Advanced oxidation of acridine orange by aqueous alkaline iodine

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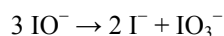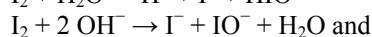Department of Chemistry, University of Karachi, Karachi, Pakistan

Abstract: The advanced oxidation process is certainly used for the dye waste water treatment. In this continuation a new advanced oxidation via aqueous alkaline iodine was developed for the oxidation of acridine orange (AO) {3, 6 –bis (dimethylamino) acridine zinc chloride double salt}. Oxidation Kinetics of AO by alkaline solution of iodine was investigated spectrophotometrically at λ_{max} 491 nm. The reaction was monitored at various operational parameters like several concentrations of dye and iodine, pH, salt electrolyte and temperature. The initial steps of oxidation kinetics followed fractional order reaction with respect to the dye while depend upon the incremental amount of iodine to certain extent whereas maximum oxidation of AO was achieved at high pH. Decline in the reaction rate in the presence of salt electrolyte suggested the presence of oppositely charged species in the rate determining step. Kinetic data revealed that the de-colorization mechanism involves triodate (I_3^-) species, instead of hypoidate (OI^-) and hypiodous acid (HOI), in alkaline medium during the photo-excitation of hydrolyzed AO. Alleviated concentration of alkali result in decreasing of rate of reaction, clearly indicate that the iodine species are active oxidizing species instead of OH radical. Activation parameters at elevated temperatures were determined which revealed that highly solvated state of dye complex existed into solution. Reaction mixture was subjected to UV/Visible and GC mass spectrum analysis that proves the secondary consecutive reaction was operative in rate determining step and finally dye complex end into smaller fragments.

Keywords: AO, iodine, oxidation, pH, fragments

INTRODUCTION

Inordinate population growth and advanced technological developments at one hand better the life of human and on the other hand it's greatly damaging the environment. It is essential to address the problems created by damaging material in an appropriate manner for safety of environment. Dyes are significant for regular life and their role cannot be overlooked but unused, untreated discharged cause serious pollution during the recent years. Various treatments processes have been developed for the treatment of dye waste water (Singh *et al.*, 2001).

The chemistry of Iodine as a sanitizer is studied very widely in aqueous medium due to its complex system of oxidation states available under ambient conditions. It showed an outstanding, quick, microbicide activity including extensive array of action that comprises nearly all significant health related microorganism such as bacterial viruses, enteric viruses and bacteria, and protozan cyst (Hoehn, 1976). Iodine also exhibits a fungicidal and trichomonicidal activity (Knolle, 1975). It generates at least ten species in water like I^-, I_2, I_3^-, I_5^- .I_6^{2-}, HOI, OI^- .HI_2O^-, I_2O^{2-}, H_2OI^+ and IO_3^-. These species are pH depended. At low pH I^-, I_2, I_3^- are effectives whereas at higher pH range it generates simple oxyacid HOI, OI^- .HI_2O^-, I_2O^{2-}, H_2OI^+ and IO_3 (Singh *et al.*, 2004)

$$I_2 + H_2O \rightleftharpoons H^+ + I^- + HIO$$
$$I_2 + 2\,OH^- \rightarrow I^- + IO^- + H_2O \text{ and}$$

$$3\,IO^- \rightarrow 2\,I^- + IO_3^-$$

General awareness about oxide and oxy-acid of iodine in chemistry is well known. It is essential for the developments of analytical methods for the detection of iodide and iodate in seawater (Wong & Cheng 2001). As Khaled *et al.,* (2011) developed four sensitive catalytic potentiometric methods for the determination of trace level of iodine based on oxidation of four dyes variamine blue (VB), Rhoda mine B (RB), methylene blue (MB) and malachite green (MG), with H_2O_2 in H_3PO_4 medium at 25±0.5°C. Perez-Ruiz *et al.*, (1984) determined micro amount of iron, phosphate and fluoride through oxidation of AO and AY in presence of oxygen. Degradation of acridine orange (AO) in aqueous solution by Fenton's reagent (Fe^{2+} and H_2O_2) was investigated by Xie *et al.* (2011). The analytical results indicated that the N-de-methylation degradation of AO dye taking place in a stepwise manner to yield mono-, di-, tri- and tetra-N-de-methylated AO species generated during the Fenton oxidation process (Chen 2009; Xie *et al.*, 2001).

AO was selected as an important staining material for microbes, cell, and blood cell commonly used in pathological laborites. It's unsafe discharged may cause coughing or mild irritation, redness and irritation when contact with skin or eye irritation. This research article will discuss the laboratory control of unused dye by alkaline iodine as numerous usual iodine products are not injurious to the ecosystem and rapidly degraded into basic elements/compounds (Perez-Ruiz et al 1984; Nazami *et al.,* 2005, Azmat & Saleem, 2011, Ahmed *et al.,* 2012). Also to our best knowledge no report was available for oxidation of AO using alkaline iodine. The effects of

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diverse operational parameters like pH of solution, ionic strength, AO and iodine concentration, which affect the efficacy of the oxidation reaction was investigated and their degraded products were analyzed by UV/Visible, GC, mass spectrometry. A mechanism of oxidation was proposed in the relevant section based on findings.

MATERIALS AND METHODS

The acridine orange dye was purchased from Sigma-Aldrich and used as received. The chemical structure formula of the orange dye is presented in fig. 1.
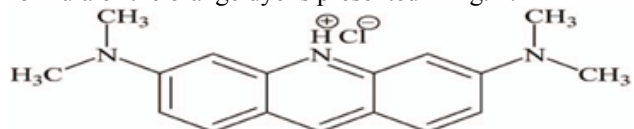
Fig. 1: Chemical formula structure of the dye (Bhagwat *et al.*, 2006)

Stock solution containing 1×10^{-3} mol.dm^{-3}, of AO dye in double deionized water was prepared and protected from light using polyethylene bottle and other solutions like sodium hydroxide of 1.5 mol. dm^{-3} and potassium chloride of 1.5 mol.dm^{-3} was prepared in deionized distilled water (Hickey *et al.,* 1997, Woittiez*,* 1991). Solution of Iodine was prepared in KI by usual method.

