A CHEMICAL AND PHOTOCHEMICAL STUDY OF RADICALIC SPECIES FORMED IN METHYLENE BLUE ACIDIC AND BASIC AQUEOUS SOLUTIONS

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abstract: Electron spin resonance spectra have been used to gain information on the mechanism of photolysis of aqueous solution of methylene blue. The identity and behaviour of the semiquinone radicals formed as intermediate reduction products were discussed for strongly acid and for alkaline solutions. In order to obtain information on the photolytic mechanism in strongly acid media, irradiation was performed in the presence of various types of scavengers: D,L-alanine, succinic acid, glucose, hydroquinone and sodium formate.

key words: methylene blue; leucodye; hyperfine splitting; unpaired electron; spin densities; photolysis

Introduction

Methylene blue (MB$^+$) is a dye belonging to the class of phenothiazine i.e. 3.7-bis-dimethylamine phenothiazine chloride ($C_{16}H_{18}N_3SCl\cdot3H_2O$), for which three mesomeric structures can be written in which the positive charge can be placed either on the amine nitrogen atom on the central nitrogen atom or on the sulfur atom. Like other compounds of the same class, the MB$^+$ dye forms with the leucobase a completely reduced form of the dye, a redox system mediated by a semiquinonic radical. In acid solution, all these species are converted into protonated forms by means of some acido-basic equilibria. This work offers some information on the behaviour of radicalic species involved in photolysis mechanisms of dye MB$^+$ in acidic and basic aqueous solutions, under various conditions. The information was obtained by ESR spectroscopy.

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Experimental

Methylene blue ("Ciech" Poland), H_2SO_4 (Merck), NaOH (Chemapol) p.a. were used as reagents. The organic substances used as scavengers (glucose, succinic acid, DL-α-alanine, hydroquinone and sodium formate) were Merck chemicals p.a without further purification.

The samples were irradiated with continous light using 4 bulbs of 250 W each mercury lamp which emitted a visible light and a flash- photolysis apparatus, "SPECTROFLASH" made by Applied Photophysics (LT) London, giving 15 kV pulses. The ESR spectra were recorded with an ART 5 spectrometer operating in the X band, with a frequency of 100 kHz. The spectra were recorded at room temperature without oxygen removal. The spectrometric measurements were made with a Pye-Unicam SP 1800 instrument.

Results and Discussion

The photolytic study of MB^+ was carried out in acidic and basic aqueous solutions and in the presence of various substances. The optical absorbtion spectra of the MB^+ aqueous solutions over a large concentration range showed significant deviations from the Lambert-Beer's low, a fact which was accounted for by the formation of molecular aggregates (di- and trimers), favoured among other factor by the large polarity of the water molecules [1]. The peak located at 664 nm observed in dilute solutions (10^{-5}-10^{-6} M) is attributable to the monomer as shown in Fig. 1.
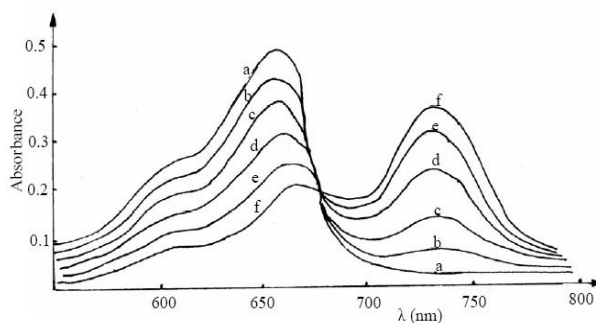

Fig. 1 *The absorption spectra of acid solutions of MB^+ ($5·10^{-6}$ M):*
a) without H_2SO_4 b) H_2SO_4 0.25 N; c) H_2SO_4 0.36 N d) H_2SO_4 0.72 N e) H_2SO_4 13N f) H_2SO_4 23N.

