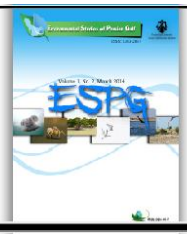
Volume 1, No.2, March 2014, Pages 93-101

Environmental Studies of Persian Gulf

journal home page: espg.pgu.ac.ir



Spectrophotometric Method for Hydrogen Peroxide Determination through Oxidation of Organic Dyes

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ARTICLE INFO

Article History:
Received
June 16, 2013
Accepted
November 16, 2013

Keywords:
Discoloration
Organic dyes
Hydrogen peroxide
Determination

ABSTRACT

Hydrogen peroxide is an important mild oxidant which is widely used in various applications including remediation of waste waters and drinking water resources. The safe level of H_2O_2 residue has to be controlled through accurate and precise measurement methods. A facile spectrophotometric method for the determination of hydrogen peroxide is based on the reaction of iodine solution with toluidine blue (TB). To increase the applicability, accuracy and sensitivity of the method, direct reactions of hydrogen peroxide and iodine solution with TB, methylene blue (MeB), methyl blue (MB) and a diazo derivative of 1,3-diamino benzene (MADA) were studied. Based on the results of this study, the method was modified using MB instead of TB. The reasons concerned single peak visible spectrum, higher extinction coefficient, linear spectrophotometric responses at three different wavelengths during its oxidation by iodine and stability at low pH(s) and higher temperatures.

1 INTRODUCTION

Due to its mild oxidation ability, H_2O_2 is widely used in different large-scale processes such as those in textile, paper, food, beverages and pharmaceutical industries (Li,1996). Hydrogen peroxide plays a key role in water treatment processes known as Advanced Oxidation Processes (Alnaizy & Akgerman, 2000). In Fenton's reaction, hydrogen peroxide is the key oxidant for converting Fe^{2+} to Fe^{3+}. This process has been widely studied for waste water treatment. It is also used and produced in a number of important biological processes (Tian et al., 2003). H_2O_2 has been employed as a food additive for controlling microorganisms' growth (Toyoda et al., 1982). However, Food and Drug Administration (FDA) regulations limit H_2O_2 residual to 0.5 $mg.l^{-1}$ in finished food packages (Ozkan et al., 2004). This is because the higher concentrations of H_2O_2 damage eyes, skin, lungs and respiratory tract (Abbas et al., 2010). Hence, the level of hydrogen peroxide residue is required to be effectively controlled within permissible limits. As a result, methods with high accuracy and precision for the determination of this substance

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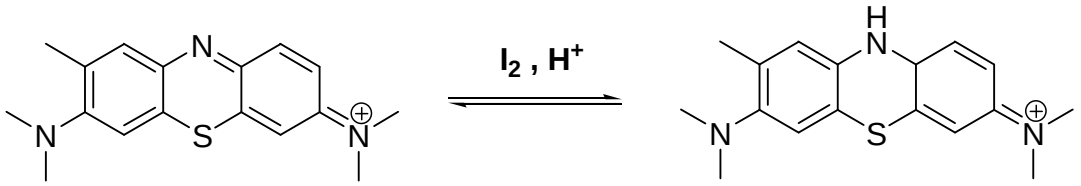

Fig. 1. Discoloration of TB in the presence of iodine in acetate buffer (2 M, pH 8).

are of major interest in various research and industrial sectors.

The analytical methods introduced for H_2O_2 determination are based on different techniques such as volumetric titration (Klassen et al., 1994), colorimetric (Eisenberg, 1943), fluorometric (Abbas et al., 2010), electrochemical (Li et al., 2011), luminescence and chemiluminescence (Hu et al., 2007; Shiang et al., 2009) measurements. However, spectrophotometric methods are preferred mainly because they are simple, fast, precise and financially viable. A review of available literature shows that three major approaches have been employed to develop spectrophotometric methods for H_2O_2 determination. The first way passes through enzymatic reaction/s (mostly peroxidase) to follow production/consumption of a chromophoric substance in the presence of hydrogen peroxide (Cosgrove et al., 1988; Fernandes et al., 2005). The second one includes those in which the presence of a metal ion is necessary (Nogueira et al., 2005) and finally, in the third approach, depletion of organic chromophores is followed during redox reactions (Sunil & Narayana, 2008; Lou, et al., 2008). Discoloration of organic dyes during oxidation seems to be a simple and straightforward procedure provided the spectrophotometric properties of the dye let reliable measurement of the added oxidant. Employing this idea, Sunil and Narayana (2008) introduced a spectrophotometric method for hydrogen peroxide determination based on the reaction of toluidine blue (TB) with iodine solution shown in equation 1 and Fig. 1.

