2 and 7.7
Figure 5.3: Spectral change of photobleaching of methylene blue with disaccharides before irradiation and after irradiation at acidity 4.2 and 7.7
5.3 THE INFRARED SPECTRA OF ISOLATED PHOTOCHEMICAL PRODUCTS

The infra red spectrum of the isolated photochemical product shows absorption bands of different groups present in the product as shown in Figures 5.4 – 5.8. Bands were observed at 1667.6 cm\(^{-1}\) showing the presence of aromatic ring which may be phenazine ring. N – H intensity band at 3699.5 cm\(^{-1}\) was observed which may be the reaction of H\(^+\) ion with N atom in heterocyclic ring and shows the formation of leuco dye. The high intensity band at 2924 cm\(^{-1}\) appeared which shows the C–H stretching in the molecules. The bands at 1728 cm\(^{-1}\) in I.R. spectra shows the presence of C – O group which may be due to the association of sugar molecule with dye molecule and it may be possible that during isolation, sugar molecule may be broken into smaller fragments which some time observed with sugar molecule. N – H stretching vibration in IR was observed at 3662 cm\(^{-1}\) while bending vibration for N – H bond exhibited around 1450 cm\(^{-1}\) in IR confirmed the presence of N – H bond for secondary amine. The IR spectrum of the compound also showed the broad band at 3463 cm\(^{-1}\) which indicated the presence of O – H bond.

The I.R spectrum showed low intensity bands due to very little quantity of the sample submitted for I.R and mass techniques. The I.R of the compound bands at 2378.5 cm\(^{-1}\) showed the presences of OH\(^-\) region which may be due to dissociation of water molecule, or OH\(^-\) ion from the base or OH\(^-\) ion from sugar molecule. This OH\(^-\) ion may attack to N – H which may result in demethylation. Demethylation [22] was observed simultaneously with leuco formation as observed in the photoreduction of dye:

\[
\begin{align*}
\text{OH} & \quad \text{(half oxidised)} \\
\text{H}^+ & \quad \text{OH} \\
\text{CH}_3 & \\
\text{N} & \\
\text{CH}_3 & \\
\text{N} & + \text{H}_2\text{O}_2 + \text{CH}_3\text{OH}
\end{align*}
\]

\[
\begin{align*}
\text{H}^+ & \quad \text{OH} \\
\text{CH}_3 & \\
\text{N} & \\
\text{CH}_3 & \\
\text{N} & + \text{H}_2\text{O}_2 + \text{CH}_3\text{OH}
\end{align*}
\]
Figure 5.5: Infrared spectrum of isolated product

[Diagram showing an infrared spectrum with labeled wavenumbers and transmittance percentages.]
Figure 5.6  Infrared spectrum of isolated product 2
Figure 5.7 Infrared spectrum of isolated product 3.
Figure 5.8 - Infrared spectrum of isolated product 4.
Figure 5.9  Infrared spectrum of isolated product 5
The dye showed very high reactivity towards $H^+$, $OH^-$ ion and $CH_3COO^-$ ion as the intensity bands of these groups are present in the I.R spectra of the products. Such scheme seems to be reasonable since demethylation will be offered some quantity of free energy, which will in turn make the decomposition of water energetically feasible.

Further $OH^-$ is from base, and this is consistent with the well known fact that demethylation easily occurs in the oxidative condition. If the transient electron transfer between the triplet dye and ground state dye occurs generally in the thiazine dye,

$$MB^* + MB \rightarrow MB^- + MB^+$$

then one may suppose that some reducing agent will selectively attack the half oxidized form. Therefore overall rate formula is expected to be approximately of the second order with respect to dye. The decomposition of water by visible light takes place by virtue of the following scheme.

$$H_2O \rightarrow H^+ + OH^-$$

$$OH^- \rightarrow e^- + OH$$

$$MB^* + 2OH^- \rightarrow MBH^- + HO_2^-$$

On the other hand, the reaction

$$2 MB^* + 2OH^- \rightarrow 2MBH^- + O_2$$

This oxygen may combine with the reductant molecule and can form $\overset{\cdot}{C} - \overset{\cdot}{O}$ which is appearing at 1219.5 cm$^{-1}$ in the I.R. Spectrum.

Since demethylation is important in case of methylene blue as $OH^-$ ion appears in the infra red spectra of isolated products of methylene blue which may be due to the detachment of one methyl group in the case of methylene blue.

The mass spectroscopy of pure compound showed molecular ion peak at m/2 365.4 and the other peaks were observed at m/2 330.3, m/2 315.3, m/2 250.3, m/2 203.3 and m/2 972 as shown in Figures 5.9 - 5.13.

The photoreduction which has been confirmed in the series of investigation would be expected to occur in other members of thiazine dyes.
Figure 5.10: Mass spectrum of isolated compound 2.
Figure 5.13: Mass spectrum of isolated compound S.
5.4 VARIATION OF ACIDITIES ON QUANTUM YIELD OF PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE IN ABSENCE OF REDUCTANT

Thiazine dyes when subjected to photon of visible light, showed reduction in absence of reductant. The photochemical reduction of methylene blue was studied in absence of reductant at different acidities. Influence of acidity on quantum yield (Φ) in presence of acetate buffer solution at concentration of \(1 \times 10^{-6}\) mol dm\(^{-3}\) in 50% aqueous methanol shows that dye reduced to leuco dye by absorption of light of 661 nm. On exposing the air, the dye oxidized to its blue color but quantum yield was very low in absence of reductant indicating that no significant reaction take place with solvent showing that reductant is necessary for redox reaction [140]. The values of quantum yields are summarized in Table 15. It was obvious from the table that quantum yield is double in lower acidity region i.e. at \(H_0 = 4.2\) as \(8.612 \times 10^{-3}\) as compared to higher acidity region \(H_0 = 7.7\) as \(4.912 \times 10^{-3}\) showing that reduction is more prominent in acidity \(H_0 = 4.2\).

5.5 INFLUENCE OF CONCENTRATION OF REDUCING SUGARS ON QUANTUM YIELD OF PHOTOREDUCTION OF METHYLENE BLUE

Photoreduction of methylene blue in 50% aqueous methanol with monochromatic light of 661 nm was investigated for about five minutes. The deflection on galvanometer of irradiation of reaction mixture was measured under inert condition. The quantum yield using equation 4.43 was determined on the basis of acidity, concentration of dye, concentration of reducing agents and their structure. The variation in Φ versus \(H_0\) was observed similar to the reported work [226, 135]. The quantum yield of photoreduction of dye is maximum i.e. 0.301 for Maltose and 0.499 for Galactose showing that quantum yield is a function of concentration of reductants [122, 135, 226]. The OH\(^-\) ion from the base oxidizes the aldehydic group into aldonic acid and H\(^+\) form aldehydic group of reducing sugars attacks on N atom in ring of methylene blue, and changes into leucomethylene blue [color less].
The presence of 4 hydroxyl group [227] greatly influenced the reaction rate and experimental evidence suggests the involvement of charge transfer mediated mechanism and participation of an initial excited encounter complex.

\[
\text{(CH}_3\text{N)}_2\text{NCH}_2\text{S} + \text{R-C-H} \xrightarrow{\text{OH A'2e}} \text{OH A'2e} \rightarrow \text{(CH}_3\text{N)}_2\text{NCH}_2\text{S} + \text{R-C-OH} \text{ (aldonic acid)}
\]

A comparative study among the reductants such as Galactose, D-Mannose, D-(+)-Ribose, Maltose and Lactose shows that quantum yield in photoreduction of methylene blue depends upon the oxidation of sugars in a similar way. The difference in quantum yield is only due to the structural difference of reducing sugars and hydrolysis of disaccharides. The configurations of aldososes examined have some bearing on oxidation of reducing sugars at which they were oxidized by methylene blue.

![ structural formulae of reducing sugars ]

Variation in the quantum yield in photoreduction process also depends on the energy of activation, since energy of activation is also related with the distance and relative orientation between dye and reductant in photochemical reaction [228-231]. Influence of concentration of dye on quantum yield shows that reduction is independent upon concentration of methylene blue for \(1 \times 10^{-6}\) and \(4.0 \times 10^{-6}\) mol/dm\(^3\) of dye. This conclusion was supported by earlier work [232] as reported in Tables 27 – 31.
Photoreduction occurs by electron transfer from the substrate to triplet excited state of dye [233] followed by proton or hydrogen atom transfer to yield the leuco dye and corresponding oxidation product.

5.6 PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE BY MONOSACCHARIDES

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% aqueous methanol in presence of acetate buffer. The values of quantum yield at different concentration of reductants are summarized in Tables 21 to 23. It is obvious from the tables that quantum yield of the photoreduction of methylene blue increases with the increase in concentration of reductants. The plots of reciprocal of concentration of reductant i.e. 1/[RCHO] against 1/φ as shown in the Figures 4.24 - 4.26 are straight lines having intercept and positive slopes. The limiting values of quantum yield at infinite concentration of reductants were found from the intercept of the plots. The quantum yield corresponding to maximum concentration of reductants were calculated as 0.496±0.001, 0.499±0.001 and 0.509±0.002 at 4.2 and 0.277±0.003, 0.275±0.002 and 0.269±0.003 at H_o = 7.7 for Galactose, D-Mannose and D(-)-Ribose. From the values of (φ) for monosaccharides it was concluded that reducing sugars are best reductants for the photoreduction of methylene blue. The reaction between dye and monosaccharides takes place very rapidly and completed in more or less 2 minutes. The reaction observed for 5 minutes reveals that hydrogen transfer reaction is taking place between monosaccharides and dye. From the plot φ versus H_o shows that reaction is greatly influenced by hydrogen ion activity as shown in Figures 4.9 - 4.11.

From the plots of φ versus H_o it was observed that quantum yield (φ) decreases as the H_o acidity of reaction medium increases. The plots of (φ₁ - φ) / (φ₁ - φ₂) against 1/H_o are straight lines passing through the origin as shown in the Figures 4.19 - 4.21. φ₁ and φ₂ are the quantum yields at low and high acidity region respectively.

The plots between 1/φ against 1/H_o are straight lines with positive slopes represented in Figures 4.14 - 4.16. These plots showed that quantum yield is controlled by equilibrium
between leuco methylene blue (color less) and oxidized from of methylene blue (blue). The values of maximum and minimum quantum yield should be regarded as a result of the equilibrium shifting towards high and low acidity regions.

Quantum yield is not a function of dye concentration as shown by the plots drawn in Figure 4.29. The plots of $\phi$ against $[MJs]$ is a straight line showing that quantum yield is independent upon the varying concentration of dye for reducing sugars. The values of quantum yield for the photoreduction of methylene blue with monosaccharides are $0.141 \pm 0.001$ for Galactose and $0.179 \pm 0.002$ for D-Mannose respectively.

5.7 PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE BY DISACCHARIDES

Disaccharides reduced methylene blue in the similar manner as observed by monosaccharides. Slight difference in values may be due to hydrolysis of disaccharides into monosaccharides as shown in Tables 24 – 25. Result shows that as acidity increases from $H_\circ$ 4.2 and 7.7, quantum yield of photoreduction of methylene blue at two different concentrations of disaccharides i.e. $8 \times 10^{-4}$ mol dm$^{-3}$ and $6 \times 10^{-3}$ mol dm$^{-3}$ decreases respectively. The representative plots between $\phi$ and $H_\circ$ are shown in the Figures 4.12 – 4.13 respectively. The plots are curved and show depression from low value of $H_\circ$ to high value of $H_\circ$ and this transient region show the process of product forming step and an equilibrium between triplet excited states.

$$\text{MB}_T^+ + H^+ \rightleftharpoons \text{MBH}_T^{++}$$

The representative plots between $1/\phi$ and $1/[\text{RCH}_2]$ as shown in Figures 4.27 – 4.28 represent the variation in quantum yield with the concentration of disaccharides i.e. Maltose and Lactose. It shows the similar behavior which was observed by earlier workers [130, 135]. The quantum yields at infinitesimal concentration of disaccharides determined at $H_\circ$ 4.2 and 7.7, are summarized in Table 26. The quantum yield of photoreduction of methylene blue was observed unchanged with the change in the concentration of dye as shown in Figure 4.29.

The plots of $1/\phi$ against $1/H_\circ$ and $(\phi_1 - \phi) / (\phi_2 - \phi_0)$ against $1/H_\circ$ shown in Figures 4.17 – 4.18 and 4.22 – 4.23 respectively, are straight line having positive slopes as a
function of reductant concentration, determined by applying linear regression method. The values of quantum yield at high concentration of reductants Maltose and Lactose are $0.508 \pm 0.001$ and $0.5160 \pm 0.002$ at $H_0=4.2$ and $0.259 \pm 0.002$ and $0.245 \pm 0.002$ at $H_0=7.7$ respectively. Varying concentration of dye has no effect on quantum yield of methylene blue indicating that quantum yield is independent upon the concentration of dye as shown in Tables 30-31.

5.8 INFLUENCE OF ACIDITY ON THE PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE BY REDUCING SUGARS

The variation of quantum yield of photoreduction of cationic dye, methylene blue by reducing sugars studied at ten different acidities $H_0$ i.e. 4.2, 4.7, 5.3, 6.6, 6.9, 7.0, 7.3, 7.4, 7.6 and 7.7 are given in Tables 16 to 20. The data interpretations of quantum yield at different acidities have been revealed that a quantum yield of photoreduction of methylene blue is a function of acidity as shown in Tables 16 to 20. The plot of $\phi$ versus $H_0$ is a typical "S" shaped curve as shown in Figures 4.9 - 4.13 for the photoreduction of methylene by reducing sugars showed that value of quantum yield 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 $\pi - \pi^*$ transition [167, 212] as compared to higher acidic medium. The two transition state of cationic dye i.e. singlet and triplet are involved in reduction process. But question is this that whether singlet state is involved in photoreduction or triplet state is taking part in the reduction. But according to mechanism singlet state could not be involved in the effect of acidity [H$_0$] 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.
\[
MB_S^+ \rightarrow MB_T^+ \quad \text{[Internal conversion to triplet state]} \quad 1
\]
\[
MB_S^+ \rightarrow MB^+ + hv \quad \text{[fluorescence]} \quad 2
\]
\[
MB_T^+ + H^+ \rightarrow MBH_T^{++} \quad 3
\]
\[
MB_T^+ + RH \rightarrow MBH_T^{+} + R \quad 4
\]

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

\[
MBH_T^{++} + RH \leftrightarrow MBH_T^{+} + RH
\]

The effect of acidity on triplet state may be due to the acid base equilibria or kinetic effect. Equation no (4) shows that increase in acidity will cause a decrease in quantum yield which is in agreement with the result \([215, 232]\) as observed in the present work.