Increase of the dye's concentration leads to molecular aggregation which is proved by new bands at 610 nm and 570 nm, assigned to the dimer [2] and to the trimer [3] respectively. Moreover, the increase of the solution acidity converts the MB^+ cations into the protonated forms MBH^{2+} and MBH_2^{3+}. Fig. 1 shows the absorption spectra of a neutral and acid dilute solution. One can see the decrease of the 664 nm peak and the increase of the 740 nm peak with the increase of acidity, an effect which was assigned to the protonated forms of the dye. The isobestic point appearing at 680 nm is due to the equilibrium established between these two forms. Among the totally oxidized (MB^+, MBH^{2+}, MBH_2^{3+}), totally reduced (MBH, MBH_2^+, MBH_3^{2+}) and the partially oxidized forms $M\dot{B}, M\dot{B}H^+, M\dot{B}H_2^{2+}, M\dot{B}H_3^{3+}$.

acido-basic equilibria such as those shown in Scheme I are established as a function of the acidity and nature of the medium.
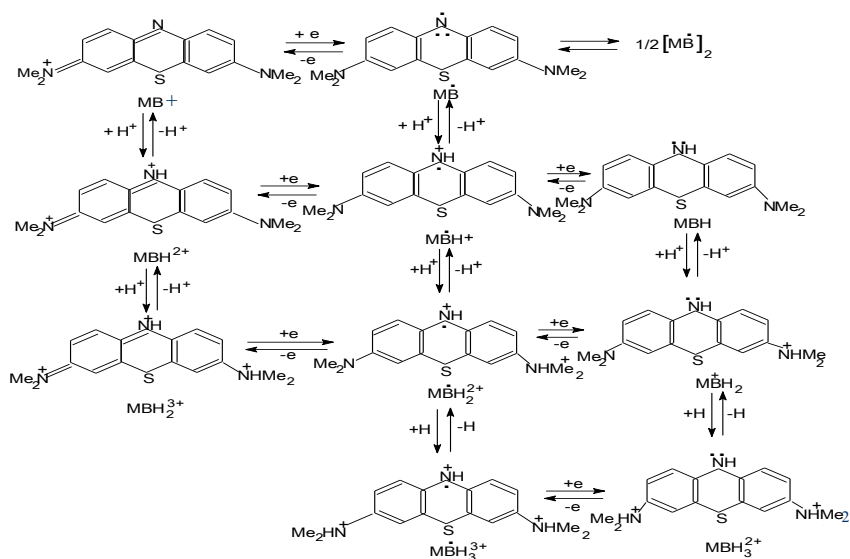
The ESR spectrum of the acid solution of MB^+ shows a four-line pattern (1:2:2:1) with the hyperfine splitting constant of 0.66 mT and a total width of 2.4 mT, and a g factor of 2.0059, as can be seen in Fig. 2.
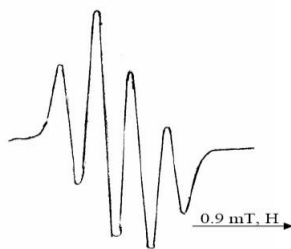

0.9 mT, H

Fig. 2 *EPR spectra of MB$^+$ solution in 13N H$_2$SO$_4$.*

This pattern is consistent with a uniform distribution of electron density of the nitrogen atom and a proton having equal high-frequency shifts, it can be assigned to anyone of the semiquinone forms: $M\dot{B}H^+$, $M\dot{B}H_2^{2+}$ or $M\dot{B}H_3^{3+}$. Since the main hyperfine interaction was localized at the central ring and is not affected by the substituent, the identity of the semiquinone radical could not be established from the ESR spectra. An increase of the protonation degree leads to a more uniform distribution of the electronic charge over the molecule as a whole. The basic solutions of MB^+ show an intense singlet-type (H=3,23 mT, g=2.0038) EPR signal. The spectrum of these solutions is due to the $M\dot{B}$ radical and it

should be a triplet (1:1:1) since the charge density of the unpaired electron is localized on the thiazinic nitrogen. The singlet form found experimentally is due to dipolar interactions between radicals, due in turn to their concentration and to the molecular aggregates from which they are formed. Some of them recombine to give the $(MB)_2$ dimer, red in colour, in equilibrium with the radicals [4]:

$$2MB^+ \underset{}{\overset{2e}{\rightleftarrows}} 2M\dot{B} \rightleftarrows (MB)_2 \qquad (1)$$
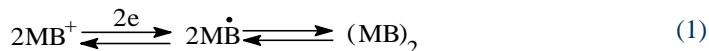
Unlike the MB^+ dye, which is soluble in polar solvents, the $M\dot{B}$ and $(MB)_2$ chargeless species are soluble in non-polar solvents. During the spectrophotometric titration of the aqueous solution of MB^+ in NaOH, we noticed that the initial blue colour changes to red by increasing the amount of base added. One also found that the intensity of the ESR singlet signal increases exponentially with the increase of hydroxide concentration from 10^{-2} M to 2M. The radical species observed in the basic solution of the dye is formed through monoelectronic reduction of MB^+ by the hydroxyl ion [5].

$$\overset{+}{M\dot{B}} + OH^- \longrightarrow M\dot{B} + \dot{O}H, \quad 2\dot{O}H \longrightarrow H_2O_2 \qquad (2)$$
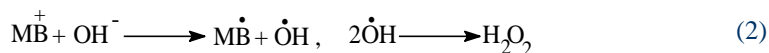
Fig. 3 illustrates the variation of ESR signal intensity (proportional to the radical concentration) versus time, recorded for a basic dye solution which contained 1M of NaOH.
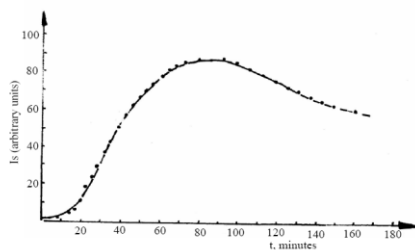

Fig. 3 *Time variation of a EPR signal intensity of MB⁺ in NaOH solutions (1M).*

We can see an increase of radical concentration up to 80-90 min. when a maximum is reached, and then a slight decrease is noticed. This behaviour proves that the process (2) is really much more complex since the radicals are formed from molecular dye aggregates and thereafter some of them take part in equilibrium (1). Beside this, one must take into account of course, the influence of O_2 from air, which decreases the radical concentration by reaction [6]:

$$M\dot{B} + O_2 \longrightarrow \overset{+}{M\dot{B}} + \dot{O}_2^- \qquad (3)$$

The decrease of the observed radical concentration take place approximately according to a second order kinetics, which would confirm the dimerization process (1). In acidic dye solutions, unlike the basic ones, mutual repulsion appear due to the positive charges of the cationic radicals, which prevent recombination and ensure the stability and accumulation of these radicals in solution. With a view to obtain a high concentration of cation radicals, the variation of the signal as a function of the concentration of H_2SO_4 was investigated. The results are presented in Fig. 4.
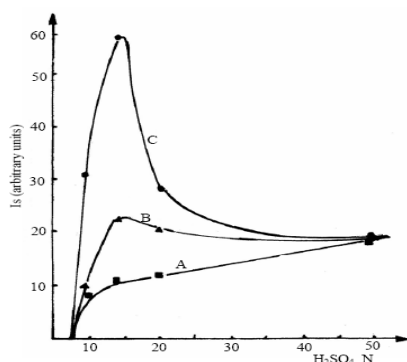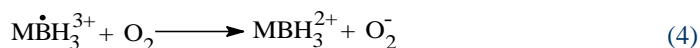
Fig. 4 *The dependence of EPR signal intensity of MB$^+$ acid solution (1.25·10^{-2} M):*
A. recorded immediately after preparing the solution,
B. A solutions irradiated with 30 successive flashes,
C. A solutions recorded after 72 hours.