$$H_2O_2 + 2KI + 2HCl \rightarrow I_2(aq) + 2KCl + 2H_2O \quad (1)$$

Precise examination of this method unveiled some concerns. To evaluate how these considerations affect the reliability of this method, oxidation of TB, methylene blue (MeB), methyl blue (MB) and a diazo derivative of 1,3-diamino benzene (MADA) with iodine solution and direct addition of H_2O_2 were studied in detail. In view of relevant literature, results of this research are presented and discussed in this paper.

2 MATERIALS AND METHODS

2.1. Reagents and solutions

All chemicals were analytical grade reagents and were used without further purification. Double distilled water was used exclusively. Diluted solutions of NaOH and HCl were used to adjust pH. Potassium iodide, toluidine blue (TB), methyl blue (MB), methylene blue (MeB), methyl orange (MO) (Fig. 2), H_2O_2 (30% wt) was purchased from Merck and 4-[(4-methoxybenzo)azo]-1,3 benzen-diamine (MADA) were prepared according to the previously introduced procedure (Haghbeen & Legge, 2009). Hydrogen peroxide stock solution (1 mM) was made by diluting H_2O_2 (10 µl) in a standard analytical flask (100 ml).

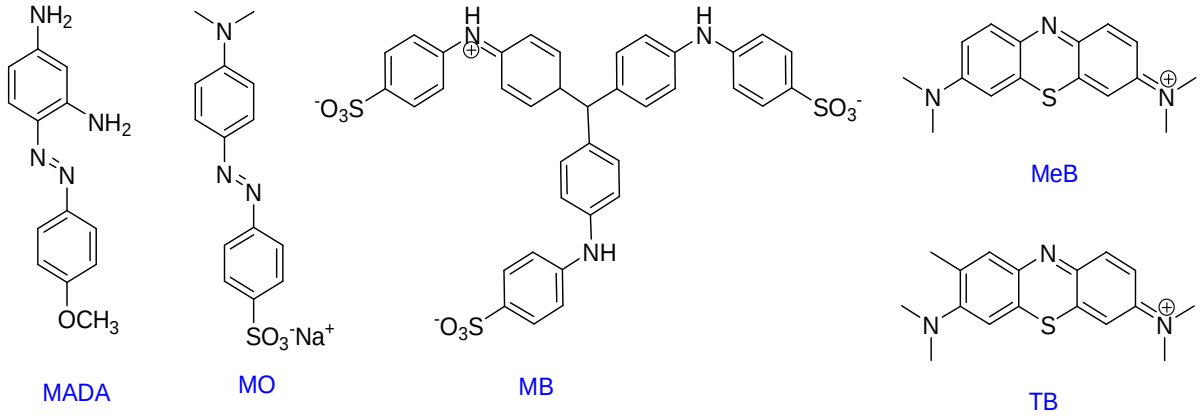

Fig. 2. Chemical structures of the organic dyes employed in the spectrophotometric studies of this research.

The stock solutions of TB, MB and MeB (0.1%) were prepared by dissolving 1 mg of each dye in 1 ml of suitable medium. The stock solution of MADA was prepared by dissolving 1 mg of MADA in ethanol (1 ml). The resultant mixture was heated until a clear solution was obtained. All stock solutions were stored in amber-glass bottles at 4 ^{o}C. Stability of the dyes solutions was checked by spectrophotometric examinations. All the spectrophotometric measurements were carried out in conventional quartz cells (1 cm path) by Analytic Jena specord 210 UV-Visible spectrophotometer (Germany) at 20 °C. Iodine solution was prepared by mixing hydrochloric acid (1 ml, 2 M) with potassium iodide (1 ml, 2%) and H_2O_2 solution (1 ml, 10 μM) according to the reaction shown in equation 1. The mixture was shaken gently until a yellow color appeared.

2.2. Oxidation of TB, MeB, MB and MADA by Iodine

TB solution (0.001 %) was prepared by diluting 30 μl of the corresponding stock solution to 3 ml by sodium acetate solution (2 M, pH 8). Then, it was reacted with certain amounts of iodine solution at room temperature. Oxidation of MeB, MB and MADA by iodine was examined using a similar procedure except the reaction medium. Reactions of MeB, MB and MADA with iodine were carried out in neutral pH (water), hydrochloric acid (2 M) and phosphate buffer solution (0.01 M, pH 2), respectively. Spectrophotometric examinations were carried out by both scanning and single wavelength methods. Spectrophotometric measurements were done using the extinction coefficient values shown in Table 1.