If effect in acidity \([H_3]\) be represented by the reaction

\[
MBH_T^{+} + H^-OH \rightarrow MBH_{2T}^{+} + OH^- \quad 5
\]
in competition with

\[
MB_T^+ + RH \rightarrow MB_T^+ + RH
\]

the shift of the point of inflexion with concentration is not satisfactorily accounted for.

Therefore the kinetic approaches become to be unsatisfactory and only the equilibrium treatment will be considered. The triplet state establishes the following equilibrium which proceeds to product.

\[
MB_T^+ + H^+ \leftrightarrow MBH_T^{++} \quad 6
\]

Another equilibrium can also be established by association of \( MBH_T^{++} \) with reductant as follows:

\[
MBH_T^{++} + RH \leftrightarrow MBH_T^{++} + RH \quad 7
\]

The equilibrium state (6) shows the correct variation of the point of inflexion with solvent and assumed to appear in the reaction steps.
5.9 ANALYSIS OF PHOTOCHEMICAL PRODUCT AND THEIR KINETICS OF REACTION MECHANISM

The reaction between methylene blue and reducing sugars is essentially an oxidation reduction reaction. Methylene blue oxidizes aldoses into aldonic acid in presence of acid base equilibria and itself reduces into leuco methylene blue but oxygen of the air oxidizes the leuco methylene blue in blue form. The system [233] was a clear solution upon passing light but changes into blue color when shaken, purple when exposed to sun light turns yellow when allowed to stand for a day. The duration of the color change depends upon the length of shaking and light. After two days, the yellow color persists and no changes in color occur by shaking, if container is opened. Then it was loaded in the column to isolate the reaction product to study the mechanism of the reaction. Five pure compounds were separated whose quantity was very minute. Only mass and I. R were possible with the help of known weight of chemical used and isolated one. It was observed that dye was highly reactive towards reagents present in the mixture and also interaction with solvent molecule is reported.

\[
\begin{align*}
\text{MB}^+ + \text{H}^+ & \quad \text{K}^+ \\
\text{MBH}^{++} & \quad \text{H}_2\text{O}
\end{align*}
\]

Sixteen fractions were collected but only five were able to purify to some extent but further vials were not able to purify which may be due to the high reactivity of the dye and TLC of each vial was showing two compound spot which may be due to equilibrium state of oxidation reduction reaction or may be dimerization and polymerization.
In simple terms, the changes between blue and colorless solution are due to redox cycle between two forms of methylene blue as shown in the reaction steps. \([234-247]\]

The possible reaction mechanism is outlined below.

**Step 1**

\[
\begin{align*}
\text{Methylene blue} & \quad \text{(oxidized form blue colour MB)} \\
\text{Mesoanhydride} & \quad \text{(reduced form colourless MBH)}
\end{align*}
\]

\[
\text{O}_{2} + \text{C}_6\text{H}_{12}\text{O}_6 + \text{OH}^{-} \quad \stackrel{\text{slow}}{\longrightarrow} \quad \text{Leucocarboxylic blue} \quad \text{(reduced form colourless MBH)}
\]

**Step 2**

\[
\frac{1}{2} \text{O}_2 + \text{Solution} \quad \stackrel{\text{slow}}{\longrightarrow} \quad \frac{1}{2} \text{O}_2 \quad \text{(air)}
\]

**Step 3**

\[
\frac{1}{2} \text{O}_2 + \text{MBH} \quad \stackrel{\text{Fast}}{\longrightarrow} \quad \text{MB} + \text{OH}^{-}
\]

Net reaction

\[
\frac{1}{2} \text{O}_2 + \text{C}_6\text{H}_{12}\text{O}_6 \quad \stackrel{\text{Very slow}}{\longrightarrow} \quad \text{C}_6\text{H}_{12}\text{O}_7 \quad \text{aldonic acid}
\]

The sugar is being on oxidation from an aldehyde to aldonic acid, steps 2 and 3 occur when system shaken and reoxidised in step 3, the MBH is oxidized to MB and the O₂ is being reduced to \(\text{OH}^{-}\).
5.10 GENERAL MECHANISM PROPOSED FOR THE PHOTO REDUCTION OF METHYLENE BLUE WITH REDUCING SUGARS

Dyes have very important application in biological and industrial fields. Some times dye issues as indicator, while oxidation property of methylene blue is used for tissue building and staining of tissues. Photochemistry of the dyes has been the subject of considerable interest throughout the nineteenth century. Various ultraviolet, visible detectors were used to study the reduction of dyes by organic and inorganic reducing agents and in the present process of photochemical reduction of dye with different reducing sugars in 50% aqueous methanol, the primary product like e', H⁺ and OH⁻ can attack the substrate molecule at different positions. Therefore these systems may show complex kinetics [225, 71]. The present work reveals a comparative study of reduction of methylene blue with reducing sugars at different parameters. The reduction processes show the similar behavior in the following respect and are well verified by the plots.

i) Plots of [ϕ] versus [H₂] are S' shaped [140, 215]
ii) Plots of l/ϕ against l/[RCHO] are straight lines [179]
iii) Plots of φ₁ - ϕ / φ₂ versus l/hₜ are straight lines [140, 215]
iv) Plots of log ϕ against l / T [236]

In general mechanism it is assumed that the dye molecule on absorption of a photon of light is raised to the singlet state with fluorescence or goes to triplet excited state by internal conversion. The equilibrium

\[ \text{MBH}_T^+ + \text{RH} \rightleftharpoons \text{MBH}_T^{+\cdot} \cdot \text{RH} \]

is established in presence of reductant molecule. The excited molecules lose their absorbed energy by collision with the reductant molecule. The triplet and the protonated triplet species are supposed to react with reductant molecule forming the semi reduced dye which on disproportionation formed the leuco methylene blue.

The general mechanism consists of following steps: The first step, when dye is subjected to photon of light, excitation of molecule of methylene blue will take place and it will go to singlet excited state, as follows

\[ \text{MB}^+ + \text{hv} \rightarrow \text{MB}_S^+ \]

Rate = \([I_a]\)
where $I_a$ is the intensity of light and $MB^+_s$ is the singlet excited state after absorbing $I_a$ of light.

De-excitation of the excited singlet [78] state is followed by the emission of fluorescence radiation or $h\nu_t$:

$$MB^+_s \xrightarrow{k_1} MB^+_r + h\nu_t \quad \text{Rate} = (1 - \alpha) [I_a]$$

Another possibility is this that $MB^+_s$ may convert into triplet excited state proved by Uddin [140, 215] and Pestemer [86].

$$MB^+_s \xrightarrow{k_2} MB^+_T \quad \text{Rate} = \alpha [I_a]$$

$k_2 =$ rate constant for inter system crossing of singlet to triplet state

$\alpha =$ fraction of light absorbed

The de-excitation of singlet excited state and triplet excited state may occur either by collision in between the unexcited dye molecule or reductant molecule which may be represented by following steps.

$$MB^+_s + MB^+ \xrightarrow{k_3} MB^+_r + MB^+ \quad \text{(de-excitation)} \quad \text{Rate} = k_3 [MB^+_T][RH]$$

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

$$MB^+_s + RH \xrightarrow{k_4} MB^+_r + RH \quad \text{(de-excitation)} \quad \text{Rate} = k_4 [MB^+_T][RH]$$

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

$$MB^+_T + RH \xrightarrow{k_5} MB^+_r + RH \quad \text{(de-excitation)} \quad \text{Rate} = k_5 [MB^+_T][RH]$$

$k_5 =$ quenching rate constant for triplet state with reductant molecule

Triplet excited state may simply transfer into ground state by releasing absorbing energy

$$MB^+_T \xrightarrow{k_6} MB^+ \quad \text{Rate} = k_6 [MB^+_T]$$

$k_6 =$ intersystem crossing of the singly charged triplet state to the ground state.
Other possibility for triplet excited state of the dye to associate with hydrogen ion [179, 237] at high acidity region which may undergo a reversible process.

\[
MB_T^+ + H^+ \xleftrightarrow{K'} MBH_T^{++}
\]

\[
K' = \frac{[MBH_T^{++}]}{[MB_T^+][H^+]}
\]

\[
K' = \frac{[MBH_T^{++}]}{[MB_T^+]^2} \times \frac{1}{\hbar_0}
\]

where \( K' \) = equilibrium constant for singly protonated state and doubly protonated state.

5.10.1 REACTION OF METHYLENE BLUE WITH REDUCING SUGARS
Reducing sugars like, Galactose, D-Mannose, D-(+)-Ribose, Maltose and Lactose when react with methylene blue, reduced methylene blue to leuco methylene blue.

when put it into air, leuco dye oxidizes to blue color, upon passing light in presence of reducing sugars dye get back to color less form. The equation for the reaction when shaken [234, 235]

\[
O_2 + MB \text{ (Colourless)} \xrightarrow{\text{light}} MB_{(ox)} \text{ (blue)}
\]

upon passing light and standing

\[
RH + OH \xrightarrow{\text{light}} R + H_2O
\]

\[
MB_{(ox)} + RH \xrightarrow{\text{light}} MB_{(red)} + \text{Aldonic acid}
\]

where \( MB_{(red)} \) is the reduced form of methylene blue, \( MB_{ox} \) is the oxidized (blue) form of methylene blue and RH represents the reducing sugars.

5.10.2 REACTION OF TRIPLET EXCITED STATE WITH REDUCTANT
Triplet excited state and protonated triplet excited state are supposed to react with reductant to from semi reduced methylene blue.

\[
MB_T^+ + RH \xrightarrow{k_7} MBH^+ + R^+
\]

Rate = \( k_7 [MB_T^+][RH] \)
\( k_7 \) = quenching rate constant for reaction of singly charged triplet excited state with reductant to form semi reduced dye.

\[
MB_T^{++} + RH \xrightarrow{k_8} MBH^{++} + R^+ \\
\text{Rate} = k_8 \ [MB_T^{++}][RH]
\]

\( k_8 \) = rate constant for the formation of \( MBH^{++} \) or reduction reaction of doubly charged triplet species with the reductant.

Deexcitation of protonated triplet excited state may take place by colliding with reductant molecule or by releasing thermal energy to the system

\[
MBH_T^{++} + RH \xrightarrow{k_9} MBH^{++} + RH \\
\text{Rate} = k_9 \ [MBH_T^{++}][RH]
\]

\( k_9 \) = quenching rate constant for doubly charged triplet state with reductant

\[
MBH_T^{++} \xrightarrow{k_{10}} MBH^{++} \\
\text{Rate} = k_{10} \ [MBH_T^{++}]
\]

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

Association of \( MBH_T^+ \) with reductant may also establish equilibrium [135,140]

\[
MBH_T^+ + RH \xrightarrow{K''} MBH_T^{++}RH \\
K'' = \frac{[MBH_T^{++}.RH]}{[MBH_T^+][RH]}
\]

\( K'' \) = equilibrium constant between doubly protonated and protonated triplet complex.

Reaction with the solvent molecule \( \{H-OH\} \) is given as [215, 232]

\[
MBH_T^{++} + \stackrel{+}{H}OH^- \xrightarrow{k_{11}} MBH_T^{++} + OH^- \\
\text{Rate} = k_{11} \ [MBH_T^{++}]
\]

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

The complex \( MBH_T^{++}.RH \) may loose its energy and converted
\[
\text{MBH}^{++} + \text{RH} \xrightarrow{k_{12}} \text{MB}^+ + H^+ + \text{RH}
\]

Rate = \(k_{12} [\text{MBH}^{++} \cdot \text{RH}]\)

\(k_{12}\) = rate constant for dissociation process.

The energy loss [140] may also occur by the collision of complex with unexcited dye molecule \(\text{MB}^+\)

\[
\text{MBH}^{++}_T + \text{MB}^+ \xrightarrow{k_{13}} \text{MBH}^{++}_2 + \text{R} + \text{MB}^+ \quad 16
\]

Rate = \(k_{13} [\text{MBH}^{++}_T \cdot \text{RH}][\text{MB}^+]\)

where \(k_{13}\) = rate constant for the formation of \(\text{MBH}^{++}_2\) = free radical.