Kinetics measurements

The effectiveness of alkaline iodine oxidation was evaluated using a Schimadzo double beam UV/vis spectrophotmeter (1800 A). The maximum absorbance wavelength (λ_{max}) of acridine oange was found at 491 nm. No interference of Degradation products with the measurement of AO concentration was observed. Therefore, the change in optical density in terms of depilation in concentration of AO in the reaction mixture at different reaction times was determined. Four sets of mixture were prepared from stock solution. In each set one solution was varied while concentrations of other three were kept fixed. Each component were mixed together and portion was transferred to the cuvette (1cm) to record a change in optical density of AO. Kinetic measurements were made at room temperature on thermostatic water bath (FJPSO UK) for determination of order of reaction(Azmat and Saleem 2011). For salt effects on alkaline oxidation of AO following Brønsted-Bjerrum equation, was used

$$\log k = \log k_o + 1.018 Z_A Z_B \sqrt{\mu}$$

### GC Mass Analysis of the reaction mixture

GC mass Analysis of the reaction mixture was performed by the method described by Azmat and Saleem (2011). A mixture of 1×10^{-3}mol.dm^{-3} iodine (10ml), 0.05 mol.dm^{-3} potassium chloride (10ml), 0.4mol.dm^{-3} sodium hydroxide (25ml) and 0.054×10^{-4}mol.dm^{-3} acridine orange (5ml) was kept for 24hr. at 35° for the complete reaction processes. Reaction mixture was extracted thrice with

ethyl acetate. The combined ethyl acetate extract was again extracted with water to remove water-soluble traces and ethyl acetate layer was evaporated under reduced pressure on rotary evaporator. Then it was subjected to GC mass analysis.
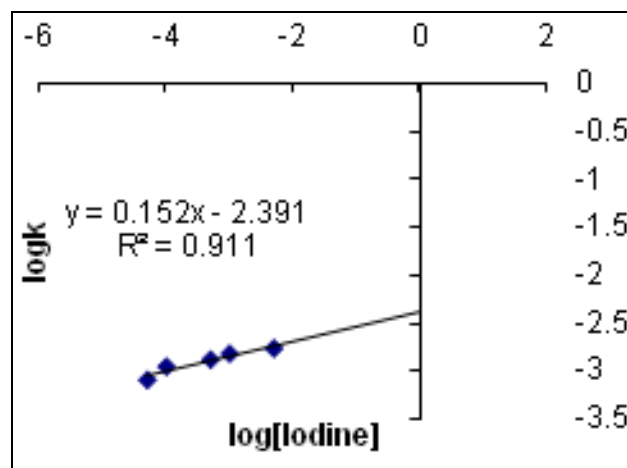
$[AO^+] = 0.054 \times 10^{-4}$mol.dm^{-3}, [Iodine] $= (0.05\text{-}5.0) \times 10^{-3}$mol.dm^3, [NaOH]$=0.4$mol.dm^{-3}, [KCl]$=0.05$mol.dm^{-3},Temperature$= 303K$

Fig. 2: A plot of log k vs log [I$_2$] for oxidation of AO$^+$ with variable concentration of Iodine.
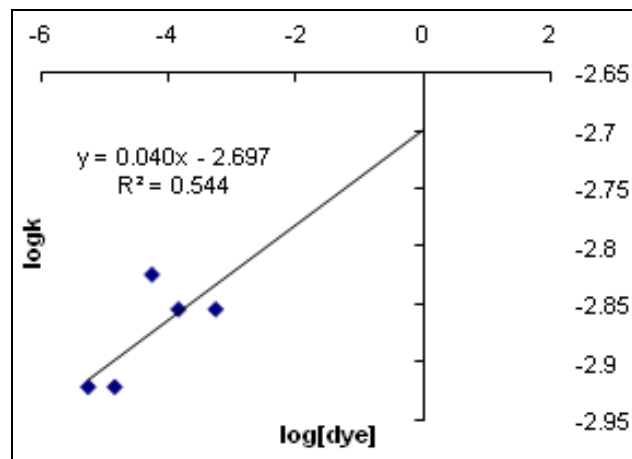
$[AO^+] = (0.054\text{-}5.4) \times 10^{-4}$mol.dm^{-3}, [Iodine] $= 1 \times 10^{-3}$mol.dm^{-3}, [NaOH] $= 0.4$ mol.dm^{-3}, [KCl] $= 0.05$ mol.dm^{-3}, Temperature $= 303K$

Fig. 3: A plot of log k. vs log dye concentration for oxidation of AO+ with Iodine.

RESULTS

Preliminary experiments

For the study of oxidation kinetics of the reactions between alkaline iodine and selected Acridine orange preliminary kinetic experiments were conducted to test the suitability of iodine as an oxidizing agent spectrophotometrically. The oxidation efficiency of the alkaline iodine process was evaluated via double beam UV/vis Schimadzo 180 A spectrometer at λ_{max}=491 nm

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and order of reaction was determined through plots of variable with respect to time for AO. No interference of oxidative products was reported throughout the experiment.
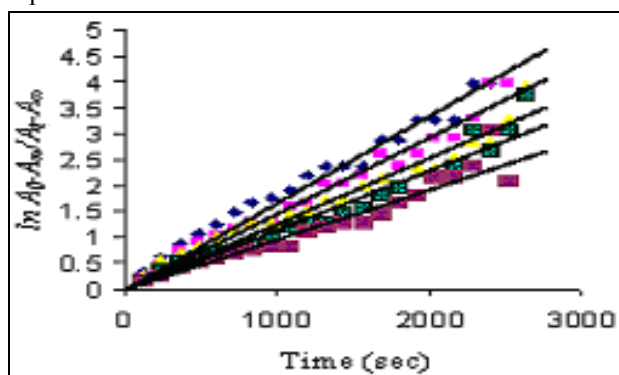
$[AO^+]$ = 0.054 × 10^{-4}M, [Iodine] = 1× 10^{-3}M, [NaOH] = (0.2-1.0) M, [KCl] = 0.05 M, Temperature = 303K