Table 1
Extinction coefficients of the dyes illustrated in Fig. 2 at 20 °C and pH(s) used in their reactions with iodine solution.

Dye	pH	λ_{max} (nm)	ε (M^{-1} cm^{-1})
TB	8	640	22480 (R^2 = 0.99)
MB	acidic	608	49100 (R^2 = 0.999)
MeB	7	665	39800 (R^2 = 0.999)
MADA	2	480	29200 (R^2 = 0.994)
MO	2.5	507	23500[a]

[a] Data comes from Tian et al., 2003.

2.3. Directoxidation of organic dyes by H_2O_2

The dye solution (containing a desired amount of the dye) was reacted with known amounts of H_2O_2 solution at room and boiling (for 5 min) temperatures. Result of each reaction was monitored and studied by spectrophotometer. It's worth mentioning that results provided in this paper are average of, at least, triplicate measurements.

3 RESULTS AND DISCUSSION

3.1. TB reaction with iodine solution

Aqueous iodine is a mild oxidant ($E°=+0.615V$) which can be produced through the reaction shown in Equation 1. The oxidizing ability of iodine has been employed in numerous applications. The yellow appearance of the iodine solution which is intensified in the presence of starch increases the applicability of this solution for analytical purposes including the well-known volumetric titrations (Klassen, et al., 1994). However, obstacles such as high toxicity and low solubility of iodine in water seriously limit the use of this reagent. Sunil and Narayana (2008) showed that iodine solution could oxidize TB at pH 8. The reaction (Fig. 1) could be followed from the decrease in the optical density of TB at 628 nm, far enough from λ_{max} of iodine in the reaction medium (Fig.s 3A and 3B). Since the iodine production is directly proportional to the hydrogen peroxide concentration (Equation 1), Sunil and Narayana (2008) used this method for H_2O_2 determination. This method produced a linear response to the added iodine over a range of 0.2 to 14 μM ($R^2 = 0.975$) with a detection limit of 1.4 μM (Sunil & Narayana, 2008)

Precise examination of TB discoloration by iodine solution in the lab revealed that despite the linear relationship between the added iodine solution and TB depletion, there was not a 1:1 stoichiometric ratio between the added iodine and the depleted dye. As a result, the precise amount of H_2O_2 had to be extracted from a standard plot. This procedure also requires preparation of TB in sodium acetate solution (2M) because of the TB instability at low pH(s) (data is not shown). In addition, the spectrophotometric behavior of TB at pH 8 is not free of problem. TB shows two close peaks between 580-630 nm. The absorption ratio of these two peaks is concentration dependent (Fig. 3A) which can affect the precision of the spectrophotometric method.

3.2. MB reaction with iodine solution

In contrast to TB, MB is stable in low pH(s) and shows a single peakwith a λ_{max} at 608 nm. This dye is also oxidized by iodine solution in an acidic solution with a pH identical to that of iodine solution (Fig. 3C) and produces a linear response to the added iodine over a range of 0.48 to 15 μM ($R^2 = 0.967$) with a detection limit of 0.6 μM (Fig. 3D). At concentrations higher than 15 μM, the reaction reaches a plateau (Fig. 3E). A similar phenomenon is observed for the reaction of TB and iodine solution. The plateau is formed as a result of interference of the oxidation product(s) absorption in the dye absorption region and is witnessed by the isosbestic points at 552 and 524 nm for TB and MB, respectively (Fig.s 3B and 3C).

Extinction coefficient of MB is two times larger than that of TB (Table 1) which renders the method more sensitive, but similar to TB, decrease in the optical density of MB at 608 nm during the reaction with iodine does not obey a 1:1 stoichiometric ratio. Therefore, a calibration curve has to be obtained prior to the determination of hydrogen peroxide. During the oxidation of MB with iodine solution, similar to TB and parallel to the decrease in absorption at 608 nm, there are increases in the optical density at 290 and 360 nm due to the formation of the oxidation product(s) (Fig. 3C). Although the optical density increase at these wavelengths follows a pretty linear pace (Fig.