5.10.3 **Oxidation – Reduction in complex**

Redox reaction is also taking place with transference of electron into complex and unexcited molecule according to [140, 215]

\[
\text{MBH}^{++}_T + \text{RH} + \text{MB}^+ + e^- \xrightarrow{k_{14}} \text{MBH}^{++} + \text{RH} + \text{MB}^+ \quad 17
\]

Rate = \(k_{14} [\text{MBH}^{++}_T \cdot \text{RH}][\text{MB}^+]\)

\(k_{14}\) = quenching rate constant for redox reaction with reductant and dye.

\[
\text{HOCH}_2(\text{CHOH})_4 \text{CHO} + 3\text{OH}^- \xrightarrow{\text{H}^+/2e^-} \text{HOCH}_2(\text{CHOH})_4 \text{CO}_2 + 2\text{H}_2\text{O} + 2e^-
\]

\(\text{MBH}^{++}_2\) disproportionates [241] to give leuco dye and \(\text{MBH}^{++}_3\) methylene blue cation \(\text{MB}^+\).

\[
2\text{MBH}^{++}_2 \xrightarrow{k_{15}} \text{MBH}^{++}_3 + H^+ + \text{MB}^+ \quad 19
\]

\(k_{15}\) = rate constant for disproportionation reaction

\[
\text{R}^+ + \text{OH}^- \xrightarrow{} \text{Aldonic Acid}
\]

The free radicals of reductant undergo oxidation with \(\text{OH}^-\) termination to form aldonic acid. In the above mentioned proposed mechanism steps, \(k\) represent the rate constants, and \(k_1\) to \(k_{15}\) show the rate constants of each process and \(K'\) and \(K''\) are the ratios of equilibrium constant.
5.10.4 OVERVIEW OF MECHANISM OF PHOTOREDUCTION OF DYE

The summary of reaction mechanism may be written in this form also

\[ \text{MB}^+ + h\nu \xrightarrow{\text{Rate } = [I]_a} \text{MB}_S^+ \]  
\[ \text{MB}^+ \xrightarrow{k_1} \text{MB}^+ + h\nu_r \xrightarrow{\text{Rate } = (1-\alpha)[I]_a} \]  
\[ \text{MB}_s^+ \xrightarrow{k_2} \text{MB}_T^+ \xrightarrow{\text{Rate } = \alpha [I]_a} \]  
\[ \text{MB}_s^+ + \text{MB}^+ \xrightarrow{k_3} \text{MB}^+ \rightleftharpoons \text{MB}^+ \rightarrow \text{MB}^+ \text{ (de-excitation)} \xrightarrow{\text{Rate } = k_4} \text{MB}^+ \rightleftharpoons \text{RH} \text{ (de-excitation)} \xrightarrow{\text{Rate } = k_5} \text{MB}^+ + \text{RH} \text{ (de-excitation)} \]  
\[ \text{MB}_T^+ \xrightarrow{k_6} \text{MB}^+ \]  
\[ \text{MB}_T^+ + H^+ \xrightarrow{K'} \xrightarrow{\text{Rate } = K'} \text{MBH}^{++}_T \]  
\[ K' = \frac{[\text{MBH}^{++}_T]}{[\text{MB}_T^+][H^+]}(\text{Rate}) \]  
\[ \text{MB}_T^+ + \text{RH} \xrightarrow{k_7} \text{MBH}^{++}_T + \text{R} \]  
\[ \text{MBH}_T^{++} + \text{RH} \xrightarrow{k_8} \text{MBH}_2^{++} + \text{R} \]  
\[ \text{MBH}_T^{++} + \text{RH} \xrightarrow{k_9} \text{MBH}^{++} + \text{RH} \]  
\[ \text{MBH}_T^{++} \xrightarrow{k_{10}} \text{MBH}^{++} \]  
\[ \text{MBH}_T^{++} + \text{RH} \xrightarrow{K''} \xrightarrow{\text{Rate } = K''} \text{MBH}^{++}_T + \text{RH} \]  
\[ K'' = \frac{[\text{MBH}^{++}_T \cdot \text{RH}]}{[\text{MBH}_T^{++}][\text{RH}]} \]
\[
\begin{align*}
\text{MBH}_{T}^{++} + \text{H}^- + \text{OH}^- & \xrightarrow{k_{11}} \text{MBH}_{2}^{++} + \text{OH}^- \\
\text{MBH}_{T}^{++}. \text{RH} & \xrightarrow{k_{12}} \text{MB}^+ + \text{H}^+ + \text{RH} \\
\text{MBH}_{T}^{++}. \text{RH} + \text{MB}^+ & \xrightarrow{k_{13}} \text{MBH}_{2}^{++} + \text{R} + \text{MB}^+ \\
\text{MBH}_{T}^{++}. \text{RH} + \text{MB}^+ + e^- & \xrightarrow{k_{14}} \text{MBH}^{++} + \text{RH} + \text{MB}^+ \\
\text{RH} + 3 \text{OH}^- & \xrightarrow{} \text{RCOOH} + 2 \text{H}_2\text{O} + 2e^- \\
\text{MB}^+ & \xrightarrow{\text{H}^+/2e^-} \text{MBH}^+ \\
\text{MBH}^+ + \text{H}^+ & \xrightarrow{} \text{MBH}_{2}^{++} \text{(semi reduced methylene blue)}^{18} \\
2\text{MBH}_{2}^{++} & \xrightarrow{k_{15}} \text{MBH}_{3}^{++} + \text{H}^+ + \text{MB}^+ \\
\text{R} + \text{OH}^- & \xrightarrow{} \text{aldonic acid}
\end{align*}
\]

5.10.5 Derivation of Rate Equations:

Let us consider the steps of the proposed reaction mechanism, assuming \([1]\)_s is the photons of light absorbed which is calculated by adding rate equation of suggested mechanism.

\[
\alpha \left[ 1 \right]_s = k_5 \left[ \text{MB}^+ \right] \left[ \text{RH} \right] + k_6 \left[ \text{MB}_{T}^{++} \right] + k_7 \left[ \text{MBH}_{T}^{++} \right] \left[ \text{RH} \right] + k_8 \left[ \text{MB}_{T}^{+} \right] \left[ \text{RH} \right] \\
+ k_9 \left[ \text{MBH}_{T}^{++} \right] \left[ \text{RH} \right] + k_{10} \left[ \text{MBH}_{T}^{++} \right] + k_{11} \left[ \text{MBH}_{T}^{++} \right] + k_{12} \left[ \text{MBH}_{T}^{++}.\text{RH} \right] \\
+ k_{13} \left[ \text{MBH}_{T}^{++}.\text{RH} \right] \left[ \text{MB}^+ \right] + k_{14} \left[ \text{MBH}_{T}^{++}.\text{RH} \right] \left[ \text{MB}^+ \right]
\]

The rate of disappearances of methylene blue is expressed as

\[
-\frac{d \left[ \text{MB}^+ \right]}{dt} = \frac{1}{2} \left[ k_7 \left[ \text{MB}^+ \right] \left[ \text{RH} \right] + k_8 \left[ \text{MB}_{T}^{++} \right] \left[ \text{RH} \right] + k_{11} \left[ \text{MBH}_{T}^{++} \right] \\
+ k_{13} \left[ \text{MBH}_{T}^{++}.\text{RH} \right] \left[ \text{MB}^+ \right] + k_{14} \left[ \text{MBH}_{T}^{++}.\text{RH} \right] \left[ \text{MB}^+ \right]
\]

Now simplify the above expression by putting
\[
[MB_T^+] = a, \quad [MBH_T^{++}] = b, \quad [MBH_T^+] \cdot RH = c,
\]
\[
[MB^+] = A, \quad [RH] = \ell \quad \text{and} \quad [H^+] = h_0
\]

substituting the symbols \( a, b, c, A, \ell \) and \( h_0 \) in equation (1) and (2)
\[
\alpha[I]_a = k_5 a \ell + k_6 a + k_7 a b + k_9 b c + k_{10} b + k_{11} b + k_{12} c + k_{13} c A + k_{14} c A
\]

\[
-\frac{d[MB^+]}{dt} = \frac{1}{2} [k_7 a \ell + k_9 b c + k_{11} b + k_{13} c A + k_{14} c A] \tag{4}
\]

\( k_{13} \) and \( k_{14} \) can be represented by only one constant because both rate constant are quenching state of complex and excited methylene blue molecule.

or \( k_{13} + k_{14} = k_{13} \)

\[
\alpha[I]_a = k_5 a \ell + k_6 a + k_7 a b + k_9 b c + k_{10} b + k_{11} b + k_{12} c + k_{13} c A
\]

\[
-\frac{d[MB^+]}{dt} = \frac{1}{2} [k_7 a \ell + k_9 b c + k_{11} b + k_{13} c A] \tag{5}
\]

Quantum yield for photoreduction of methylene blue is given by
\[
\phi = -\frac{d[MB^+]}{dt} \tag{6}
\]

Substituting the value from equation No. (5) and (6) in equation (7) we get following expression.
\[
\phi = \frac{\alpha}{2} \cdot \frac{k_7 a \ell + k_9 b c + k_{11} b + k_{13} c A}{k_5 a \ell + k_6 a + k_7 a b + k_9 b c + k_{10} b + k_{11} b + k_{12} c + k_{13} c A} \tag{7}
\]

Now consider the equilibrium expressions appear in the mechanism
\[
MB_T^+ + H^+ \xrightleftharpoons[K']{} MBH_T^{++} \tag{8}
\]

and
\[
MBH_T^+ + RH \xrightleftharpoons[K'']{\text{RH}} MBH_T^- \cdot RH \tag{9}
\]

Using \( a, b, c, A, \ell \) and \( h_0 \) in equilibrium expression
\[ a + h^0 \xrightarrow{K'} b \]

and
\[ b + \ell \xrightarrow{K''} c \]
or
\[ K' = \frac{b}{a \cdot h_0} \]
\[ b = K' \cdot a \cdot h_0 \]
\[ K'' = \frac{c}{b \cdot \ell} \]
\[ c = K'' \cdot b \cdot \ell \]

Put the value of \( b \) from equation (12) in (14) we get
\[ c = K'' \cdot K' \cdot a \cdot h_0 \cdot \ell \]

Substituting the value of \( b \) and \( c \) from equation (12) and (15) in equation (8) we have
\[ \phi = \frac{\alpha}{2} \cdot \frac{k_7 \cdot a \cdot \ell + k_7 \cdot K' \cdot a \cdot h_0 \cdot \ell + k_{11} \cdot K' \cdot a \cdot h_0 + k_{13} \cdot K'' \cdot K' \cdot a \cdot h_0 \cdot \ell \cdot A}{[k_5 \cdot a \cdot \ell + k_6 \cdot a + k_7 \cdot a \cdot \ell + k_9 \cdot K' \cdot a \cdot h_0 \cdot \ell + k_{10} \cdot K' \cdot a \cdot h_0 + k_{11} \cdot K' \cdot a \cdot h_0 + k_{12} \cdot K'' \cdot K' \cdot a \cdot h_0 \cdot \ell \cdot A]} \]
\[ \phi = \frac{\alpha}{2} \cdot \frac{a \cdot [k_7 \cdot \ell + K' \cdot h_0 (k_8 \cdot \ell + k_{11}) + K'' \cdot k_{13} \cdot h_0 \cdot \ell \cdot A]}{a \cdot [k_5 \cdot \ell + k_6 + k_7 \cdot \ell + K' \cdot h_0 (k_8 \cdot \ell + k_9 \cdot \ell + k_{10} + k_{11} + K'' \cdot k_{12} + K'' \cdot k_{13} \cdot \ell \cdot A)]} \]
\[ \phi = \frac{\alpha}{2} \cdot \frac{k_7 \cdot \ell + [K' \cdot h_0 (k_8 \cdot \ell + k_{11}) + K'' \cdot k_{13} \cdot \ell \cdot A]}{[k_5 \cdot \ell + k_6 + k_7 \cdot \ell + K' \cdot h_0 (k_8 \cdot \ell + k_9 \cdot \ell + k_{10} + k_{11} + K'' \cdot k_{12} + K'' \cdot k_{13} \cdot \ell \cdot A)]} \]

let \( \phi = \phi_1 \) at high acidity region
\( \phi = \phi_2 \) at lower acidity region

for high acidity, species \( \{ MB^- T \} \) have been neglected, therefore omitting the steps from the mechanism involving rate expression \( k_5, k_6, k_7 \); equation (17) reduced to,
\[ \phi_1 = \frac{\alpha}{2} \cdot \frac{K' h_o (k_8 \ell + k_{11}) + K'' k_{13} \ell A}{K' h_o (k_8 \ell + k_9 \ell + k_{10}^+ k_{11} + K'' k_{12} \ell + K'' k_{13} \ell A)} \]

when \( \ell = 0 \) then equation 18 reduced to

\[ \phi_1 \text{ (solvent)} = \frac{\alpha}{2} \cdot \frac{k_{11}}{k_{10}^+ k_{11}} \]

at low acidity \( \phi = \phi_2 \) then [MBH\(^{++}_F \)] [MBH\(^{++}_R \), RH] species are not present, therefore steps involving in reaction mechanism like \( k_8, k_9, k_{10}, k_{11}, k_{12}, k_{13} \) are eliminated, then equation (17) reduced to

\[ \phi_2 = \frac{\alpha}{2} \cdot \frac{k_7 \ell}{k_5 \ell + k_6 + k_7 \ell} \]

Taking inverse of (19) and (20)

\[ \frac{1}{\phi_1} = \frac{2}{\alpha} \cdot \frac{k_{10} + k_{11}}{k_{11}} \]

\[ \frac{1}{\phi_2} = \frac{2}{\alpha} \cdot \frac{k_5 \ell + k_6 + k_7 \ell}{k_7 \ell} \]

Taking inverse of equation (18) and adding with equation (22) we get

\[ \frac{\alpha}{2} \left( \frac{1}{\phi_2} + \frac{1}{\phi_1} \right) K' h_o (k_8 \ell + k_{11} + K'' k_{11} \ell A) = [k_5 \ell + k_6 \ell + k_7 \ell + K' h_o (k_8 \ell + k_6 \ell + k_{10}^+ k_{11} + K'' k_{13} \ell A) \]

Multiply by \( \phi \)

\[ \frac{\alpha}{2} \left( \frac{\phi}{\phi_2} + \frac{\phi}{\phi_1} \right) K' h_o (k_8 \ell + k_{11} + K'' k_{11} \ell A) = [k_5 \ell + k_6 \ell + k_7 \ell + K' h_o (k_8 \ell + k_6 \ell + k_{10}^+ k_{11} + K'' k_{13} \ell A) \]

\[ \frac{\phi_1 - \phi}{\phi - \phi_2} = \frac{\phi_1}{\phi_2} \cdot \frac{k_7 \ell}{K' h_o (k_8 \ell + k_{11} + K'' k_{13} \ell A)} \]

Neglecting \( k_{11} \) from the denominator

\[ \frac{\phi_1 - \phi}{\phi - \phi_2} = \frac{\phi_1}{\phi_2} \cdot \frac{k_7 \ell}{K' (k_8 + K'' k_{13} \ell A)} \cdot \frac{1}{h_o} \]
Equation (25) shows the relationship between \( \phi_1 - \phi / \phi - \phi_2 \) and \( 1 / h_o \). The plot of this equation will be straight line passing through origin.