Fig. 4: A plot of ln A_0-A_∞/A_t-A_∞ vs Time for oxidation of AO+ with variable concentration of NaOH.
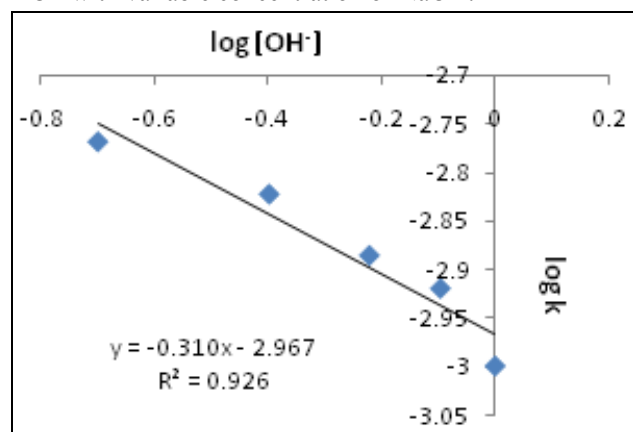

$[AO^+]$ =(0.054-5.4) ×10^{-4}mol.dm^{-3}, [Iodine] =1×10^{-3}mol.dm^{-3}, [NaOH] =0.4 mol.dm^{-3}, [KCl] =0.05 mol.dm^{-3}, Temperature =303K

**Fig. 5**: A plot of log k vs log[OH$^-$] showed linear dependence rate constant on OH$^-$ion concentration

Rate constants of reactions between iodine and dye

The redox reaction of AO^+ was carried out under conditions having an aqueous alkaline iodine solution with $[AO^+]$ =5.4x10^{-5} mol.dm^{-3}, $[I_2]$ =1x10^{-3} mol.dm^{-3}, [NaOH] =0.4mol.dm^{-3} and [KCl] =0.05mol.dm^{-3}. The concentration of iodine was varied over a wide range, while other reactants were keep constants. Plot of rate constant log k versus log $[I_2]$ (table 1, fig. 2 Kindly put Fig 2 and Table here) is found to be a straight line with intercept, showed fractional order kinetics. This fractional order may be due to the oxy species of iodine in alkaline medium. The low value of the rate constant may be related with fragmentation of the dye complex into small component during oxidation (Azmat *et al.,* 2013). The plot of $1/k_{obs}$ against 1/ $[I_2]$ gives straight line with intercept (fig. 3 Kindly put Fig 3 here) showed the formation of intermediate in the reaction which later on

confirmed by GC mass Analysis. The decoloration kinetics of AO was studied at various initial concentrations of AO as an application point of view of this investigation, and the results were shown in table 2. It can be seen that the rate constant is almost independent of the concentration of dye as AO concentration increased from 0.054 to 0.54 × 10^{-4} mol.dm^{-3}, the decolorization efficiency of AO within 60 min of reaction fell from 65.0% to 34.6%. A plot of the rate constant k of reaction versus the concentration of the AO solution showed a fractional order for AO (0.544) which indicated that the rate of reaction is partially depend upon the concentration of the AO^+ solution (Woittiez *et al.,* 1991). Bichsel and Gunten 1999, reported that the kinetics of oxidation of hypoiodous acid with phenolic compound in aqueous solution in the pH range of 2-12 followed second order whereas present reaction showed approximate second order reaction (first order with respect to iodine and fractional order with respect to dye), it can be assumed that a similar behavior of iodine in alkaline medium with AO like bromination of phenolic compound was reported (Oswald *et al.*, 1989).
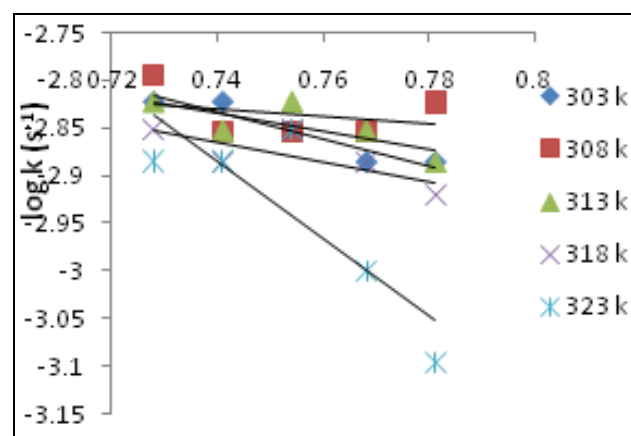

Fig. 6: A plot of logk vs √μ for oxidation of AO+ with Iodine at various temperatures.
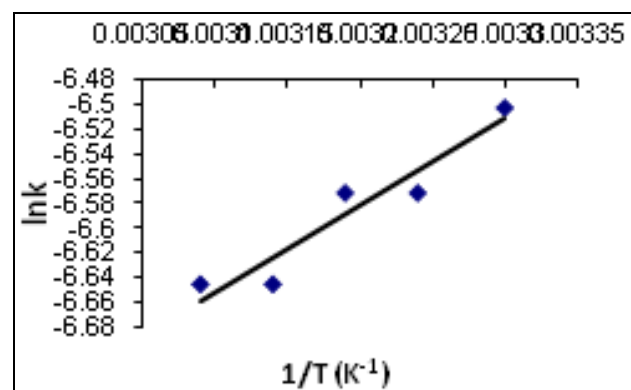
**Fig. 7**: A plot of lnk and I/T for oxidation of AO with aqueous alakine Iodine