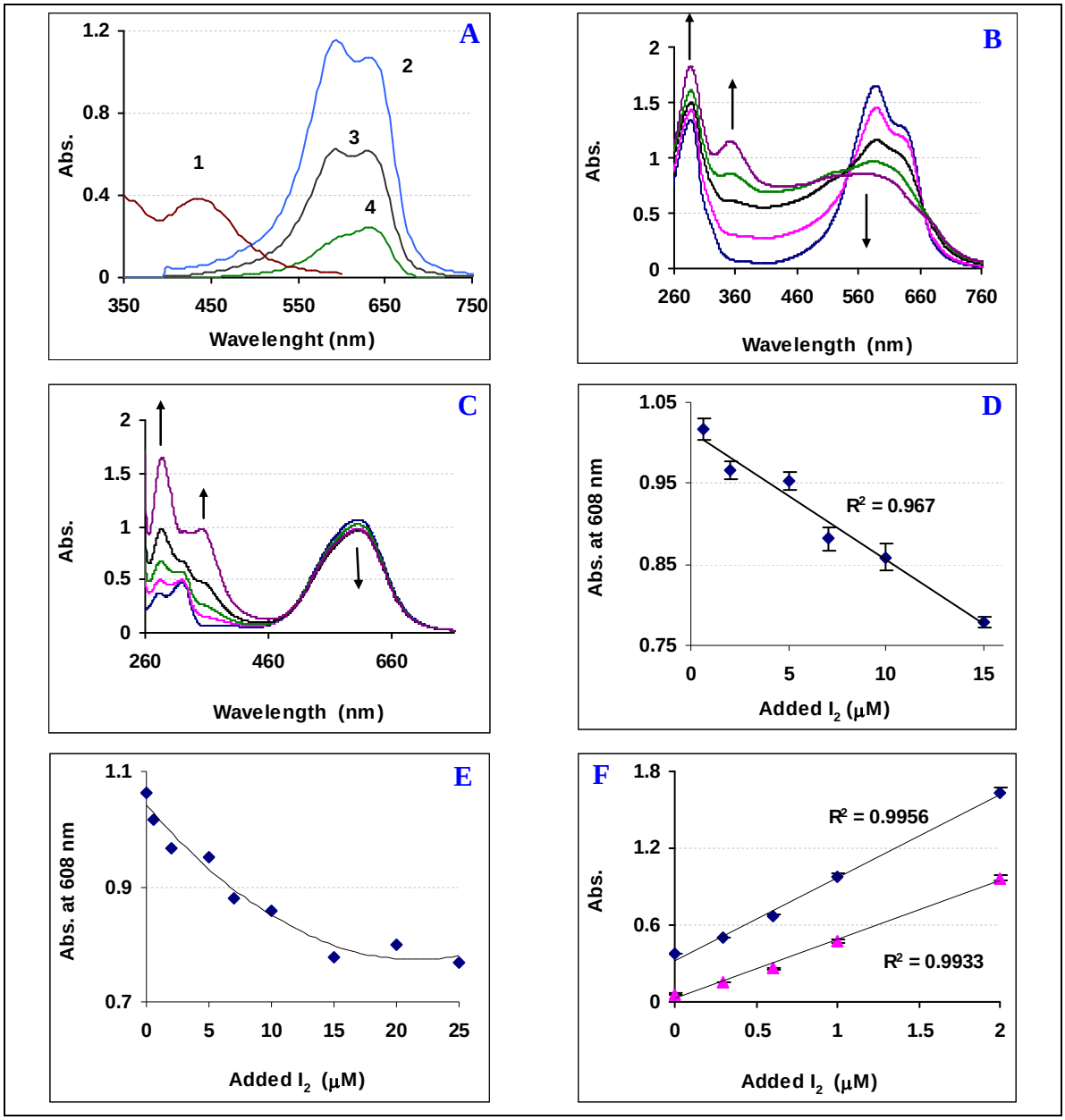

Fig. 3. UV-Visible spectra of **A)** acidic iodine solution (1) and TB at three different concentrations (2, 3, and 4) in acetate buffer (2 M, pH 8). Overlaid spectra of the reaction mixture of iodine solution with **B)** TB, **C)** MB. Changes in the optical density of **D, E)** MB at 608 nm, and **F**) MB at [■] 290 and [▲] 360 nm solutions after successive addition of iodine at room temperature. Reactions were carried out at pH(s) summarized in Table 1.

3F), its applicability in the determination of hydrogen peroxide is limited to concentrations lower than 2.5 μM mainly because of the large extinction coefficient of the product(s) at these wavelengths.