Curve A shows the variation of the ESR signals recorded immediately after the preparation of the acid dye solutions. An increase of the semiquinone radical concentration with the increase of acidity was noticed. Curve C shows the signals recorded for the same solutions 72 hours after preparation. Curve B should be compared with A because it was drawn immediately after irradiation by 30 successive flash pulse (one flash 15 kV). Consequently curve B expresses radical formation by both chemical and photochemical processes. This experiment revealed a maximal intensity of the signal at 15 M H$_2$SO$_4$, where according to Matsumoto [7], the radical is present mainly as $M\dot{B}H_3^{3+}$. All subsequent experiments were carried out at this concentration. The time variation of the radical concentration proved that the chemical process of their formation is actually very complex, since both the radicals and the molecular species from which they originate have different degrees of protonation and coexist in acido-basic equilibria, as shown in Scheme I. In addition, should also be considered the reaction with O$_2$ from air [8], consisting of:

$$M\dot{B}H_3^{3+} + O_2 \longrightarrow MBH_3^{2+} + O_2^- \qquad (4)$$

$$O_2^- + H^+ \longrightarrow HO_2 \qquad\qquad 2HO_2 \longrightarrow H_2O_2 + O_2 \qquad (5)$$

In order to obtain more information on the photolysis mechanism, one performed experiments in presence of: glucose, DL-α-alanine and succinic acid. Samples of methylene blue in acid solutions which contained one of these substances (at a concentration of 0.5 M) displayed the same spectrum as the blank sample, but diminished by 30-40%. The intensity of the signal decreased just after adding one of these substances down to a constant value which was reached after 50-60 min. This value was, as mentioned, constant, which proves that the chemical reaction was then over. In order to avoid a superposition of the chemical and photochemical effects, the investigated samples, with the mentioned substances added, were prepared some days before irradiation. The results show that these substances can induce the conversion of the semiquinone species $M\dot{B}H_3^{3+}$ into leucodye MBH_3^{2+}, even

in absence of irradiation. Upon continuous irradiation with visible light of these solutions the results illustrated in Fig. 5 were obtained.
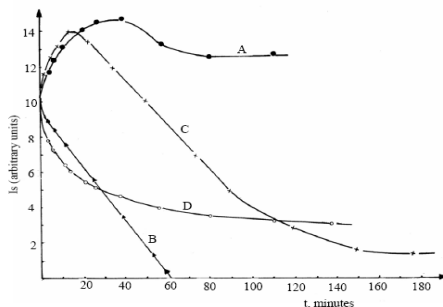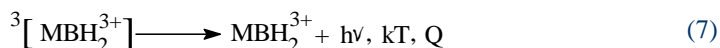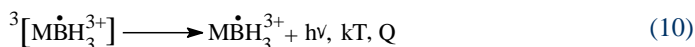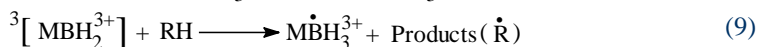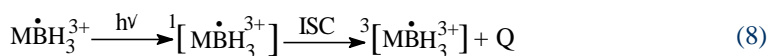

Fig. 5 *The EPR signal intensity variation with irradiation time (integral visibile light) for MB$^+$ (1.25·10^{-2} M): A) without added, B) with alanine (0.5M), C) with succinic acid (0.5 M), D) with glucose (0.5 M).*

Curve A represents the behaviour of the acid dye solution after irradiation, in absence of the mentioned substances. An increase of about 50% of the signal intensity can be noticed from the start, then a maximum is reached which begins afterwards to decrease, reaching a constant value. A similar behaviour was also noticed in the presence of succinic acid (curve C).When glucose was added the signal intensity decreased exponentially (curve D), whereas in the case of DL-α-alanine the decrease was linear (curve B). It has been definitely established that under light action the dye molecules are excited to a triplet state by a non-radiative process of internal system crossing (ISC) of states [9]. Bonneau et al. [10] have found, after studying the mechanism of thiazinic dyes photoreduction that the reactivity of the triplet state increases at the same extent with the protonation degree of the corresponding species. In agreement with this observation the excited triplet states of the MBH_2^{3+}, $M\dot{B}H_3^{3+}$ and MBH_3^{2+} species are the most reactive in electron transfer phenomena. Increase of the reactivity with the protonation degree has been accounted for by a decrease of electron density in the aromatic rings as a consequence of protonation [11]. As suggested in the literature [12] the mechanism of photolysis process involves several steps in which both the dye molecules and the semiquinonic radicals formed initially by a chemical path and involved thereby in acido-basic equilibria (Scheme I) take part. The first step consists of the excitation of these species to their triplet states. In the second step these states disappear through two competitive processes: spontaneous deactivation by any of the available pathways (emission of radiation hυ, thermal heat loss kT, heat Q) and reduction by electrons provided by the reducing agent present in the system (denoted by RH) [12], presented in Fig. 5.