Regression analysis based on the pseudo-first order reaction kinetics for the decolorization of AO in alkaline

iodine solution conducted and the results were shown in (table 2, fig. 3). The apparent 1st order rate constant exhibited a pH-dependence, which might be elucidated by the species of iodine only in alkaline aqueous solutions, and it can be hypothesized that the initial oxidation of AO was 1st order reaction with respect to iodine. The low value of the rate constant may be related with fragmentation of the dye complex into small component (table 2) (Nazami et al 2005). It can be seen that the decolorization efficiency of AO fell as AO concentration rose. This is because increasing concentration of the dye molecule may absorb all photon of light or may be due to a relatively lower concentration of •OH results from the increasing concentration of AO or scavenging of iodine decreasing of the decolorization efficiency of AO.
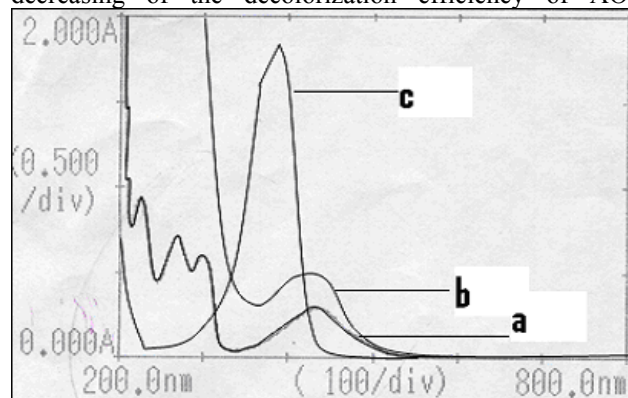
Fig. 8: Spectral evideance of demathylation of acridine orange with alkaline iodine
a= AO, b= AO M, c = (Intermediate complex AO,MM I_3)
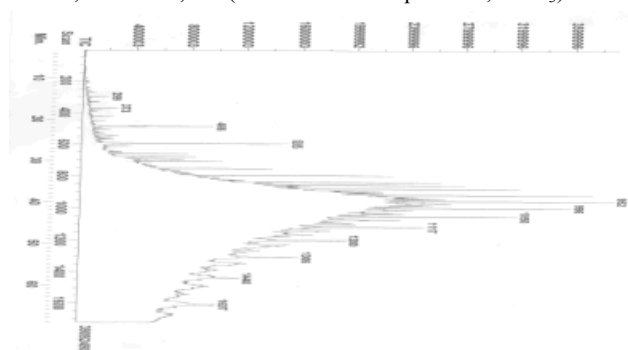

Fig. 9: GC chromatogram of reaction mixture of AO and I_2

Effect of the pH on oxidation

The effect of the pH was monitored by adding HCl and NaOH (table 3) but no reaction was observed in the low pH range therefore the possibility of IO_3^- in the rate determiming step is role out because IO_3^- was effective oxidizing species at low pH. Literature reported three main species (IO_3. HOI and I_3^-) existed in alkaline solution in which HOI was the most effective for oxidizing the organic compounds like sugar (Knolle 1975; Ruscic & Berkowitz 1994; Wong & Cheng 2001; Chen et al 2009; Khaled et al 2011). This species analogy to HOCl and very effective at high pH where as triodate act

as a reservoir of oxidation capacity (Woittiez *et al.,* 1991) and HOI immediately begins to disproportionate according to following equation.

$$3HOI \leftrightarrow IO_3^- + 2I^- + 3H^+ \text{ (disproportionation)} \quad (1)$$
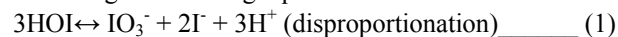
leads to iodide and proton and reverse reaction is not possible where it generates molecular iodine because reaction mixture was color less at the end of reaction attributed to that of the hypoiodous acid dissociates into ($HIO = H^+ + IO^-$), dye dissociated into phenoxide ion (PhO^-), and total concentrations of iodine ($[I_2]_t$) (Woittiez *et al.,* 1991)

$$I_2 = [IO_3] + [HOI] + [I_3] \quad (2)$$

It was observed that when concentration of alkali increased from 0.2 to 1.0 M (fig. 4), decolorization of AO^+ was significantly influenced by the medium of the reaction, and best decolorization efficiency was obtained at pH (13.602). Whereas the values of the rate constant "k "were decreases with increase in concentration of alkali. This is due to in higher alkaline medium; the concentration of hypoiodite is increases which is a slower oxidizing agent than hypoiodous acid and the rate of disproportionation of hypoiodite decreases. Moreover the values of the rate constant (k) were decreases with increase in decoloration efficiency of the dye, which may be due to degradation of the AO showing that alkaline iodine was effective in oxidation leading to the degradation of dye. Plot of $[OH^+]$ versus 'k' is linear (fig. 5 put fig 5 here) showing the dependence of oxidation on HOI. Plot of log $[OH^-]$ versus log k is linear and order with respect to $[OH^-]$ is fractional (fig. 5) reflecting complex nature of reaction and the dependence of oxidation on HOI.

Effect of ionic strength on oxidation of AO

The influence of ionic strength on the rate constant is also examined by adding fluctuating concentrations of KCl to the reaction mixture, keeping all other parameters constant and results presented in the table 4 put table 4 here. Effect of salt electrolyte on the decoloration kinetics of AO salts play an important role in dyes reaction and they also coexisted in the dyes effluent and impact on dye waste water treatment (Azmat *et al.,* 2013). In the presence of iodine in alkaline medium the decoloration of AO showed inverse relation where decolorizing efficiency of reaction decreases as a consequence of increasing concentration of salt electrolyte from 0.01-0.09 mol.dm^{-3}. The inhibitive effect of salt electrolyte on the decoloration of AO can be explained by the scavenging of I_3^- species in complex formation with the AO^+ according to the following equation. The plot of log k against $\sqrt{\mu}$ at different temperature in the range of 303K to 323K is linear as shown in (fig. 6 put fig 6 here) with slop equal to 1.02 $Z_A Z_B$. The sign and value of the slope was evaluated and it equal to -1. The value of the slope suggests that in the rate determining step two singly oppositely charged species are involved which may be of I_3^- and AO^+ as triodide ion partially present in iodine and iodide ion.