3.3. MeB reaction with iodine solution

The oxidation of MeB by iodine solution was also examined. MeB dissolves in water and its fresh solution shows no auto-oxidation at 20 °C in dark. The UV-Visible spectrum of MeB in water shows a twin peak at 618 and 665 nm.

In contrast to TB, the ratio of these two peaks is independent of the MeB concentration. Upon reaction with the iodine solution (Fig. 4A), oxidation of MeB proceeds through a linear course over a range of 0.3 to 17 μM (Fig. 4B) until it reaches a plateau. However, similar to what was observed for TB and MB, a 1:1 stoichiometric ratio between the added iodine and the depleted dye is not concluded from the spectrophotometric measurements.

3.4. MADA reaction with iodine solution

In a different spectrophotometric method, MO was directly reacted with H_2O_2 in the presence of Fe(II) (Lou et al., 2008). Reaction proceeded through the depletion of MO at 507 nm (Fig. 4C) over a range of 0.5 to 100 μM of the added hydrogen peroxide. There is a significant structural difference between MO with TB, MB and MeB. MO is a diazo dye, but none of these dyes carries any free amino group. Therefore, reaction of MADA with iodine solution was also studied. Diazo derivatives of meta-diaminobenzene are easily synthesized through the coupling reaction of a diazonium salt with meta-diaminobenzene and are not susceptible to auto-oxidation (Haghbeen & Legge, 2009). MADA is stable in low pH(s) and is oxidized by iodine solution at pH 2 and 20 °C. However, the progress of the reaction can not be followed spectrophotometrically due to the progressive overlap of the absorption of MADA and product(s) of the reaction (Fig. 4D).

3.5. Direct reactions of TB, MB, MeB, and MADA with H_2O_2

Experiments revealed that TB, MeB, MB and MADA can be oxidized by direct addition of H_2O_2, but reactions seemed to be slow at room temperature. To accelerate the reaction, the mixture of the organic dye and hydrogen peroxide had to be heated. However, it was only the acidic solution of MB which was stable even at boiling temperature. Examining the direct oxidation of MB by H_2O_2 using boiling conditions, (Fig. 4E) proved that the method is applicable for hydrogen peroxide determination at concentrations higher than 70 μM (> 2.3 ppm) (Fig. 4F). However, comparing Fig. 4E with Fig. 3C indicates that the direct oxidation of MB by hydrogen peroxide proceeds through a different mechanism and it is worthwhile to be studied in a different study.

3.6. Stoichiometry and ion interferences

As mentioned, from a spectrophotometric point of view, none of the reactions of TB, MeB, MB and MADA with iodine solution follows a 1:1 stoichiometry. Sunil and Narayana (2008) suggests that the oxidation of TB by I_2 solution proceeds through the reaction shown in Fig. 1. The number of isosbestic points in the spectra illustrated in Fig.s 3B, 3C and 4A hardly suggests the existence of more than 2 different molecules in the reaction mixture of each case. Nevertheless, the overlap between the starting material and the oxidation product absorption is evident, an inevitable phenomenon that destroys the stoichiometric ratio between the added iodine and the depleted dye.

Although it is beyond the scope of this paper to compare the efficacy of this method with those which are based on oxidation of an organic dye in the presence of H_2O_2 and a metallic ion such as MO reaction with H_2O_2 in the presence of Fe(II), it is instructive to know that the stoichiometric ratio between the added hydrogen peroxide and the decrease in the optical density of MO was not observed in this method either (data is not shown). Interestingly, the direct reaction of H_2O_2 with MB at boiling temperature also fails to obey a 1:1 stoichiometric ratio. It seems that finding a chromophoric substance with proper reducing ability and non-interfering oxidation product(s) is a common challenge for improving these