Now consider the equation (18)

\[
\frac{\phi_1}{2} = \frac{K' h_o (k_8 \ell + k_{11}) + K'' k_{13} \ell A}{K' h_o (k_8 \ell + k_{10} + k_{11} + K'' k_{12} \ell + K'' k_{13} \ell A)}
\]

Taking inverse of equation (18)

\[
\frac{1}{\phi_1} = \frac{2}{\alpha} \cdot \frac{K' h_o (k_8 \ell + k_9 \ell + k_{10} + k_{11} + K'' k_{12} \ell + K'' k_{13} \ell A)}{[K' h_o (k_8 \ell + k_{11}) + K'' k_{13} \ell A]}
\]

Neglecting \( k_{11} \) and rearranging the equation

\[
\frac{1}{\phi_1} = \frac{2}{\alpha} \cdot \frac{k_8 + k_9 + K'' k_{12} + K'' k_{13} A}{k_8 + K'' k_{13} A} + \frac{2}{\alpha} \cdot \frac{k_{10}}{k_9 + K'' k_{13} A} \cdot \frac{1}{\ell}
\]

now consider equation (22)

\[
\frac{1}{\phi_2} = \frac{2}{\alpha} \cdot \frac{k_5 + k_7}{k_7} + \frac{2}{\alpha} \cdot \frac{k_6}{k_7} \cdot \frac{1}{\ell}
\]

Equations (26) and (27) are straight line equation having both slope and intercept and show the relationship between quantum yield and reductant concentration (1) at high acidity and low acidity.

Now consider equation (17)

\[
\phi = \frac{\alpha}{2} \cdot \frac{k_5 \ell + [K' h_o (k_8 \ell + k_{11}) + K'' k_{13} \ell A]}{[k_5 \ell + k_8 + k_7 \ell + K' h_o (k_8 \ell + k_9 \ell + k_{10} + k_{11} + K'' k_{12} \ell + K'' k_{13} \ell A)]}
\]

Neglecting \( k_{11}, k_7 \) and inversing the above equation following relation is established

\[
1 = \frac{\alpha}{2} \cdot \frac{k_8 \ell + k_9 \ell + k_{10} + K'' k_{12} \ell + K'' k_{13} \ell A}{k_5 \ell + K'' k_{13} \ell A} + \frac{\alpha}{2} \cdot \frac{k_5 \ell + k_6}{K' h_o (k_8 \ell + K'' k_{13} \ell A)}
\]

Neglecting \( k_6 \) from the above relation, equation reduced to

\[
\frac{l}{\phi} = \frac{2}{\alpha} \cdot \frac{k_8 \ell + k_9 + k_{10} + K'' k_{12} \ell + K'' k_{13} \ell A}{k_8 \ell + K'' k_{13} \ell A} + \frac{\alpha}{2} \cdot \frac{1}{k_8 \ell + K'' k_{13} \ell A} \cdot \frac{1}{h_o}
\]
Equation (29) shows the relationship between \( 1 / \phi \) and \( 1 / h_o \). It is a straight line plots having slope

\[
\frac{\alpha}{2} \cdot \frac{k_5}{K' (k_8 + K'' k_{13} A)}
\]

and intercept

\[
\frac{2}{\alpha} \cdot \frac{k_8 \ell + k_9 + k_{10} + K'' k_{12} \ell + K'' k_{13} \ell A}{k_8 \ell + K'' k_{13} \ell A}
\]

Now consider equation (28) at constant concentration of reactant, \( k_5, k_8 \) and \( k_9 \) can be neglected

\[
\frac{1}{\phi} = \frac{2}{\alpha} \cdot \frac{k_{10} + K'' k_{12} + K'' k_{13} A}{k_8 + K'' k_{13} A} + \frac{2}{\alpha} \cdot \frac{k_5}{K' (k_8 + K'' k_{13} A)} \cdot \frac{1}{h_o}
\]

Neglecting internal conversion process (\( k_{10} \) and \( k_{12} \)) then above relation reduced to

\[
\phi = \frac{2}{\alpha} \cdot \frac{K'' k_{13} A}{k_8 + K'' k_{13} A} + \frac{2}{\alpha} \cdot \frac{k_5}{K' (k_8 + K'' k_{13} A)} \cdot \frac{1}{h_o}
\]

Equation (31) represents the straight line relationship between \( 1 / \phi \) and \( 1 / h_o \) having slope

\[
\frac{2}{\alpha} \cdot \frac{k_6}{K' (k_8 + K'' k_{13} A)}
\]

and intercept

\[
\frac{2}{\alpha} \cdot \frac{K'' k_{13} A}{k_8 + K'' k_{13} A}
\]

Relationship between \( 1 / \phi \) and \( 1 / l \) or \( 1 / [RCHO] \).

Consider equation no. (8)

\[
\phi = \frac{\alpha}{2} \cdot \frac{k_7 a \ell + k_8 b \ell + k_{11} b + k_{13} c A}{k_5 a \ell + k_6 a + k_7 a \ell + k_8 b \ell + k_9 b \ell + k_{10} b + k_{11} b + k_{12} c + k_{13} c A}
\]

put the values of \( b \) and \( c \) in above equation and inverting the relation.

\[
\frac{1}{\phi} = \frac{2}{\alpha} \left[ \frac{1 + \frac{(k_5 \ell + k_6)}{k_9 \ell + K' h_o (\ell k_8 + k_{11}) + K' h_o K'' k_{12}}}{k_8 \ell + K' h_o (\ell k_8 + k_{11}) + K' h_o K'' k_{13} A} \right]
\]
Neglecting $k_{12}$ and suppose that $[M^{i+}]$ is not present thus from above equation, following relation will be obtained.

$$
\frac{1}{\phi} = \frac{2}{\alpha} \left[ \frac{k_9 + K'' k_{12}}{k_8 + K'' k_{13} A} + \frac{k_{10}}{k_9 + K'' k_{13} A} \cdot \frac{1}{\ell} \right]
$$

$$
\frac{1}{\phi} = \frac{2}{\alpha} + \frac{2}{\alpha} \frac{k_9 + K'' k_{12}}{k_8 + K'' k_{13} A} + \frac{2}{\alpha} \frac{k_{10}}{k_8 + K'' k_{13} A} \cdot \frac{1}{\ell}
$$

The above equation shows the straight line relationship between $1/\phi$ and $1/[RCHO]$ having slope and intercept as

$$
\text{Slope} = \frac{2}{\alpha} \cdot \frac{k_{10}}{k_8 + K'' k_{13} A}
$$

$$
\text{Intercept} = \frac{2}{\alpha} + \frac{2}{\alpha} \frac{k_9 + K'' k_{12}}{k_8 + K'' k_{13} A}
$$

The intercept shows the quantum yield at infinite concentration, therefore equation 33 can be written as

$$
\frac{1}{\phi} = \frac{1}{\phi_o} + \frac{2}{\alpha} \frac{k_{10}}{k_8 + K'' k_{13} A} + \frac{1}{\ell}
$$

5.10.6 DETERMINATION OF THE RATIOS OF THE RATE CONSTANTS OF PROPOSED GENERAL MECHANISM OF PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE BY REDUCING SUGARS

The ratios of the rate constants were calculated by intercepts and slopes of the different linear equations and plots of $1/\phi$ versus $1/h_o$, $(\phi_1 - \phi / \phi - \phi_2)$ versus $1/h_o$ and $1/\phi$ versus $1/[RCHO]$ applying least square method. These plots of different equations showed in the Figures 4.14 – 4.18, 4.19 – 4.23 and 4.24 – 4.28 respectively and the corresponding values are tabulated in Tables 40-42.

The plots of $(\phi_1 - \phi / \phi - \phi_2)$ versus $1/h_o$ are straight lines passing through origin with slopes $\frac{k_7}{K' (k_8 + K'' k_{13} A)}$ having no intercept as shown in equation no. (25).
Table - 40
THE VALUES OF INTERCEPTS AND SLOPES OF PLOT $1/\phi$ VS $1/[RCHO]$ FOR PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE WITH REDUCING SUGARS

<table>
<thead>
<tr>
<th>Reductants</th>
<th>$H_o$</th>
<th>Intercepts</th>
<th>Slope $\times 10^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose</td>
<td>4.2</td>
<td>$2.2680 \pm 0.15$</td>
<td>$4.0171 \pm 0.2$</td>
</tr>
<tr>
<td></td>
<td>7.7</td>
<td>$4.3292 \pm 0.01$</td>
<td>$5.1145 \pm 0.3$</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>4.2</td>
<td>$2.1342 \pm 0.01$</td>
<td>$4.2998 \pm 0.1$</td>
</tr>
<tr>
<td></td>
<td>7.7</td>
<td>$3.8185 \pm 0.12$</td>
<td>$4.9801 \pm 0.2$</td>
</tr>
<tr>
<td>D-(+)-Ribose</td>
<td>4.2</td>
<td>$2.0754 \pm 0.11$</td>
<td>$3.6658 \pm 0.3$</td>
</tr>
<tr>
<td></td>
<td>7.7</td>
<td>$3.4095 \pm 0.10$</td>
<td>$5.4742 \pm 0.1$</td>
</tr>
<tr>
<td>Maltose</td>
<td>4.2</td>
<td>$2.2330 \pm 0.01$</td>
<td>$3.9050 \pm 0.3$</td>
</tr>
<tr>
<td></td>
<td>7.7</td>
<td>$4.1835 \pm 0.01$</td>
<td>$6.1925 \pm 0.2$</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.2</td>
<td>$2.1342 \pm 0.2$</td>
<td>$4.2998 \pm 0.2$</td>
</tr>
<tr>
<td></td>
<td>7.7</td>
<td>$1.0843 \pm 0.3$</td>
<td>$5.7590 \pm 0.1$</td>
</tr>
</tbody>
</table>
Table - 41

THE VALUES OF INTERCEPTS AND SLOPES OF PLOT $1/\phi$ VS $1/b_0$
FOR PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE WITH
REDUCING SUGARS

<table>
<thead>
<tr>
<th>Reductants</th>
<th>$[\text{RCHO}] \cdot 10^3$</th>
<th>Intercepts</th>
<th>Slope $\cdot 10^6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose</td>
<td>0.8</td>
<td>7.7413 ± 1.12</td>
<td>0.0605 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>2.4396 ± 0.5</td>
<td>0.0301 ± 0.01</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>0.8</td>
<td>5.5890 ± 1.3</td>
<td>0.4174 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>1.9896 ± 1.1</td>
<td>0.1855 ± 0.03</td>
</tr>
<tr>
<td>D-(+)-Ribose</td>
<td>0.8</td>
<td>7.6344 ± 1.3</td>
<td>0.0504 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>2.3875 ± 0.8</td>
<td>0.0442 ± 0.01</td>
</tr>
<tr>
<td>Maltose</td>
<td>0.8</td>
<td>7.6322 ± 1.5</td>
<td>0.0816 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>2.2190 ± 0.9</td>
<td>0.0382 ± 0.03</td>
</tr>
<tr>
<td>Lactose</td>
<td>0.8</td>
<td>7.3184 ± 1.1</td>
<td>0.071 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>2.2267 ± 0.7</td>
<td>0.0398 ± 0.01</td>
</tr>
</tbody>
</table>
Table  - 42
THE VALUES OF INTERCEPTS AND SLOPES OF PLOT \((\phi_1 - \phi / \phi - \phi_2) VS 1/t_o\) FOR PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE WITH REDUCING SUGARS

<table>
<thead>
<tr>
<th>Reductants</th>
<th>[RCHO] ( \times 10^4 ) mol dm(^{-3} )</th>
<th>Slope ( \times 10^6 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose</td>
<td>0.8</td>
<td>0.6264 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>1.0261 ± 0.3</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>0.8</td>
<td>0.4891 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>1.2003 ± 0.1</td>
</tr>
<tr>
<td>D-(-)-Ribose</td>
<td>0.8</td>
<td>0.5308 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>1.9265 ± 0.1</td>
</tr>
<tr>
<td>Maltose</td>
<td>0.8</td>
<td>0.5395 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.8550 ± 0.1</td>
</tr>
<tr>
<td>Lactose</td>
<td>0.8</td>
<td>0.2318 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0.2809 ± 0.1</td>
</tr>
</tbody>
</table>
Ratio of rate constants were calculated by slopes of the equations by fixing some limitation and neglecting some less contributing rate constants. $k_7$ is associated with formation of semi reduced dye through singly protonated triplet state of methylene blue while $k_9$ is associated with doubly protonated triplet state of methylene blue. Form these steps of $k_7$ and $k_9$ one can get the idea about the contribution of singly protonated or doubly protonated triplet excited state of methylene blue in the proposed mechanism of photoreduction of dye.

The ratios given in equation no. (27) i.e.

$$\frac{2}{\alpha} \frac{k_5}{k_7} \quad \text{and} \quad \frac{2}{\alpha} \frac{k_5 + k_7}{k_7}$$

were calculated through the slopes and intercepts of the plots of $1/\phi$ versus $1/[\text{RCHO}]$. The respective plots are shown in Figures 4.14 – 4.18 at low acidity and the values of intercepts and slopes are tabulated in Table 40.

In these ratios $k_5$ is the quenching specific rate constant for triplet state with reductant and follows second order kinetics where $k_6$ corresponds to the rate constants of deexcitation of triplet excited state of methylene blue and follows first order reaction and $k_7$ is concerned with reduced state of methylene blue. The triplet states of methylene blue react with reducing agent, as two molecules are taking part therefore order of reaction determined here is second.

From equation (26), the ratio

$$\frac{k_8 + k_9 + \frac{K''}{k_{12}} k_{12} + \frac{K'''}{k_{13}} A}{k_8 + \frac{K''}{k_{13}} A}$$

was obtained through the intercept of plot of $1/\phi$ versus $1/[\text{RCHO}]$ shown in Figures 4.24 – 4.28. In the above ratios, constant $k_{13}$ represents the complex formation with triplet excited state and reductant molecule. In above ratio constant $k_{12}$ shows the dissociation of the complex molecule $\text{MBH}^{++}_2\text{T}. \text{RH}$, order of reaction determined here is first. The chance of deexcitation by release in thermal energy is maximum i.e. $k_9$ less effective than $k_8$ shows the reaction of protonated triplet state of dye with reductant molecule.