Table 1: Effect of concentration of Iodine on Rate of Oxidation of Acridine Orange.

Concentration of Iodine x 10^3 mol dm⁻³	Specific rate constant (k_{obs}) x10^3s⁻¹	Velocity υ x 10^6	% decoloration	$1/(k_{obs})$	log k_{obs}	log [Iodine]
0.05	0.8	-3	10	1250	-3.09	-4.30
0.1	1.1	-7	19.6	909.09	-2.95	-4
0.5	1.3	-10	39.6	769.23	-2.88	-3.30
1.0	1.5	-9	34.6	666.66	-2.82	-3
5.0	1.7	-8	29.32	588.23	-2.76	-2.30

[AO⁺] =0.054×10^{-4}mol.dm⁻³, [Iodine] =(0.05-5.0)×10^{-3}mol.dm⁻³, [NaOH]=0.4mol.dm⁻³, [KCl]=0.05mol.dm⁻³, Temperature =303K

Table 2: Kinetic parameters (rate of reaction, rate constants and % decoloration) for Iodine oxidation of acridine orange at various initial concentrations of dye

Concentration of [AO⁺]¹x10^4 mol dm⁻³	Specific rate constant (k_{obs}) x10^3s⁻¹	Velocity υ x 10^5	% decoloration	$1/(k_{obs})$	log k_{obs}	log [AO⁺]
0.054	1.2	-0.4	65	833.33	-2.92	-5.26
0.14	1.3	-0.5	60	769.23	-2.88	-4.85
0.54	1.5	-1.0	44.95	666.66	-2.82	-4.26
1.4	1.4	-2.0	34.56	714.28	-2.85	-3.85
5.4	1.4	-30	45.77	714.28	-2.85	-3.26

Table 3: Kinetic parameters (rate of reaction, rate constants and % decoloration) for Iodine oxidation of acridine orange at various initial concentrations of Alkali

Concentration of NaOH mol dm⁻³	Specific rate constant (k_{obs})x10^3s⁻¹	Velocity υ x 10^5	% decoloration	$1/(k_{obs})$	[H⁺] $\times 10^{-13}$
0.2	1.7	-1	45.68	588.23	2.00
0.4	1.5	-1	43.2	666.66	3.99
0.6	1.3	-1	44.34	769.23	5.99
0.8	1.2	-1	40.18	833.33	7.99
`1.0	1.0	-1	33.85	1000	10.00

Table 4: Effect of Ionic Strength on Rate of Oxidation of AO with Iodine.

Concentration of KCl mol dm³	Specific rate constant (k_{obs}) x10^3s⁻¹	Velocity υ x 10^5 mol/dm³/s	% decoloration	$1/(k_{obs})$	log k_{obs}	$\sqrt{\mu}$ mol$^{0.5}$.dm$^{-3/2}$
0.01	1.5	-1	45.45	666.66	-2.82	0.728
0.03	1.5	-1	42.97	666.66	-2.82	0.741
0.05	1.4	-1	42.85	714.28	-2.85	0.754
0.07	1.3	-1	47.27	769.23	-2.88	0.768
0.09	1.3	-1	45.09	769.23	-2.88	0.781

[AO⁺]=0.054 × 10^{-4}mol.dm⁻³, [Iodine] =1× 10^{-3}mol.dm⁻³, [NaOH] =(0.2-1.0) mol.dm⁻³, [KCl] =0.05 mol.dm⁻³, Temperature=303K

Table 5: Thermodynamics Activation parameters at various ionic strength for oxidation of AO

$\sqrt{\mu}$ (mol.dm⁻³)$^{0.5}$	E_a kJ.mol⁻¹	ΔH^* kJ.mol⁻¹	ΔS^* J.mol⁻¹.K⁻¹	ΔG^* kJ.mol⁻¹
0.728	-6.537	0.0325	-197.526	59.882
0.741	-5.786	0.0365	-197.514	59.883
0.754	-42.368	0.0528	-197.462	59.830
0.768	-9.250	0.0325	-197.530	59.884
0.781	-18.572	-1719x10^{-3}	-197.641	59.885

Table 6: Constant for effect of salt electrolyte at various Temperatures

Temperature (K)	Slope	$Z^A Z^B$
303	-1.4247	-1.396
308	-0.4144	-0.406
313	-0.9506	-0.931
318	-1.0111	-0.991
323	-4.0411	-3.961

Table 7: -N-demethylation Intermediates

GC chromatogram Peaks	N-demethylation Intermediates	Abbreviation	ESI-MS Peak (m/z)
I	Acridine Orange	AO	265
II	N-de-mono-methyl Acridine Orange	AO-M	251
III	N-N'-de-dimethyl Acridine Orange	AO-MM	237
IV	N,N,N'-de-trimethyl Acridine Orange	AO-DM	223
V	N,N,N',N'-de-tetramethyl Acridine Orange	AO-DD	209