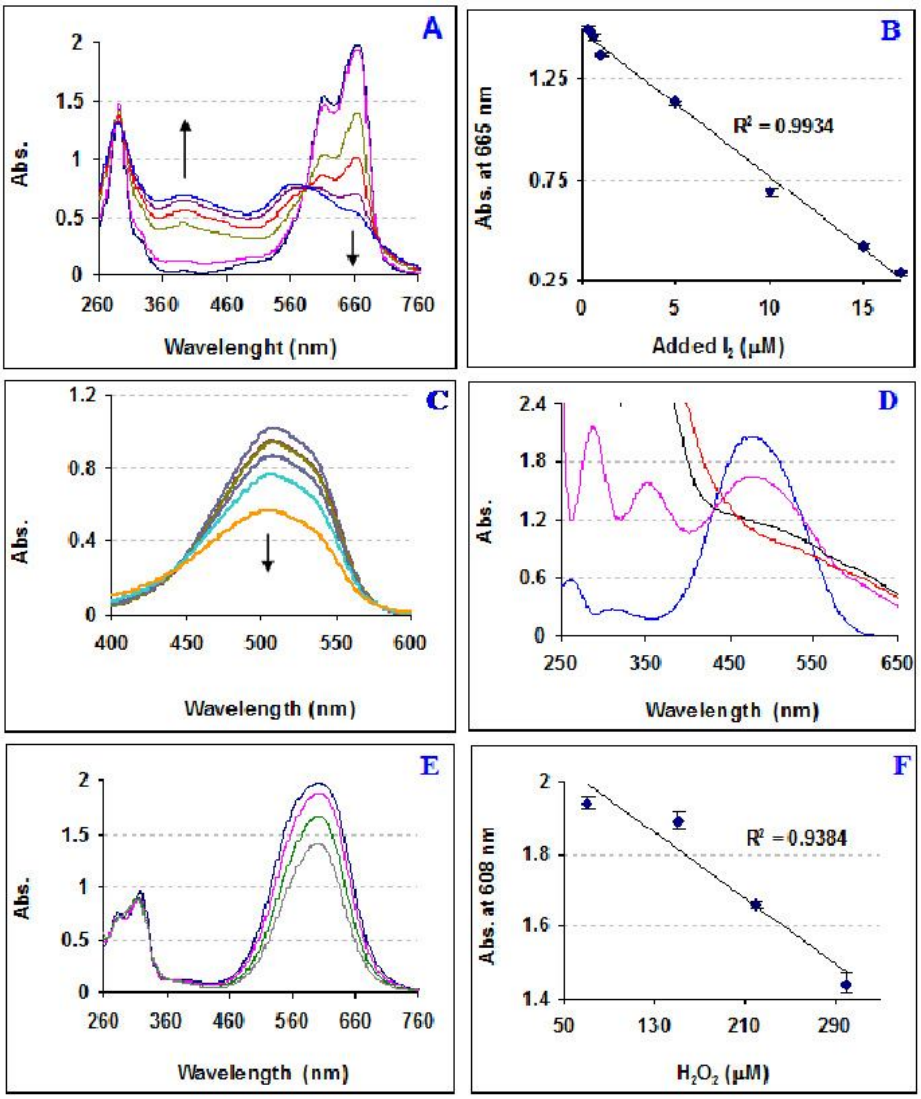

Fig. 4. Overlaid spectra of the reaction mixture of iodine solution with **A)** MeB and **D)** MADA. Overlaid spectra of the direct reaction of H_2O_2 with **C)** MO in the presence of Fe(II) at room temperature and **E)** MB at boiling temperature. Changes in the optical density of **B)** MeB at 665 nm at room temperature and **F)** MB at 608 nm at boiling temperature. Reactions were carried out at pH(s) summarized in Table 1.

methods.

In agreement with the results reported by Sunil and Narayana (2008), all the examined cations in this work including Cr^{3+}, Ni^{2+}, Fe^{2+}, Co^{2+}, Cu^{2+}, Ca^{2+}, Mg^{2+} Na^{+}, K^{+}, and Ce^{4+} could change the intensity of the optical density of MB at 290, 360 and 608 nm (data is not shown). It means that the cations were able to interact with both MB and its oxidation product.

4 CONCLUSION

Spectrophotometric examination of TB, MeB, MB, and MADA reactions with iodine solution revealed progressive interference of the oxidation product(s) with the dyes' absorption. As a result, the whole course of the reaction can not be followed in a linear pace as the decrease

in the optical density of the dyes reaches plateau. The interference also obstructs correct assessment of the added iodine by reading the changes in the dyes absorption. These studies also disclosed that the optical density intensities of both dyes and their oxidation products are affected by the presence of various metallic ions. This makes the analysis of unknown samples even more difficult. Yet, this method can be used for hydrogen peroxide determination provided a calibration plot is obtained prior to the analysis. Based on the results of this study, a more accurate and precise method was developed by using MB instead of TB. MB has a single peak visible spectrum, higher extinction coefficient, and shows linear spectrophotometric responses at three different wavelengths during oxidation and stability at low pH(s) and higher temperatures.

ACKNOWLEDGEMENTS

The authors would like to appreciate the Enovironmental Protection Organization (Alborz Province, Karaj, Islamic Republic of Iran) for funding support on this project.

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