$$MBH_2^{3+} \xrightarrow{\ h\upsilon\ } {}^1\!\left[MBH_2^{3+}\right] \xrightarrow{\ ISC\ } {}^3\!\left[MBH_2^{3+}\right] + Q \tag{6}$$

$$^3\!\left[MBH_2^{3+}\right] \longrightarrow MBH_2^{3+} + h\upsilon,\ kT,\ Q \tag{7}$$

$$M\dot{B}H_3^{3+} \xrightarrow{h\nu} {}^1\left[M\dot{B}H_3^{3+}\right] \xrightarrow{ISC} {}^3\left[M\dot{B}H_3^{3+}\right] + Q \tag{8}$$

$${}^3\left[MBH_2^{3+}\right] + RH \longrightarrow M\dot{B}H_3^{3+} + Products(\dot{R}) \tag{9}$$

$${}^3\left[M\dot{B}H_3^{3+}\right] \longrightarrow M\dot{B}H_3^{3+} + h\nu, kT, Q \tag{10}$$

$${}^3\left[M\dot{B}H_3^{3+}\right] + RH \longrightarrow MBH_3^{2+} + Products(\dot{R}+H^+) \tag{11}$$

This observation is consistent with the following equilibrium in acidity:

$$2 M\dot{B}H_3^{3+} \rightleftarrows MBH_3^{2+} + MBH_2^{3+} + H^+ \tag{12}$$

Therefore, an increase of the acidity produces an increase of the semiquinone radical concentration [13]. Because in all our experiments we worked in the presence of oxygen, reactions (4) and (5) must also be included into the reaction. One can see that the radicals species followed by ESR are formed in some reactions and disappears in others, thus modifying the initial concentration generated chemically. The presented mechanism only involves the reactions in which the investigated species plays a role, but it is obvious that other species could also be implied in Scheme I. However, the reactions included in this mechanism may allow an interpretation of the results presented in Fig. 5. In the case of the curve 5A, RH is H_2O or Cl^-. The shape of the curves (Fig. 5) suggests that irradiation in presence of the mentioned substances leads to a preponderance of radicals disappearance process over the formation process. Unlike the substances mentioned above, addition of hydroquinone (0.5 M) leads to a modification of the ESR spectrum as a function of the irradiation time. Upon irradiation under selected monochromatic light (λ=660 nm) of a dye acid solution containing 0.5 M hydroquinone the quartet signal was converted into triplet (1:1:1) at H=3.23 mT, g=2.0039, as shown in Fig. 6.

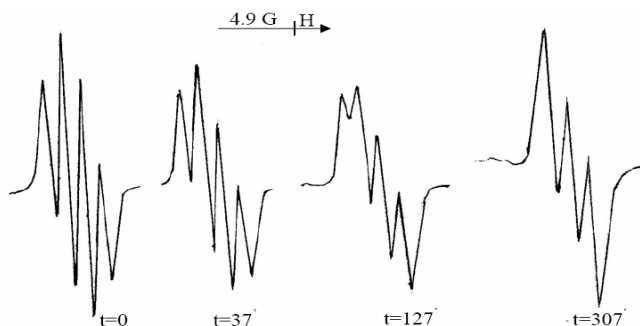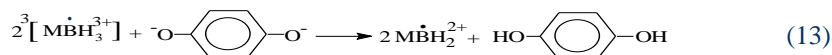Fig. 6 *Time variation of EPR spectra hyperfine structure of MB^+ solution ($1.25 \cdot 10^{-2}$ M) with hydroquinone 0.5 M irradiated with selected monochromatic light (λ=660 nm).*