Table 8: Degradation Products of Acridine Orange

GC chromatogram Peaks	Degradation Products	ESI-MS Peak (m/z)
A	N,N-dimethyl hydrogen Iodide	172
B	Methyl Iodide	142
C	Ethyl Iodide	156
D	Methyl Diiodide	268
E	Propyl Iodide	170
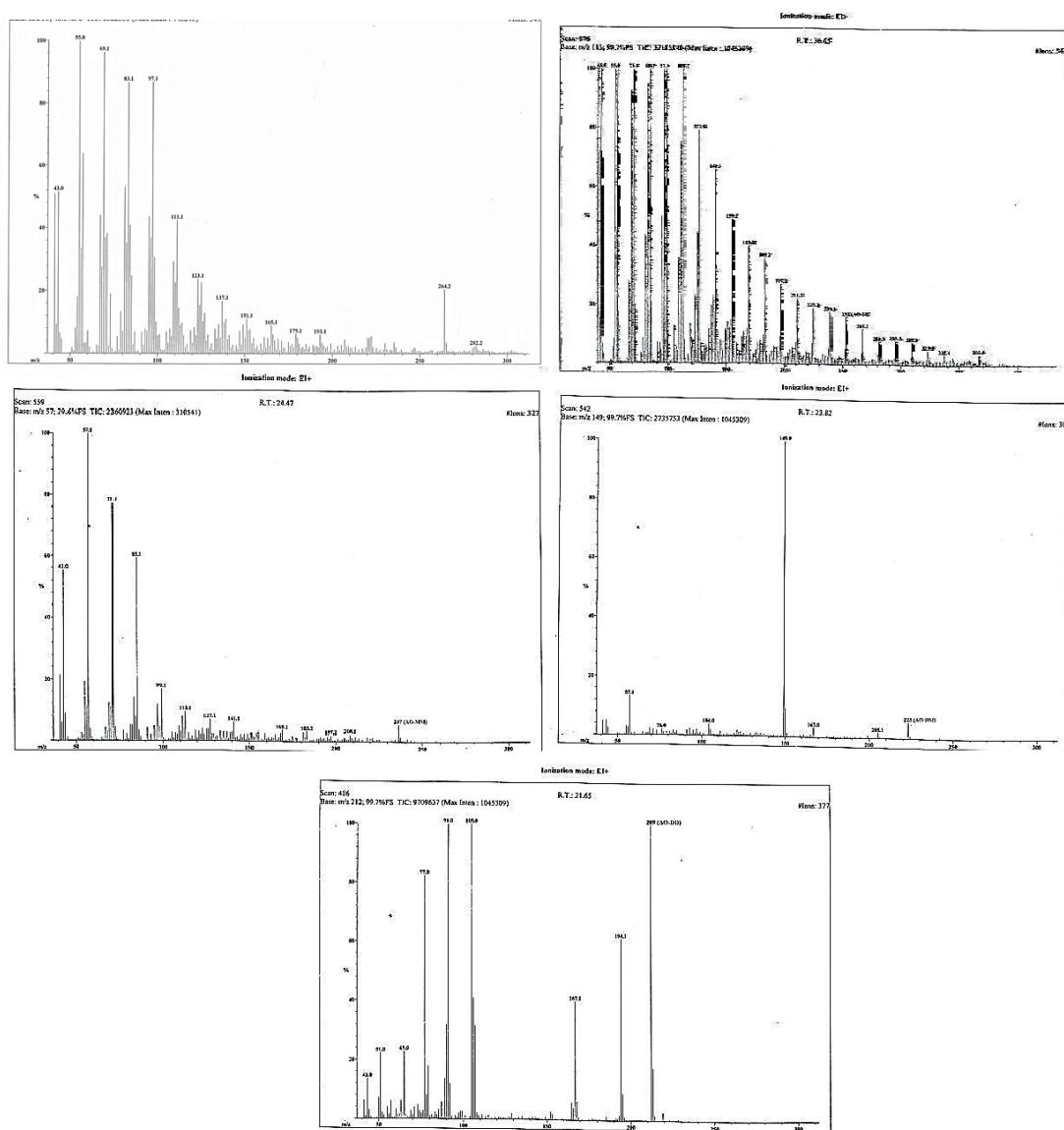
Fig. 10: Mass chromatogram of N-demethylation products

Fig. 11: Mass chromatogram of degradation products

$$\overset{+}{AO} + \overset{-}{I_3} \xrightarrow{\ k_{10}\ } [\ \overset{+}{AO} \text{----------} \overset{-}{I_3}\ ]$$

The effect of ionic strength on the rate constant was also investigated at different temperature in the range of 303K to 323K (table 5 put table 5 here) and plotted as log k versus $\sqrt{\mu}$ (fig. 6 put fig 6 here) showing is linear one. The value of $Z_A Z_B$ were calculated from the slopes are given in table 6 put table 6 here. These values also indicate that the reaction is in between two singly oppositely charged species.

The oxidation reaction of AO was monitored at temperatures range of 303K to 323K and a plot ln k versus 1/T, using an Arrhenius equation was shown in (fig. 7 put table 7 here). The graph is linear and, the value of E_a is calculated from the slope. The values of activation parameters such as energy of activation (E_a), entropy of activation (ΔS), enthalpy (ΔH) and Gibbs free energy (ΔG) of activation were determined at various ionic strengths and presented in the table 5 fig. 7. The value of

entropy of activation is negative which may be attributed that entropy decreases upon accomplishing the transition state. Hence the transition state in the region of activated complex state has a more ordered or more rigid structure than the reactants in the ground state, while positive value of ΔG indicated that product have more free energy than reactants and supposed to as energy storing molecules. The values of thermodynamics parameters showed highly solvated state of the dye complex which indicates stronger hydration for AO ---I complex which later on dissociated into several smaller components due to which nonlinear values of activation parameters were reported. These values related with oxidation of the dye including demethylation as well as iodo-formation of methyl group, which indicate that iodine is major participant of oxidation kinetic of AO instead of OH radical. Absorption spectrum of dye mixture recorded and presented in fig. 7 put fig.7 here clearly indicates the decolouring efficiency of aqueous alkaline iodine followed by complex formation.