Thus

$$\frac{k_8 + k_9 + \frac{K''}{k_{12}} k_{12} + \frac{K'''}{k_{13}} A}{k_8 + \frac{K''}{k_{13}} A}$$

is important factor in controlling the quantum yield in the region of high acidity.
The ratio \( \frac{2}{\alpha} \cdot \frac{k_{10}}{k_8 + K'' k_{13} A} \) and \( \frac{2}{\alpha} \cdot \frac{k_9 + K'' k_{12}}{k_8 + K'' k_{13} A} \) were determined from the slopes of plots of \( I \phi \) versus \( 1/\text{RCHO} \). According to equation no. (30) the ratios of rate constants were obtained from the plots of \( I \phi \) versus \( 1/\text{h}_0 \) having

intercept \( \frac{2}{\alpha} \cdot \frac{k_{10} + K'' k_{12} + K'' k_{13} A}{k_8 + K'' k_{13} A} \) and

slope \( \frac{2}{\alpha} \cdot \frac{k_6}{K' (k_8 + K'' k_{13} A)} \).

The representative plots are given in the Figures 4.14 – 4.18. The values obtained by the slopes and intercepts are summarized in Table 41.

The ratio \( \frac{\alpha}{2} \cdot \frac{k_5}{K' (k_8 + K'' k_{13} A)} \) shows that steps of mechanism gives

the balance point between triplet excited and protonated triplet excited state of dye. The values of slopes indicated that reaction between triplet excited state and reductant is predominating and formation of leuco dye is taking place. It is assumed that step (7) and (12) are rate determining steps and deexcitation of dye is not taking place by releasing thermal energy. Therefore the rate constant \( k_{10} \) can be neglected and thus the

ratios will become \( \frac{2}{\alpha} \cdot \frac{k_8 + k_9 + K'' k_{12} + K'' k_{13} A}{k_8 + K'' k_{13} A} \).

In this ratio equilibrium constant \( K'' \) is appearing which shows the equilibrium state in between the MBH^{++}T and reductant molecule. This ratio shows the extent of contribution of the complex MBH^{++}RH in the quantum yield of this reduction. The value of \( K'' \) is given in table 45. According to equation (31), the plots between \( I \phi \) versus \( 1/\text{h}_0 \) drawn are shown in Figures 4.14 – 4.18 having

intercept \( \frac{2}{\alpha} \cdot \frac{K'' k_{13} A}{k_8 + K'' k_{13} A} \)

and slope \( \frac{2}{\alpha} \cdot \frac{k_6}{K' (k_8 + K'' k_{13} A)} \).

The ratio of rate constants shows quenching of complex with unexcited methylene blue.
molecule, where \( \frac{2}{\alpha} \cdot \frac{k_6}{K' (k_8 + K'' k_{13} A)} \) shows the ratio of rate constant of transfer of triplet state into ground state by releasing thermal energy. The life time of triplet transient species corresponds to 10.1 μs.

5.10.7 JUSTIFICATION OF THE PROPOSED MECHANISM:

The values of different rate constant and constant for equilibrium state for photoreduction of methylene blue with reducing sugars were calculated on the basis of assumptions given by Uddin [140], Kayser and Young [237] Faure and Dubein [238, 239], Rabinowitch [153], Solar et al [240-241]. The values of rate constants of photoreduction are summarized in Tables 43-45.

Following tests were put forward to justify the reaction mechanism.

The value of \( \frac{2}{\alpha} \) represents the minimum value of 1/ϕ when the concentration of the reductant is maximum. The minimum value of 1/ϕ as 2.0128 and the value of k₆ = 10⁵ s⁻¹ as reported by Parker [76] provided the concentration of methylene blue A = 1 × 10⁻⁶ mol dm⁻³. The value of equilibrium constant \( K' = 1 \times 10^{-6}, k_8 \) and \( k_{13} \) reported by Uddin [140] and Uddin et al [215] are 1.0 × 10¹¹ mol dm⁻³ s⁻¹ and 10¹⁰ respectively. The value of slope calculated by the plots of 1/ϕ vs 1/na as shown in Figures 4.14 – 4.18 is 6.05 × 10⁻⁸. Using above values the relation becomes as follows

\[
\frac{2}{\alpha} \cdot \frac{k_6}{K' (k_8 + K'' k_{13} A)} = 6.05 \times 10^{-8}
\]

\[
\frac{2 \cdot 10^5}{2.0128 \cdot 10^{-6} (10^{11} + K'' \times 10^{10} \times 10^{-6})} = 6.05 \times 10^{-8}
\]

\[
\frac{10^{11}}{10^{11} + K'' \times 10^4} = 6.05 \times 10^{-8} \times 1.0064
\]

\[
10^{11} = 6.088 \times 10^{-8} (10^{11} + K'' \times 10^4)
\]

\[
K'' = 1.642 \times 10^{14} \text{ mol dm}^{-3} \text{ s}^{-1}
\]

The values of \( K'' \) calculated for present work comes out to be 1.642 × 10¹⁴ mol dm⁻³ s⁻¹ which is according to the reported values by Uddin [140, 215] and Epstein and Rabinowitch [153].
5.10.8 Calculation and verification of rate constants:

By using all above values, the rate constant for the proposed mechanism can be obtained by different rate equations

\[ \frac{2}{\alpha} \cdot \frac{k_5}{K' \left( k_8 + K'' k_{13} A \right)} \]

\[ \frac{2}{\phi_1} \cdot \frac{k_6}{K' \left( k_8 + K'' k_{13} A \right)} \]

\[ \frac{2}{\phi_2} \cdot \frac{k_9}{K' \left( k_8 + K'' k_{13} A \right)} \]

\[ \frac{2}{\alpha} + \frac{2}{\alpha} \cdot \frac{k_9}{k_8 + K'' k_{13} A} \]

\[ \alpha \cdot \frac{k_{10}}{k_8 + K'' k_{13} A} \]

\[ \frac{2}{\alpha} + \frac{2}{\alpha} \cdot \frac{K'' k_{12}}{K'' k_{12}} \]

\[ \frac{2}{\alpha} \cdot \frac{K'' k_{13} A}{k_8 + K'' k_{13} A} \]

As \(( k_8 + K'' k_{13} A )\) is common in above expressions, the value can be obtained by putting all the given values

\[
\left( 10^{11} + 1.642 \times 10^{14} \times 10^{10} \times 10^6 \right) \\
\left( 10^{11} + 1.642 \times 10^{24} \times 10^6 \right) \\
\left( 10^{17} + 1.642 \times 10^{18} \right) \\
1.642 \times 10^{18}
\]
Calculation of rate constant for $k_5$

\[
\frac{2}{\alpha} \frac{k_5}{K' (k_8 + K'' k_{13} A)} = 3.01 \times 10^{-8}
\]

\[
\frac{2}{2.0128} \frac{10^5}{10^{-6} (1.624 \times 10^{18})} = 3.01 \times 10^{-8}
\]

\[
\frac{k_5}{10^{-6} (1.624 \times 10^{18})} = 3.01 \times 10^{-8} \times 1.0064
\]

\[
k_5 = 3.01 \times 10^{-8} \times 1.0064 \times 1.642 \times 10^{12} = 5.122 \times 10^4
\]

\[
k_5 = 5.122 \times 10^4 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}
\]

Calculation of rate constant for $k_6$

\[
\frac{2}{\alpha} \frac{k_6}{K' (k_8 + K'' k_{13} A)} = 6.05 \times 10^{-8}
\]

\[
\frac{2}{2.0128} \frac{k_6}{10^{-6} (1.624 \times 10^{18})} = 6.05 \times 10^{-8}
\]

\[
k_6 = 6.05 \times 10^{-8} \times 1.0064 \times 1.642 \times 10^{12}
\]

\[
k_6 = 9.99 \times 10^4 \text{ s}^{-1}
\]
Table 43
RATIOS OF RATE CONSTANT OF PHOTOREDUCTION OF METHYLENE BLUE WITH MONOSACCHARIDES

<table>
<thead>
<tr>
<th>Ratios</th>
<th>Galactose</th>
<th>D-Mannose</th>
<th>D(+)-Ribose</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \frac{2}{\alpha} \left( \frac{k_5 + k_7}{k_7} \right) )</td>
<td>4.3292 ± 0.01</td>
<td>3.8185 ± 0.12</td>
<td>3.4095 ± 0.1</td>
</tr>
<tr>
<td>( \frac{2}{\alpha} \left( \frac{k_6}{k_9} \right) \cdot 10^3 )</td>
<td>5.1145 ± 0.3</td>
<td>4.9801 ± 0.2</td>
<td>5.4742 ± 0.1</td>
</tr>
<tr>
<td>( \frac{2}{\alpha} \left( \frac{k_{10}}{k_8 + K''/k_{13}A} \right) \cdot 10^3 )</td>
<td>4.0171 ± 0.2</td>
<td>4.2998 ± 0.1</td>
<td>3.6658 ± 0.3</td>
</tr>
<tr>
<td>( \frac{2}{\alpha} \left( \frac{k_8 + k_9 + K''k_{12} + K''/k_{13}A}{k_8 + K''/k_{13}A} \right) )</td>
<td>2.2680 ± 0.15</td>
<td>2.1342 ± 0.01</td>
<td>2.0754 ± 0.11</td>
</tr>
<tr>
<td>( \frac{2}{\alpha} \left( \frac{k_5}{k_8 + K''/k_{13}A} \right) \cdot 10^6 )</td>
<td>2.4396 ± 0.5</td>
<td>1.9896 ± 1.1</td>
<td>2.3875 ± 0.8</td>
</tr>
<tr>
<td>( \frac{2}{\alpha} \left( \frac{k_8}{k_8 + K''/k_{13}A} \right) \cdot 10^6 )</td>
<td>0.0301 ± 0.01</td>
<td>0.1855 ± 0.03</td>
<td>0.0442 ± 0.01</td>
</tr>
<tr>
<td>( \frac{2}{\alpha} \left( \frac{k_6}{k_8 + K''/k_{13}A} \right) \cdot 10^6 )</td>
<td>0.0605 ± 0.01</td>
<td>0.4174 ± 0.02</td>
<td>0.0504 ± 0.01</td>
</tr>
<tr>
<td>( \frac{2}{\alpha} k_{13} A )</td>
<td>7.7413 ± 1.12</td>
<td>5.5890 ± 1.3</td>
<td>7.6344 ± 1.3</td>
</tr>
<tr>
<td>( \frac{k_7}{K' (k_8 + K''/k_{13}A)} \cdot 10^6 )</td>
<td>0.6264 ± 0.2</td>
<td>0.4891 ± 0.2</td>
<td>0.5308 ± 0.2</td>
</tr>
<tr>
<td>( \frac{k_7}{K' (k_8 + K''/k_{13}A)} \cdot 10^6 )</td>
<td>1.0261 ± 0.3</td>
<td>1.2003 ± 0.1</td>
<td>1.9265 ± 0.1</td>
</tr>
<tr>
<td>( \frac{k_8 + k_9 + K''/k_{12} + K''/k_{13}A}{K''/k_{13}A} )</td>
<td>0.3220 ± 0.01</td>
<td>0.3553 ± 0.01</td>
<td>0.31272 ± 0.01</td>
</tr>
</tbody>
</table>

(at low con. of red)

(at high con. of red)
### Table - 44

**RATIOS OF RATE CONSTANT OF PHOTOREDUCTION OF METHYLENE BLUE WITH DIASACCHARIDES**

<table>
<thead>
<tr>
<th>Ratios</th>
<th>Maltose</th>
<th>Lactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \frac{2}{\alpha} \cdot \frac{k_5 + k_7}{k_7} )</td>
<td>4.1835 ± 0.01</td>
<td>4.0843 ± 0.30</td>
</tr>
<tr>
<td>( \frac{2}{\alpha} \cdot \frac{k_6}{k_9} \cdot 10^3 )</td>
<td>6.1925 ± 0.20</td>
<td>5.7590 ± 0.10</td>
</tr>
<tr>
<td>( \frac{2}{\alpha} \cdot \frac{k_{10}}{k_8 + K''<em>{k</em>{13}} A} \cdot 10^4 )</td>
<td>3.9050 ± 0.30</td>
<td>4.2998 ± 0.20</td>
</tr>
<tr>
<td>( \frac{2}{\alpha} \cdot \frac{k_{12} + K'' k_{12} + K'' k_{13} A}{k_8 + K'' k_{13} A} )</td>
<td>2.2330 ± 0.01</td>
<td>2.1342 ± 0.20</td>
</tr>
<tr>
<td>( \frac{k_9 + k_9 + K'' k_{12} + K'' k_{13} A}{k_8 + K'' k_{13} A} )</td>
<td>2.2190 ± 0.90</td>
<td>2.2670 ± 0.70</td>
</tr>
<tr>
<td>( \frac{2}{\alpha} \cdot \frac{k_5}{K' (k_8 + K'' k_{13} A)} \cdot 10^6 )</td>
<td>0.0382 ± 0.03</td>
<td>0.0398 ± 0.01</td>
</tr>
<tr>
<td>( \frac{2}{\alpha} \cdot \frac{k_6}{K' (k_8 + K'' k_{13} A)} )</td>
<td>0.0816 ± 0.04</td>
<td>0.0710 ± 0.02</td>
</tr>
<tr>
<td>( \frac{2}{\alpha} \cdot \frac{K' k_{13} A}{k_8 + K'' k_{13} A} )</td>
<td>7.6322 ± 1.5</td>
<td>7.3184 ± 1.1</td>
</tr>
<tr>
<td>( \frac{\phi_1}{\phi_2} \cdot \frac{k_7}{K' (k_8 + K'' k_{13} A)} \cdot 10^3 )</td>
<td>0.5395 ± 0.20</td>
<td>0.2318 ± 0.20</td>
</tr>
<tr>
<td>( \frac{k_7}{K' (k_8 + K'' k_{13} A)} \cdot 10^6 )</td>
<td>0.8550 ± 0.10</td>
<td>0.2809 ± 0.10</td>
</tr>
<tr>
<td>( \frac{k_9 + k_9 + K'' k_{12} + K'' k_{13} A}{K'' k_{13} A} )</td>
<td>0.2907 ± 0.10</td>
<td>0.3097 ± 0.10</td>
</tr>
</tbody>
</table>
Calculation of rate constant for quenching rate of $\text{MB}_T^+$

$$\frac{\phi_1}{\phi_2} \frac{k_7}{K' (k_8 + K'' k_{13})} = 0.4968 \frac{k_7}{10^{-6} (1.624 \times 10^{18})} = 1.0261 \times 10^{-6}$$

$$k_7 = 1.0261 \times 10^{-6} \times 0.5581 \times 1.642 \times 10^{12}$$

$$k_7 = 9.403 \times 10^5 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$$