Fig. 6 illustrates the shape of the spectra at several irradiation times. This behaviour can only be explained by admitting that at such a high hydroquinone concentration (0.5 M) the thiazinic nitrogen atom of the central ring was deprotonated as a consequence of the action of hydroquinone anion:

$$2\,{}^{3}_{2}[\,\mathrm{M\dot{B}H_3^{3+}}\,] + {}^{-}\mathrm{O}\!-\!\!\!\bigcirc\!\!\!-\!\mathrm{O}^{-} \longrightarrow 2\,\mathrm{M\dot{B}H_2^{2+}} + \mathrm{HO}\!-\!\!\!\bigcirc\!\!\!-\!\mathrm{OH} \tag{13}$$

However, if the same solution is irradiated with unselected visible light, the initial quartet disappears after a few tenths of seconds and a singlet forms instead, which increases in intensity during irradiation. The shape and parameters of the spectrum are identical to those of the signal recorded with the alkaline solution. The shape of the ESR spectra at several irradiation times are illustrated in Fig. 7.
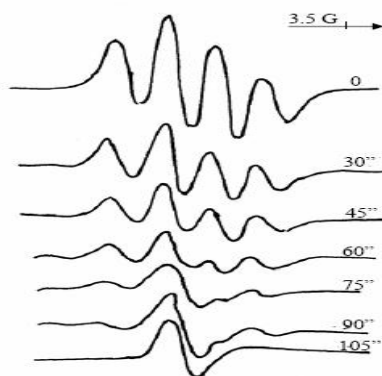

Fig. 7 *The changes of EPR spectra with irradiation time with visible integral light, of a MB⁺ solution(1.25·10⁻²M) with hydroquinone 0.5 M.*

One can see that a signal overlaps a much weaker triplet. This result was interpreted by admitting that, upon irradiation with visible light, hydroquinone acts in favour of increasing the deprotonation degree of the excited radicals, finally generating $\mathrm{M\dot{B}}$ radicals (Scheme I). We should make clear that the signals illustrated in Figs. 6 and 7 are not caused by hydroquinone, but by the dye: we checked that the acid solutions of hydroquinone, both irradiated and non-irradiated give no ESR signal. The disappearance of the triplet hyperfine structure and transformation in to a singlet is due to the overlapping of the spectral lines produced by the radicals dipolar interaction. Upon irradiation with visible light of the acid solutions of MB⁺ in presence of HCOONa (0.5 M) an 8 to 10 time increase of the intensity of the ESR signal was obtained, compared to the non-irradiated solution. This increase can be accounted for by the reducing effect of the $\mathrm{CO_2^-}$ radical ion upon the dye, under the acidic conditions prevailing in the formate photolysis [14].

Conclusions

The EPR spectra of MB⁺ acidic solutions consists in four components of 1:2:2:1 intensity, attributed to one of semiquinonic forms: $\mathrm{M\dot{B}H^+}$, $\mathrm{M\dot{B}H_2^{2+}}$, $\mathrm{M\dot{B}H_3^{3+}}$. MB⁺ basic solutions present one intensive singlet attributed to $\mathrm{M\dot{B}}$ radical by reduction of the MB⁺ by OH⁻ ion. It has been found the radicals concentration decreasing due to their reaction with O_2 from

atmosphere. Irradiation with light of the acid dye solutions in the absence and presence of added (glucose, DL-α-alanine, succinic acid) was studied. In the photolysis process are involved the triplet excited states of MB^+ protonated which disappear either by deactivation or by reduction of the substances mentioned above. By irradiation with monochromatic light (λ=660 nm) of MB^+ acidic solution in the presence of hydroquinone, the quartet is converted into triplet and by irradiation with integral visible light is turned into singlet. This behaviour proves that hydroquinone increases the excited radicals deprotonation degree.

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