### Separation and identification of the dye fragments
In order to understand the role of aqueous alkaline iodine in oxidation of AO, reaction mixture was subjected to GC Mass analysis (figs. 8-11 put fig 8-11 here). The oxidation pathway of acridine fragmentation of ethyl acetate extracted compound display in figs. GC chromatogram showed of 60 minutes scan and reported at least 12 main compounds several different molecular ions peak whereas mass peaks showed a very complex kinetics and reported numerous intermediates and the intensities of the some peaks increased at first and subsequently decreased, indicating the formation of complex and their degradation. Spectral analysis of some peaks showed that a series of N de-methylated intermediates (Azmat *et al.,* 2013, Chen *et al.,* 2002) were confirmed through their molecular masses and absorption spectrum(fig.8). Mass spectrum of scan 1306 showed the low intensity peak of 442.4 that related to that of demethylated AO (Nidheesh *et al.,* 2013) with iodine species (table 7 put table 7 here). Similarly scan of 1200, 117,1050, 996, 951-953,914 m/z, showed mass peaks ranges from 470,400 showed complex reaction kinetics and solvated dye molecule with iodide species. UV/ Visible spectrum also showed a complex formation followed by degradation of complex in to several components (table 8 put table 8 here)

Scan 299 showed high intensity peaks at 162 and 147 m/z may confirm the smaller fragment of AO. That may be of methyl and ethyl iodide in the clear reaction mixture at the end of reaction. These are the compounds, produced in enormous amounts valued to be more than 214,000 tons annually via algae and help in the world's moderate seawaters and in reduced quantities on terrestrial due to native fungi and bacteria use in organic synthesis and approved as a pesticide by the United States Environmental Protection Agency in 2007 (Knolle 1975; Ruscic & Berkowitz 1994; Wong & Cheng 2001; Khaled

2011). Iodomethane which may be apart of complex degradation of AO-I_3 is also a pre-plant biocide commonly used to control insects, plant parasitic nematodes soil borne pathogens, and weed seeds. The compound is registered for use as a pre plant soil treatment for field grown strawberries, peppers, tomatoes, stone fruits, tree nuts, grape vines, ornamentals and turf and nursery grown strawberries, stone fruits, tree nuts, and conifer trees as reported in literature (Wong & Cheng 2011,). Methyl iodide is used as an intermediate in the manufacture of some pharmaceuticals and pesticides, in methylation processes, and in the field of microscopy (www.scribd.com/doc/30121795/Iodine-and-Iodine-Compounds*).*
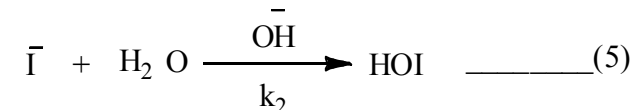
Reaction pathway for alkaline iodine oxidation of AO
The possible reaction mechanism involve between I_2 and its species in aqueous alkaline medium with AO may be as follows
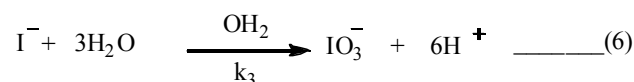
$$I_2 + \text{N---H} \xrightarrow{k_1} \text{N---I} + \overset{+}{H} + \overset{-}{I} \quad (3)$$
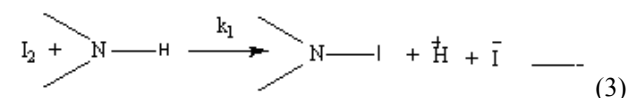
In aqueous alkaline medium, total concentration of iodine is

$$I_2 = [\ I\overline{O}_3] + [HOI] + [\overline{I}_3] \qquad (4)$$

And

$$\overline{I} + H_2O \xrightarrow[k_2]{\overline{OH}} HOI \qquad (5)$$

HOI is reported as strong oxidizing agent at high pH

$$I^- + 3H_2O \xrightarrow[k_3]{OH_2} IO_3^- + 6H^+ \qquad (6)$$
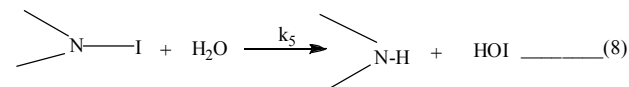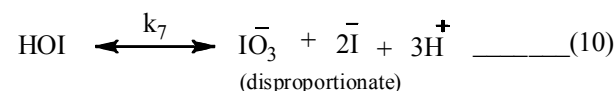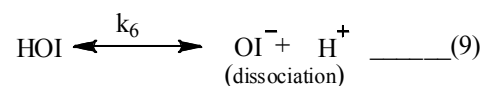
IO_3^- work as oxidizing agent at low pH

The total triiodide in the reaction mixture was existed in equilibrium state with molecular iodine and iodide ion

$$I^- + I_2 \xrightarrow{k_4} \overline{I}_3 \qquad (7)$$
$$\text{(act as a reservoir of oxidation)}$$
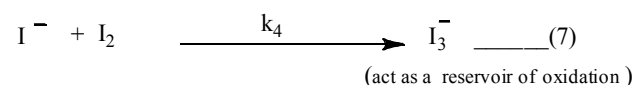
Hydrolysis of N__I gives

$$\text{N---I} + H_2O \xrightarrow{k_5} \text{N-H} + HOI \qquad (8)$$

Disproportionate of HOI gives

$$HOI \xrightleftharpoons{k_6} O\overline{I} + H^+ \qquad (9)$$
$$\text{(dissociation)}$$

$$HOI \xrightleftharpoons{k_7} I\overline{O}_3 + 2\overline{I} + 3H^+ \qquad (10)$$
$$\text{(disproportionate)}$$

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Oxidation of AO take place via complex formation with various species of iodine proved through GC mass analysis which also showed a complex kinetics