Calculation of rate constant for deexcitation of dye with reductant molecule i.e $k_9$

$$\frac{2}{\alpha} + \frac{2}{\alpha} \frac{k_9}{k_8 + K'' k_{13}} = \frac{2}{2.0128} + \frac{2}{2.0128} \cdot \frac{k_9}{1.642 \times 10^{18}} = 2.268$$

$$0.9936 k_9 = 2.268 \cdot 0.9936$$

$$k_9 = \frac{1.2744 \times 1.642 \times 10^{18}}{0.9936}$$

$$k_9 = 2.106 \times 10^{18} \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$$

Calculation of rate constant by releasing thermal energy i.e $k_{10}$

$$\frac{2}{\alpha} \frac{k_{10}}{k_8 + K' k_{15} A} = \frac{2}{2.0128} \frac{k_{10}}{(1.624 \times 10^{18})} = 4.0171 \times 10^{-3}$$

$$k_{10} = 4.0171 \times 10^{-3} \times 1.0064 \times 1.642 \times 10^{18}$$

$$k_{10} = 6.638 \times 10^{15} \text{ s}^{-1}$$
Calculation of rate constant for dissociation process of the complex MBH₇⁺⁺. RH
i.e. k₁₂

\[ \frac{2}{\alpha} + \frac{2}{\alpha} \cdot \frac{K'' k_{12}}{k_8 + K'' k_{13} A} = \frac{2.0128}{1.642 \times 10^{18}} \cdot \frac{1.642 \times 10^{14} k_{12}}{1.642 \times 10^{18}} = 2.268 - 0.9936 \]

\[ k_{12} = \frac{1.2744 \times 1.0064 \times 1.642 \times 10^{18}}{1.642 \times 10^{14}} \]

\[ k_{12} = 1.2825 \times 10^{4} \text{ s}^{-1} \]
<table>
<thead>
<tr>
<th>Specific rate constants</th>
<th>Reductant Monosaccharides</th>
<th>Reductant Diasaccharides</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_5$ (mol$^{-1}$ dm$^3$ s$^{-1}$)</td>
<td>$5.122 \times 10^4$</td>
<td>$4.4339 \times 10^4$</td>
</tr>
<tr>
<td>$k_6$ (s$^{-1}$)</td>
<td>$9.99 \times 10^4$</td>
<td>$9.4980 \times 10^4$</td>
</tr>
<tr>
<td>$k_7$ (mol$^{-1}$ dm$^3$ s$^{-1}$)</td>
<td>$9.403 \times 10^5$</td>
<td>$5.146 \times 10^5$</td>
</tr>
<tr>
<td>$k_9$ (mol$^{-1}$ dm$^3$ s$^{-1}$)</td>
<td>$4.99 \times 10^{18}$</td>
<td>$3.352 \times 10^{18}$</td>
</tr>
<tr>
<td>$k_{10}$ (s$^{-1}$)</td>
<td>$3.747 \times 10^{15}$</td>
<td>$2.599 \times 10^{15}$</td>
</tr>
<tr>
<td>$k_{12}$ (s$^{-1}$)</td>
<td>$3.0428 \times 10^3$</td>
<td>$2.841 \times 10^4$</td>
</tr>
<tr>
<td>$k_{13}$ (mol$^{-1}$ dm$^3$ s$^{-1}$)</td>
<td>$7.790 \times 10^{10}$</td>
<td>$7.750 \times 10^{10}$</td>
</tr>
</tbody>
</table>

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

| $K'$ | $0.914 \times 10^{-6}$ | $1.237 \times 10^{-6}$ |
| $K''$ | $1.642 \times 10^{14}$ | $1.180 \times 10^{14}$ |
Calculation of rate constant of disproportionation of complex i.e k₁₃

\[ \frac{2}{\alpha} \cdot \frac{K'' k₁₃ A}{k₈ + K'' k₁₃ A} \]

Substituting the value

\[ \frac{2}{2.0128} \cdot \frac{k₁₃ \times 1.642 \times 10^{14} \times 1 \times 10^{-6}}{1.642 \times 10^{18}} = 7.7413 \]

\[ k₁₃ = \frac{7.7413 \times 1.8064 \times 1.642 \times 10^{18}}{1.642 \times 10^{8}} \]

\[ k₁₃ = 7.790 \times 10^{10} \text{ mol}^{-1} \text{ dm}^{3} \text{ s}^{-1} \]

Calculation of equilibrium constant K'

\[ \frac{k₇}{K' (k₈ + K'' k₁₃ A)} \]

By putting the values

\[ \frac{k₇}{K' (k₈ + K'' k₁₃ A)} = 6.264 \times 10^{-7} \]

Calculation

\[ K' = \frac{9.403 \times 10^5}{6.264 \times 10^{-7} \times 1.642 \times 10^{18}} \]

\[ K' = 9.14 \times 10^{-7} \]

or \[ K' = 0.914 \times 10^6 \]

5.11 INFLUENCE OF TEMPERATURE ON PHOTOCHEMICAL REDUCTION OF METHYLENE BY REDUCING SUGARS

The effects of temperature on photochemical reactions are quite different from those thermal reactions. For the later an increase of 10⁰ C in temperature generally leads to two or three fold increase in the rate. In photochemical reaction, however, the same increase in temperature results as a rule in only a very small increase in the rate. For most reactions the temperature coefficient is not far from unity.
In present work, the influence of temperature from 20°C to 45°C on quantum yield of photoreduction of methylene at concentration of dye $1 \times 10^{-6}$ mol dm$^{-3}$ and reductant concentration $8 \times 10^{-4}$ mol dm$^{-3}$ was studied. It was found that quantum yield in inert reaction mixture along with reducing sugar is affected by change in temperature. The average value of temperature coefficient is found to be 1.150 and 1.184 at two different acidities i.e. $H_o = 4.2$ and 7.7 for Galactose. The quantum yield of photoreduction of methylene blue with monosaccharides and disaccharides increases gradually as temperature rises in similar way. The difference in values could be correlated with structural difference of OH group of reducing sugars [227]. The lower value of quantum yield at $[H_o]$ 7.7 is also in accordance with the other studies [242-246]. The values of intercept and slope of Arrhenius plots are tabulated in Table 46.
Table - 46

THE VALUES OF INTERCEPTS AND SLOPES OBTAINED BY ARRHENIUS
PLOT FOR PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE IN
PERESENCES OF REDUCING SUGAR

\[
\begin{align*}
[RCHO] &= 8 \times 10^{-4} \text{ mol dm}^{-3}, & [MB] &= 1 \times 10^{-6} \text{ mol dm}^{-3}, \\
\text{Buffer} &= \text{Acetate} & \text{Solvent} &= 50\% \text{ aqueous methanol}
\end{align*}
\]

<table>
<thead>
<tr>
<th>Reductants</th>
<th>Intercepts</th>
<th>Slope $\times 10^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose</td>
<td>2.9291 ± 0.01</td>
<td>-1.1140 ± 0.002</td>
</tr>
<tr>
<td>4.2</td>
<td>2.7501 ± 0.01</td>
<td>-1.1364 ± 0.003</td>
</tr>
<tr>
<td>7.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Mannose</td>
<td>2.7291 ± 0.01</td>
<td>-1.0395 ± 0.001</td>
</tr>
<tr>
<td>4.2</td>
<td>3.314 ± 0.01</td>
<td>-1.2764 ± 0.01</td>
</tr>
<tr>
<td>7.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-(+)-Ribose</td>
<td>3.2635 ± 0.01</td>
<td>-1.2058 ± 0.02</td>
</tr>
<tr>
<td>4.2</td>
<td>3.3672 ± 0.01</td>
<td>-1.2994 ± 0.01</td>
</tr>
<tr>
<td>7.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maltose</td>
<td>3.2583 ± 0.01</td>
<td>-1.2125 ± 0.01</td>
</tr>
<tr>
<td>4.2</td>
<td>3.4557 ± 0.01</td>
<td>-1.3314 ± 0.01</td>
</tr>
<tr>
<td>7.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactose</td>
<td>3.7719 ± 0.01</td>
<td>-1.3710 ± 0.01</td>
</tr>
<tr>
<td>4.2</td>
<td>3.9532 ± 0.01</td>
<td>-1.4809 ± 0.01</td>
</tr>
<tr>
<td>7.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5.12 THERMODYNAMICS ACTIVATION PARAMETERS OF
PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE
WITH REDUCING SUGARS

Thermodynamics activation parameters were determined at different temperatures and at
two different acidities of reaction mixture. As the rate of reaction in terms of quantum
yield depends on concentration of reductants i.e. reducing sugars, the temperature
dependence of photoreduction can be determined by Arrhenius relation [246].

\[ k = A e^{-E_a/RT} \]

or

\[ \phi = A e^{-E_a/RT} \]

In photoreduction of methylene blue quantum yield \( \phi \) is taken in place of \( k \) i.e. rate of
reaction, \( A \) is frequency factor, \( E_a \) is the energy of activation, \( T \) is absolute temperature in
K°, and \( R \) is the gas constant. The plots of log \( \phi \) against 1/T in presence of reducing
sugars and at different acidities 4.2 and 7.7 are shown in Figures 4.30 – 4.34. The plots
are linear over the accessible temperature range. The Arrhenius parameters \( E_a \) and \( A \) were
determined from the straight line equation of plot. The values of \( E_a \) are summarized in
Table 47. The values of thermodynamics quantities like \( \Delta H^* \) activation enthalpy change,
\( \Delta S^* \) activation entropy change and \( \Delta G^* \) free energy change of activation calculated by
usual relations [246] are tabulated in Tables 48 – 53. Enthalpy change (\( \Delta H^* \)) is related to
the potential energy barrier for the reaction.

\[ \Delta H^* = E_a - RT \]

The larger value of enthalpy change shows the slower rate of reaction. In the
interpretation of the pre-exponential factor [A]

\[ A = \frac{KT e^{\Delta S^*/R}}{h} \]

By the transition state theory, a small factor indicates an improbable complex as the
values of \( \Delta S^* \) obtained were negative from the above relation, where \( k \) is Boltzmann's
constant, \( h \) is the Planck's constant:

Free energy change of activation (\( \Delta G^* \)) is determined by relation

\[ \Delta S^* = \frac{\Delta H^* - \Delta G^*}{T} \]
<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>Change in Enthalpy of activation [$\Delta H^*$] (KJ mol$^{-1}$)</th>
<th>Change in free entropy of activation [$\Delta S^*$] (JK$^{-1}$ mol$^{-1}$)</th>
<th>Change in free energy of activation [$\Delta G^*$] (KJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>293</td>
<td>17.46 ± 1.1</td>
<td>-26.32 ± 1.2</td>
<td>25.88 ± 0.1</td>
</tr>
<tr>
<td>298</td>
<td>17.42 ± 1.0</td>
<td>-26.33 ± 1.3</td>
<td>25.27 ± 0.2</td>
</tr>
<tr>
<td>303</td>
<td>17.38 ± 0.9</td>
<td>-26.35 ± 1.2</td>
<td>25.37 ± 0.1</td>
</tr>
<tr>
<td>308</td>
<td>17.34 ± 0.8</td>
<td>-26.37 ± 1.1</td>
<td>25.46 ± 0.1</td>
</tr>
<tr>
<td>313</td>
<td>17.30 ± 0.2</td>
<td>-26.38 ± 1.0</td>
<td>25.56 ± 0.1</td>
</tr>
<tr>
<td>318</td>
<td>17.26 ± 0.7</td>
<td>-26.40 ± 1.3</td>
<td>25.65 ± 0.3</td>
</tr>
</tbody>
</table>

$H_0 = 4.2$

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>Change in Enthalpy of activation [$\Delta H^*$] (KJ mol$^{-1}$)</th>
<th>Change in free entropy of activation [$\Delta S^*$] (JK$^{-1}$ mol$^{-1}$)</th>
<th>Change in free energy of activation [$\Delta G^*$] (KJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>293</td>
<td>22.00 ± 0.2</td>
<td>-26.12 ± 0.9</td>
<td>29.65 ± 0.1</td>
</tr>
<tr>
<td>298</td>
<td>21.96 ± 0.1</td>
<td>-26.14 ± 0.8</td>
<td>29.75 ± 0.1</td>
</tr>
<tr>
<td>303</td>
<td>21.92 ± 0.2</td>
<td>-26.16 ± 0.9</td>
<td>29.84 ± 0.1</td>
</tr>
<tr>
<td>308</td>
<td>21.87 ± 0.1</td>
<td>-26.17 ± 0.9</td>
<td>29.94 ± 0.1</td>
</tr>
<tr>
<td>313</td>
<td>21.83 ± 0.1</td>
<td>-26.19 ± 0.9</td>
<td>30.03 ± 0.1</td>
</tr>
<tr>
<td>318</td>
<td>21.79 ± 0.1</td>
<td>-26.21 ± 0.1</td>
<td>30.13 ± 0.1</td>
</tr>
</tbody>
</table>

$H_0 = 7.7$
### Table 50

**THERMODYNAMICS ACTIVATION PARAMETERS FOR PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE WITH REDUCTANT D-(+)-RIBOSE AT DIFFERENT TEMPERATURE AND ACIDITY [H0]**

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>Change in Enthalpy of activation [ΔH°′] (KJ mol⁻¹)</th>
<th>Change in free entropy of activation [ΔS°′] (JK⁻¹ mol⁻¹)</th>
<th>Change in free energy of activation [ΔG°′] (KJ mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>293</td>
<td>20.65 ± 0.1</td>
<td>-26.14 ± 1.0</td>
<td>28.31 ± 0.1</td>
</tr>
<tr>
<td>298</td>
<td>20.61 ± 0.2</td>
<td>-26.16 ± 0.9</td>
<td>28.44 ± 0.2</td>
</tr>
<tr>
<td>303</td>
<td>20.56 ± 0.1</td>
<td>-26.17 ± 0.3</td>
<td>28.58 ± 0.2</td>
</tr>
<tr>
<td>308</td>
<td>20.52 ± 0.2</td>
<td>-26.19 ± 0.4</td>
<td>28.71 ± 0.01</td>
</tr>
<tr>
<td>313</td>
<td>20.48 ± 0.4</td>
<td>-26.26 ± 0.5</td>
<td>28.87 ± 0.1</td>
</tr>
<tr>
<td>318</td>
<td>20.44 ± 0.4</td>
<td>-26.28 ± 0.1</td>
<td>29.00 ± 0.1</td>
</tr>
</tbody>
</table>