Therefore I$_3$ species was the highly effective species in rate determining step and it also act as reservoir of iodine for oxidation of various organic compounds
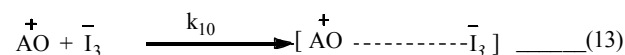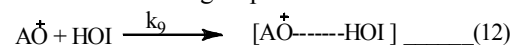
Reaction pathway for degradation of Acridine orange **This the reaction pathway put this before conclusion**
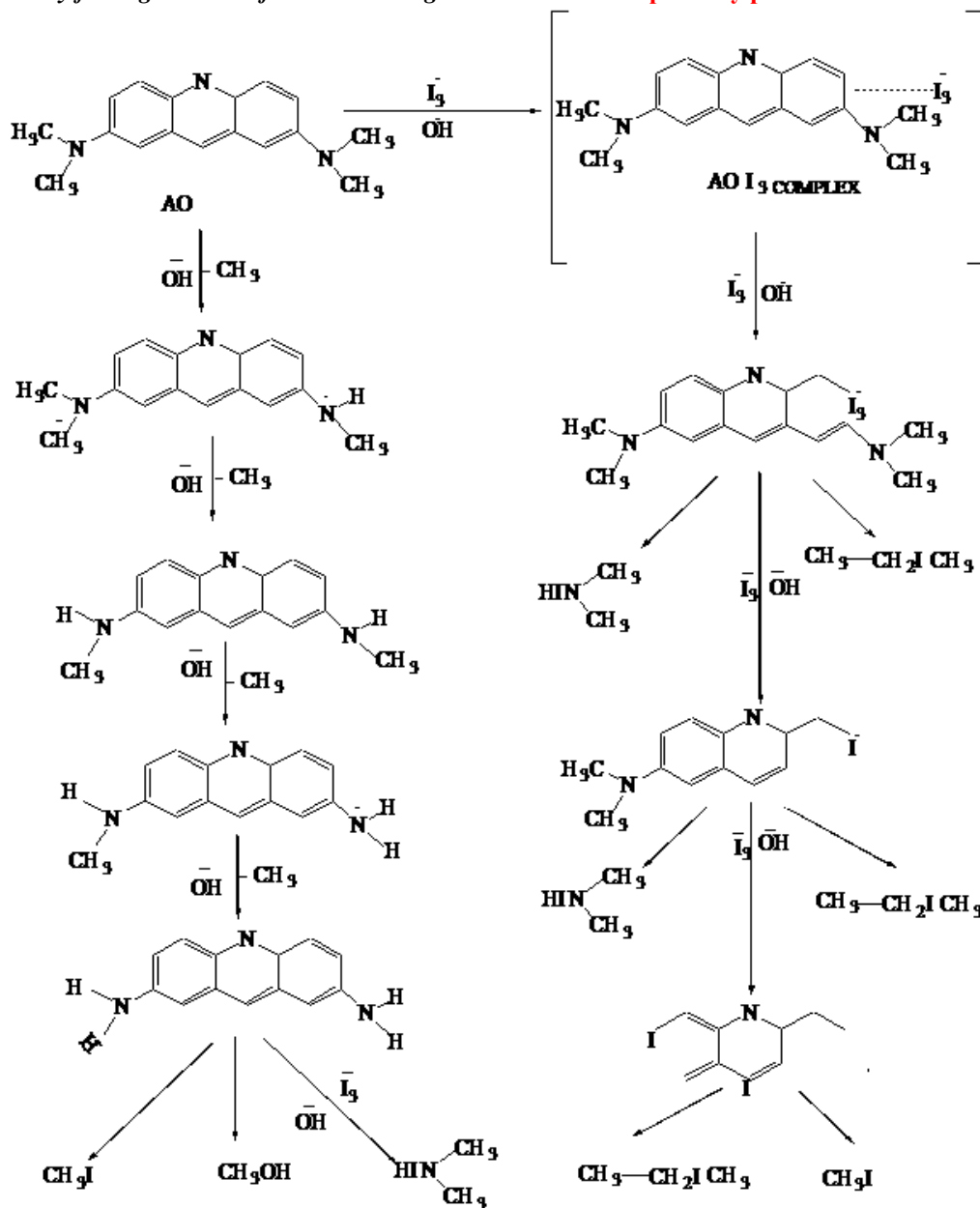
The probability of Acridine orange may react with IO$_3^-$ to form complex is less because this species is effective at low pH therefore we cannot consider this species as a highly reactive negative species in rate determining step

$$\overset{+}{AO} + \overset{-}{IO_3} \xrightarrow{k_8} [\; \overset{+}{AO} \text{----------} \overset{-}{IO_3} \;] \quad \underline{\quad}(11)$$
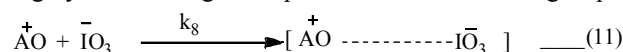
As HOI is a molecular effective oxidizing species in rate determining step but salt effect suggest no neutral species in rate determining step

$$A\overset{+}{O} + HOI \xrightarrow{k_9} [A\overset{+}{O}\text{------}HOI] \underline{\quad\quad}(12)$$

$$\overset{+}{AO} + \overset{-}{I_3} \xrightarrow{k_{10}} [\; \overset{+}{AO} \text{----------}\overset{-}{I_3} \;] \underline{\quad\quad}(13)$$

Rate law

Rate law for the oxidation of acridine is now proposed based on above data

$$- d[I_2]/dt = - k_8[A\overset{+}{O}]^{1/2} [\overset{-}{IO_3}] - k_9[A\overset{+}{O}]^{1/2} [HOI] - k_{10}[A\overset{+}{O}]^{1/2} [\overset{-}{I_3}] \quad\quad\underline{\quad}(14)$$

The two species of iodine in alkaline medium are less effective therefor the rate law become

$$- \{d[I_2]/dt = - [A\overset{+}{O}]^{1/2}\{k_8\;[\overset{-}{IO_3}] + k_9[HOI] + k_{10}\;[\overset{-}{I_3}]\} \underline{\quad\quad}(15)$$

$$- \{d[I_2]/dt = - k_{10} [A\overset{+}{O}]^{1/2} [\bar{I}_3]\} \quad \underline{\hspace{2cm}}(15)$$

$$\{d[I_2]/ [I_3] = - dt.k_{10} [A\overset{+}{O}]^{1/2}\} \quad \underline{\hspace{2cm}}(16)$$

Change in concentration of I_3 was directly proportional to AO concentration this completely showed that dye formed complex with iodide ion and dissociated in to the smaller through phenazine ring. Following pathway based on GC mass analysis as described by Xie (2011). Kindly put reaction pathway here

CONCLUSION

It was concluded that oxidation of AO in alkaline iodine aqueous medium appears to have the capability to entirely decolourize the dye followed by degradation in a nontoxic components. The outcomes of this research suggested that more searches should be under taken with other dyes to validate the reaction credibility as a best future advanced oxidation technique, which can save the running streams from colorant material without sludge.

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