**H0 = 4.2**

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>Change in Enthalpy of activation [ΔH°′] (KJ mol⁻¹)</th>
<th>Change in free entropy of activation [ΔS°′] (JK⁻¹ mol⁻¹)</th>
<th>Change in free energy of activation [ΔG°′] (KJ mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>293</td>
<td>22.44 ± 0.2</td>
<td>-25.11 ± 0.7</td>
<td>30.09 ± 0.04</td>
</tr>
<tr>
<td>298</td>
<td>22.40 ± 0.3</td>
<td>-25.13 ± 0.2</td>
<td>30.23 ± 0.02</td>
</tr>
<tr>
<td>303</td>
<td>22.36 ± 0.1</td>
<td>-25.14 ± 0.3</td>
<td>30.36 ± 0.01</td>
</tr>
<tr>
<td>308</td>
<td>22.31 ± 0.2</td>
<td>-26.16 ± 0.1</td>
<td>30.50 ± 0.01</td>
</tr>
<tr>
<td>313</td>
<td>22.27 ± 0.1</td>
<td>-26.17 ± 0.1</td>
<td>30.63 ± 0.1</td>
</tr>
<tr>
<td>318</td>
<td>22.23 ± 0.2</td>
<td>-26.19 ± 0.1</td>
<td>30.77 ± 0.02</td>
</tr>
</tbody>
</table>

**H0 = 7.7**
<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>Change in Enthalpy of activation [\Delta H^*] (KJ mol(^{-1}))</th>
<th>Change in free entropy of activation [\Delta S^*] (JK(^{-1})mol(^{-1}))</th>
<th>Change in free energy of activation [\Delta G^*] (KJ mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>293</td>
<td>20.82 ± 0.08</td>
<td>-26.14 ± 0.05</td>
<td>28.48 ± 0.01</td>
</tr>
<tr>
<td>298</td>
<td>20.78 ± 0.07</td>
<td>-26.16 ± 0.01</td>
<td>28.44 ± 0.01</td>
</tr>
<tr>
<td>303</td>
<td>20.74 ± 0.02</td>
<td>-26.18 ± 0.02</td>
<td>28.67 ± 0.01</td>
</tr>
<tr>
<td>308</td>
<td>20.70 ± 0.01</td>
<td>-26.19 ± 0.09</td>
<td>28.77 ± 0.10</td>
</tr>
<tr>
<td>313</td>
<td>20.66 ± 0.01</td>
<td>-26.21 ± 0.05</td>
<td>28.86 ± 0.10</td>
</tr>
<tr>
<td>318</td>
<td>20.62 ± 0.02</td>
<td>-26.23 ± 0.01</td>
<td>28.96 ± 0.10</td>
</tr>
</tbody>
</table>

\[\text{H}_0 = 4.2\]

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>Change in Enthalpy of activation [\Delta H^*] (KJ mol(^{-1}))</th>
<th>Change in free entropy of activation [\Delta S^*] (JK(^{-1})mol(^{-1}))</th>
<th>Change in free energy of activation [\Delta G^*] (KJ mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>293</td>
<td>23.05 ± 0.01</td>
<td>-26.09 ± 0.01</td>
<td>30.70 ± 0.02</td>
</tr>
<tr>
<td>298</td>
<td>23.01 ± 0.01</td>
<td>-26.10 ± 0.07</td>
<td>30.79 ± 0.01</td>
</tr>
<tr>
<td>303</td>
<td>22.97 ± 0.02</td>
<td>-26.12 ± 0.04</td>
<td>30.88 ± 0.01</td>
</tr>
<tr>
<td>308</td>
<td>22.93 ± 0.03</td>
<td>-26.14 ± 0.01</td>
<td>30.98 ± 0.01</td>
</tr>
<tr>
<td>313</td>
<td>22.98 ± 0.02</td>
<td>-26.15 ± 0.01</td>
<td>31.07 ± 0.01</td>
</tr>
<tr>
<td>318</td>
<td>22.84 ± 0.01</td>
<td>-26.17 ± 0.01</td>
<td>31.17 ± 0.06</td>
</tr>
</tbody>
</table>

\[\text{H}_0 = 7.7\]
<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>Change in Enthalpy of activation ($\Delta H^*$) (KJ mol$^{-1}$)</th>
<th>Change in free entropy of activation ($\Delta S^*$) (JK$^{-1}$ mol$^{-1}$)</th>
<th>Change in free energy of activation ($\Delta G^*$) (KJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>293</td>
<td>23.81 ± 0.01</td>
<td>-26.03 ± 0.01</td>
<td>31.43 ± 0.01</td>
</tr>
<tr>
<td>298</td>
<td>23.77 ± 0.01</td>
<td>-26.02 ± 0.02</td>
<td>31.52 ± 0.2</td>
</tr>
<tr>
<td>303</td>
<td>23.73 ± 0.02</td>
<td>-26.03 ± 0.1</td>
<td>31.62 ± 0.1</td>
</tr>
<tr>
<td>308</td>
<td>23.68 ± 0.09</td>
<td>-26.05 ± 0.1</td>
<td>31.71 ± 0.1</td>
</tr>
<tr>
<td>313</td>
<td>23.64 ± 0.08</td>
<td>-26.06 ± 0.2</td>
<td>31.87 ± 0.2</td>
</tr>
<tr>
<td>318</td>
<td>23.60 ± 0.02</td>
<td>-26.08 ± 0.2</td>
<td>31.90 ± 0.1</td>
</tr>
<tr>
<td>293</td>
<td>25.91 ± 0.01</td>
<td>-25.95 ± 0.1</td>
<td>33.52 ± 0.04</td>
</tr>
<tr>
<td>298</td>
<td>25.87 ± 0.1</td>
<td>25.97 ± 0.12</td>
<td>33.61 ± 0.07</td>
</tr>
<tr>
<td>303</td>
<td>25.83 ± 0.02</td>
<td>-25.98 ± 0.03</td>
<td>33.71 ± 0.01</td>
</tr>
<tr>
<td>308</td>
<td>25.79 ± 0.03</td>
<td>-26.00 ± 0.01</td>
<td>33.80 ± 0.01</td>
</tr>
<tr>
<td>313</td>
<td>25.75 ± 0.04</td>
<td>-26.02 ± 0.02</td>
<td>33.89 ± 1.1</td>
</tr>
<tr>
<td>318</td>
<td>25.71 ± 0.01</td>
<td>-26.03 ± 0.08</td>
<td>33.99 ± 1.1</td>
</tr>
<tr>
<td>Reductants</td>
<td>Activation Parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>-----------------------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td></td>
<td>$E_a$ (KJ mol$^{-1}$)</td>
<td>$\Delta H^*$ (KJ mol$^{-1}$)</td>
<td>$\Delta S^*$ (JK mol$^{-1}$)</td>
</tr>
<tr>
<td>Galactose</td>
<td>21.33 ± 0.51</td>
<td>18.85 ± 0.3</td>
<td>-26.26 ± 1.1</td>
</tr>
<tr>
<td>D-mannose</td>
<td>19.90 ± 0.61</td>
<td>17.42 ± 1.0</td>
<td>-26.33 ± 1.3</td>
</tr>
<tr>
<td>D-(+)-Ribose</td>
<td>23.08 ± 0.1</td>
<td>20.61 ± 0.2</td>
<td>-26.16 ± 0.9</td>
</tr>
<tr>
<td>Maltose</td>
<td>23.26 ± 0.1</td>
<td>20.78 ± 0.2</td>
<td>-26.16 ± 0.1</td>
</tr>
<tr>
<td>Lactose</td>
<td>26.25 ± 0.1</td>
<td>23.77 ± 0.1</td>
<td>-26.02 ± 0.1</td>
</tr>
<tr>
<td>$H_0 = 4.2$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>21.75 ± 0.51</td>
<td>19.28 ± 0.2</td>
<td>-26.33 ± 1.3</td>
</tr>
<tr>
<td>D-mannose</td>
<td>24.44 ± 0.07</td>
<td>21.96 ± 0.1</td>
<td>-26.14 ± 0.8</td>
</tr>
<tr>
<td>D-(+)-Ribose</td>
<td>24.88 ± 0.2</td>
<td>22.40 ± 0.3</td>
<td>-25.13 ± 0.2</td>
</tr>
<tr>
<td>Maltose</td>
<td>25.49 ± 0.1</td>
<td>23.01 ± 0.3</td>
<td>-26.10 ± 0.1</td>
</tr>
<tr>
<td>Lactose</td>
<td>28.35 ± 0.1</td>
<td>25.87 ± 0.1</td>
<td>-25.973 ± 0.1</td>
</tr>
<tr>
<td>$H_0 = 7.7$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
\[ \Delta G^* = \Delta H^* - T \Delta S^* \]

where \( \Delta G^* \) is change in free energy of activation, \( \Delta H^* \) is the enthalpy change of activation, \( \Delta S^* \) is the entropy change of activation. It was observed from the values of energy of activation that at high acidity \( H_o = 4.2 \), the value of \( E_a \) is less as compared to that at low acidity region \( H_o = 7.7 \). At \( H_o = 7.7 \). An increase in the values of \( E_a \) indicate that reaction is slower at acidity of \( H_o = 7.7 \). The value of energy of activation \( E_a \) for Galactose and D-Mannose are \( 21.33 \pm 0.51 \) kJ mol\(^{-1}\) and \( 19.90 \pm 0.61 \) kJ mol\(^{-1}\) at acidity \( H_o = 4.2 \).

The difference of values can be interpreted in term of structural difference of the two monosaccharides. The value of D-(+)-Ribose is \( 23.08 \pm 0.1 \) at \( H_o = 4.2 \) which may be due to the fact that one carbon atom is less in Ribose as compared to other monosaccharides. The value of energy of activation of Maltose and Lactose at \( H_o = 4.2 \) are \( 23.26 \pm 0.01 \) and \( 26.25 \pm 0.1 \) respectively. The difference in values of two disaccharides could be the difference in rate of hydrolysis of two disaccharides and their structural difference of hydrolysis products. It was observed from the results that there is no remarkable difference in the values of activation parameters of reducing sugar, indicating that photoreduction of methylene blue belongs to oxidation of carbonyl group \( \overset{\text{C}}{\overset{\text{O}}{\text{C}} = \text{O}} \) and difference is due to difference in position of \( \text{OH}^+ \) groups attached at \( C \) atom of mono, and disaccharides. [227,233-236]

The lower value of \( \Delta H^* \) enthalpy change of activation show that reduction of methylene blue in presence of reducing sugars is fast reaction at acidity 4.2. The values of entropy of activation at 4.2 are higher as compared to that at \( H_o = 7.7 \) which show that reduction is more favorable at acidity 4.2.
5.13 COMPARISON WITH EARLIER WORK

The results of photoreduction of methylene blue in presence of reducing sugars can be compared with earlier work in many respects. The results are compared with respect to influence of nitrogen, acidity, dye concentration, reductant concentration and temperature, rate of the chemical reaction in term of quantum yield. Present work was not reported for the photoreduction of methylene blue in presences of carbohydrates in 50% aqueous methanol with acetate buffer, although a lot of research work was carried out on photoreduction of methylene in presence of organic and inorganic reductants. [257-303]. Therefore present work can be compared to the photoreduction of methylene blue and thiazine dye.

5.13.1 MOLAR ABSORPTIVITY COEFFICIENT

Bonneau and Dubien [57] studied the photolysis of methylene blue in neutral solution in the presence of paraphenyl diamine as a reductant and observed the formation of semiquinone at $\lambda_{max} = 880$ nm and $\varepsilon_{380} = 17000$ m$^2$ mol$^{-1}$. Solar et al [241] studied the kinetics of multiple H-attack on methylene blue in aqueous solution by flash photolysis for semiquinone $\lambda_{max} = 380$ nm and $\varepsilon_{380} = 740$ m$^2$ mol$^{-1}$ at pH = 2 and the rate constant for disproportionation was obtained as $2k = 1.5 \times 10^9$ mol$^{-1}$ S$^{-1}$. The photoreduction of thionine with anethole and alkylthiourea was investigated by Pestemer [86] and Sawkar [80]. Koiizumi et al [23] studied the photoreduction of thiazine dyes and found the $\lambda_{max}$ lies at 275 and 650 nm for methylene green in aqueous solution and rate increases in buffer. The quantum yield of bleaching was $3 \times 10^4$. They concluded that in suitable cases the photoreduction process of thiazine dyes is simple second order with regard to the dye. Uddin et al [215] observed $\lambda_{max}$ for photochemical reduction of methylene blue with trimethylamine in absolute methanol was 656 nm and $\varepsilon_{556} = 4 \times 10^4$ mol$^{-1}$ dm$^3$ cm$^{-1}$. Photochemical reduction of methylene blue in 50% isopropanol with allylurea in acetate buffer was studied by Khalid [242] and reported $\lambda_{max} = 660$ nm and $\varepsilon_{660} = 6.533 \times 10^4$ mol$^{-1}$ dm$^3$ cm$^{-1}$.

Present studies were carried out for photochemical reduction of methylene blue at concentration of $1 \times 10^{-6}$ mol dm$^{-3}$ in 50% methanol at $\lambda_{max} = 661$ nm and $\varepsilon_{661} = 8.21 \times 10^4$ mol$^{-1}$ dm$^3$ cm$^{-1}$. 
Table - 54

A COMPARISON OF $\lambda_{\text{max}}$ and $\varepsilon_{\text{max}}$ OF METHYLENE IN 50% AQUEOUS METHANOL WITH EARLIER WORKERS

<table>
<thead>
<tr>
<th>Dye</th>
<th>Solvent</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>$\varepsilon_{\text{max}}$ (mol$^{-1}$ dm$^3$ cm$^{-1}$)</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylene blue</td>
<td>Neutral solution</td>
<td>880</td>
<td>17000</td>
<td>[57]</td>
</tr>
<tr>
<td>Methylene blue</td>
<td>Water</td>
<td>380</td>
<td>740</td>
<td>[241]</td>
</tr>
</tbody>
</table>
| Thiazone        | Water                  | 275
650
|               |                        |                            | [22]                                                   |
| Methylene blue  | Absolute methanol      | 656                        | $4 \times 10^4$                                         | [215]      |
| Methylene blue  | 50% isopropanol        | 660                        | $6.533 \times 10^4$                                      | [242]      |
| Methylene green | 50% isopropanol        | 657.5                      | $1.400 \times 10^5$                                      | [247]      |
| Methylene blue  |                        | 665                        | $8.16 \times 10^4$                                      | [78]       |
| Methylene blue  |                        | 420                        | $2.25 \times 10^4$                                      | [237]      |
| Methylene blue  | H$_2$O                 | 665                        | $8.400 \times 10^4$                                      | [Present]  |
| Methylene blue  | Absolute methanol      | 653                        | $3.59 \times 10^5$                                      | [Present]  |
| Methylene blue  | 50% aqueous methanol   | 661                        | $8.21 \times 10^4$                                      | [Present]  |

5.13.2 A COMPARISON OF INERT ATMOSPHERE

Methylene blue shows blue color in the presence of oxygen, therefore, it is necessary to carry out its photoreduction in deoxygenated atmospheric system by creating inert atmosphere by passing nitrogen through the reaction mixture. Earlier workers reported the time of passing of nitrogen for complete deoxygenated system [144] that an inert atmosphere is necessary for photoreduction of methylene blue with trimethylamine in acetate buffer solution. He founds that a complete inert system required forty minutes for
purgning nitrogen in the reaction mixture. Koizumi et al [23] and Koizumi and Usui [206] found that methylene blue in suitable buffer solution without any reducing agent is photoreduced in deoxygenated atmosphere [248]. Kato et al [249] reported that methylene blue is photoreduced in inert phosphate buffer solution. Present work shows that maximum quantum yield was obtained for photoreduction of methylene blue with reducing sugars in inert atmosphere. Upon exposure to light, blue color disappears. It was also observed that maximum quantum yield was obtained when time of passing of nitrogen was forty minutes which is comparable with earlier researchers. [22, 215, 250]

5.13.3 A COMPARISON OF PHOTOCHEMICAL KINETICS OF METHYLENE BLUE WITH EARLIER SIMILAR REACTIONS

The maximum quantum yield obtained in present photochemical reduction of methylene blue with reducing sugars in acetate buffer for monosaccharides and disaccharides are 0.496 ± 0.001 and 0.516 ± 0.002 respectively at acidity $H_0 = 4.2$, and 0.277 ± 0.003 and 0.245±0.002 at $H_0 = 7.7$ respectively. These values may be compared with the values obtained by Uddin [140] for photochemical reduction of methylene blue with triethylamine (0.446) and (0.35) and thionine with N-Phenylglycine.

However the Pestemer [86] reported $\phi$ as 0.27 for the reaction of thionine with allythiourea. The maximum quantum yield obtained by Sawkar [80] and Ahmed [188] for the reaction of thionine with allythiourea and anethole were 0.22 and 0.458 respectively which is also comparable with the present work. The values of quantum yield for photochemical reduction of methylene blue with reducing sugars at different acidities are slightly different with each other showing that proton transfer reaction takes place from aldehydic group in each sugar.

The linear plots of $\phi_1 - \phi / \phi \sim \phi_2$ versus $1/H_0$ passing through origin obtained in the present work are comparable to those obtained by Uddin [135, 140]. The plots of $\phi$ against $H_0$ are similar to those reported by Uddin [215], Pestemer [86] and Ahmed [188]. The comparisons of straight line plots of $1/\phi$ against $1/H_0$ are also similar as reported by Pestemer [86], Ahmed [188] and Uddin and Hasnain [232]. The influence of change in concentration of reductants also found to be similar as reported by earlier worker [244]. The variations of quantum yield with acidities were also verified by the Swakar [80] and Uddin [232]. While Matsumoto [78] and Ahmed [188] reported that quantum yield is
independent upon the concentration of dye which was also observed in the present work. The quantum yield of photoreduction of methylene blue in absence of reducing sugar with acetate buffer in 50% aqueous methanol was $8.012 \times 10^{-3}$ which may be compared with quantum yield [250] in phosphate buffer with water as solvent as $9.2 \times 10^{-4}$ and borate buffer in water as $3.0 \times 10^{-4}$. The life time of triplet transient specie in present work in 50% aqueous methanol was found to be $10.1 \pm 0.1 \mu s$ which is comparable with $11 \pm 2 \mu s$ for methanol as reported earlier [72] in methanol and 14 $\mu s$ in acetonitrile [70].

5.13.4 COMPARISON OF EFFECT OF TEMPERATURE WITH EARLIER WORKER

Chibisov et al [251] studied the effect of temperature on the reduction of methylene blue with paraphenyl diamine and determined the temperature coefficient 1.26 which is comparable with present photochemical reduction of methylene blue with reducing sugars as (1.16 and 1.15). The value obtained by Uddin was approximately equal to 1.3 in the reaction of thionine and thiourea [140]. Similarly Kostryukova [219] reported the value as 1.2 for the reaction of thionine and thiourea, which is comparable with present work as 1.2 for methylene blue and Lactose.

Vonnach and Getoff [186], Fausto et al [246] obi and Makino [172] studied the temperature effect on the rate of reaction. The influence of temperature on the reaction of thionine with thiourea and alkylthiourea was also found by Sternes et al [252]. They found that the number of effective collision of excited thionine with the reductant increases with the rise in temperature and concentration of reductants. Present work shows the influence of temperature on photochemical reduction of methylene blue by reducing sugars and revealed that reduction increased by increase in temperature which results in the increase of quantum yield for photoreduction of methylene blue. The values of temperature coefficients for photochemical reduction of methylene blue in presence of monosaccharides at $H_o = 4.2$ is 1.1508 and 1.1841 at $H_o = 7.7$. For diaaccharides 1.1647 at $H_o = 4.2$ and 1.1704 for $H_o = 7.7$.

5.13.5 A COMPARISON OF ACTIVATION PARAMETERS

In the present work an attempt has been made to evaluate the activation parameters for the photoreduction of methylene blue with reducing sugars in presence of acetate buffer in 50% aqueous methanol. Thermodynamics activation parameters were determined at two different acidities $H_o = 4.2$ and 7.1 at reductant concentration of $8 \times 10^{-4}$ mol $dm^{-3}$. A
investigation also reveals the same pattern and ratios of rate constant were determined and found in the similar patterns as mentioned by earlier scientists.

**Table - 55**

**COMPARISON OF THE VALUE OF RATE CONSTANTS FOR PHOTOCHEMICAL REDUCTION OF METHYLENE BLUE BY REDUCING SUGARS WITH LITERATURE VALUES FOR THIAZINE DYES WITH REDUCTANTS**

<table>
<thead>
<tr>
<th>Sp. rate constants</th>
<th>Literature value</th>
<th>Value for proposed mechanism (present work)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_3 ) (mol(^{-1}) dm(^3) s(^{-1}))</td>
<td>( 9.5 \times 10^7 ) [215] ( 6.26 \times 10^5 ) [135], ( 3.2 \times 10^8 ) [144], ( 5.0 \times 10^6 ) [215]</td>
<td>( 5.122 \times 10^4 )</td>
</tr>
<tr>
<td>( k_6 ) (s(^{-1}))</td>
<td>( 10^5 ) [239] ( 4.0 \times 10^4 ) [145], ( 7.0 \times 10^6 ) [116]</td>
<td>( 9.99 \times 10^4 )</td>
</tr>
<tr>
<td>( k_7 ) (mol(^{-1}) dm(^3) s(^{-1}))</td>
<td>( 3.0 \times 10^7 ) [215] ( 3.35 \times 10^6 ) [135], ( 10^6 ) [116]</td>
<td>( 9.403 \times 10^5 )</td>
</tr>
<tr>
<td>( k_9 ) (mol(^{-1}) dm(^3) s(^{-1}))</td>
<td>( 7.85 \times 10^{17} ) [144]</td>
<td>( 2.106 \times 10^{18} )</td>
</tr>
<tr>
<td>( k_{10} ) (s(^{-1}))</td>
<td>( 2.75 \times 10^{15} ) [144]</td>
<td>( 6.63 \times 10^{15} )</td>
</tr>
<tr>
<td>$k_{12}$ (s$^{-1}$)</td>
<td>$9.99 \times 10^3$ [144]</td>
<td>$1.2825 \times 10^4$</td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>$k_{13}$ (mol$^{-1}$ dm$^3$ s$^{-1}$)</td>
<td>$10^9$ [256]</td>
<td>$7.790 \times 10^{10}$</td>
</tr>
</tbody>
</table>

**Equilibrium Constants**

<table>
<thead>
<tr>
<th>$K'$</th>
<th>$10^{-6}$ [144]</th>
<th>$0.914 \times 10^{-6}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K''$</td>
<td>$10^{14}$ [215]</td>
<td>$1 \times 10^{14}$ [144]</td>
</tr>
</tbody>
</table>
5.14 CONCLUSIONS

Present investigation deals with kinetics of photochemical reduction of methylene blue with reducing sugars such as Galactose, D-Mannose, D-(+)-Ribose, Maltose and Lactose in 50% aqueous methanol with acetate buffer, as these reductant were not studied yet, in acidity region $\text{H}^0 = 4.2$ and 7.7. Experimental results were obtained under concentration of the influence of different parameters like concentration of dye, reductants, acidity of reaction mixture and temperature. Mechanism was Proposed and an attempt was also be made to analyse the reaction mixture. It was concluded that mechanism of the reaction shows following important steps of photoreduction.

1) Reaction takes place in between the triplet excited state and aldehydic group of reducing sugars.

$$\text{MBH}^{++}_T + \text{RH} \rightleftharpoons \text{MBH}^{++}_T . \text{RH}$$

2) Conversion of protonated triplet excited state methylene blue and reductant takes place into leuco dye through electron transfer process and hydrogen transfer process.

$$\text{HOCH}_2(\text{CHOH})_4\text{CHO} + 3\text{OH}^- \rightleftharpoons \text{HOCH}_2(\text{CHOH})_4\text{CO}_2^- + 2\text{H}_2\text{O} + 2\text{e}^-$$

3) Two equilibrium states have been established with solvent and dye molecule.

$$\text{MB}^+_T + \text{H}^+ \rightleftharpoons \text{MBH}^{++}_T$$

$$\text{MBH}^{++}_T + \text{RH} \rightleftharpoons \text{MBH}^{++}_T . \text{RH}$$

4) Quantum yield for the photoreduction of methylene blue was found to be the function of acidity, concentration of reductants (monosaccharides and disaccharides) and temperature.

5) Quantum yield was independent upon the concentration of dye.

6) Thermodynamic study of photoreduction of methylene blue reveals that reaction in between methylene blue and reducing sugars are fast reactions.
7) Many side reaction of the dye has been observed during the analysis of reaction mixture by column chromatographic technique showing the high reactivity of the dye towards different reagents present in the mixture which can be observed in mass spectrum of the purified compound.

8) Infra red spectrum of the isolated pure compound from reaction mixture shows the bands of OH region and N – H group.

9) Photoreduction of methylene blue follow second order kinetics with respect to dye and reductant.

10) Triplet transition decay follows first order reaction and life time of triplet species was 10.1 μs.

5.15 RESEARCH APPLICATIONS AND IMPORTANCE

1) The topic of this thesis is the kinetics of methylene blue by organic reductants such as Galactose, D-Mannose, D-(+)-Ribose, Maltose and Lactose. The importance and application of reduction of dyes is very important according to industrial point of view that some dyes are insoluble in water and cannot be used directly for dyeing, but on reduction to leuco form, they become soluble in presence of alkali and acquire affinity for cellulose fibers. A solution of a leuco compound can be applied for dyeing or printing and on reoxidation (usually exposure to air) the original insoluble dye is formed with in the structure of fiber. A final treatment with hot soap or other detergent brings about aggregation or crystallization so that the particle of pigments becomes firmly fixed and the shade is fully developed.

2) Oxidation and Reduction process of dye involves in solar cell where currents were produced by the reduction of dye [1,2].

3) Dye reduction processes were used to check the purity of milk, as bacteria absorbs oxygen and converts methylene blue to colorless dye.

4) Methylene blue dye test are used for the testing of industrial products.
5.16 FUTURE PROSPECTIVES

Although a lot of work on oxidation and reduction of methylene has been reported by several researchers and in spite of all its biological and photochemical applications, more investigations on reduction with organic and inorganic reducing agent with following spect can be made in future.

1) Methylene blue solar cell can be prepared like thionine solar cell with Fe$^{++}$. [149]

2) Effect of inorganic reducing agent like PbS$_2$, CdS$_2$ and ZnS on photoreduction of methylene blue can be carried out on the same optical bench as described in this work and spectrophotometrically.

3) Reduction process of methylene blue with organic and inorganic reducing agents can be used to prepare the voltaic cell.

4) Photochemical reduction process of methylene blue with organic and inorganic reductants can be carried out in presence of high intensity of light obtained by halogen bulb, laser technique and modern flash photolysis techniques on the same bench.

5) Reaction of dye with different class of fiber may explain the reduction process of dye with fabrics.

6) The isolated photochemical product of methylene blue with organic and inorganic oxidizing agent can be treated for further research by oxidation of leuco dye.

7) Comparison of reduction of methylene blue with the other dyes of same class and other class of dyes like xanthane dye and oxazine dye can be made.

8) Reduction of other members of thiazine dye with polymer like PVC can be tested. [190]

9) The photochemical reduction of thiazine dye family should be performed in presence of methane, hydrogen, carbon dioxide gas and ammonia gas atmosphere.

10) Reduction of other members of thiazine dye family can be tested with bacteria and other micro organism.

11) U.V radiation from deuterium and xenone blub can be used to study the photoreduction of dye.
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