Bull. Chem. Soc. Ethiop. 2005, 19(1), 103-116.
Printed in Ethiopia
ISSN 1011-3924

KINETICS AND MECHANISM OF REACTION OF ACIDIC CHLORITE WITH PHENOXAZINE DYES, NILE BLUE AND MELDOLA'S BLUE

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(Received July 8, 2004; revised January 28, 2005)

ABSTRACT. The kinetics and mechanism of the oxidation of two phenoxazine dyes namely Nile blue (7-amino-3-diethylamino-8,9-benzo phenoxazine chloride, NB^+) and Meldola's blue (3-dimethylamino-8,9-benzo phenoxazine chloride, MB^+) with acidic chlorite and hypochlorous acid have been investigated using a UV-visible and a stopped flow equipment. For both Nile blue and Meldola's blue reactions the rates have first-order dependence on each substrate, chlorite and acid. Both reactions showed negative salt effect indicating the reaction is between the oppositely charged species, likely the substrate cation and chlorite anion. The acidic chlorite reaction with MB^+ was very slow compared with NB^+ and was studied at higher temperature of 40 °C. The overall third order rate constants for the reaction of acidic chlorite with Nile blue and Meldola's blue were (0.363 ± 0.005) $M^{-2}\ s^{-1}$ at 25 °C and (3.09 ± 0.08) x $10^{-3}\ M^{-2}\ s^{-1}$ at 40 °C, respectively. The energy parameters for NB^+ reaction were E_a = 47.8 kJ mol^{-1}, $\Delta H^{\neq}$ = 40.4 kJ mol^{-1} and $\Delta S^{\neq}$ = -233 J $K^{-1}\ mol^{-1}$, while the corresponding values for MB^+ reaction were 62.4 kJ mol^{-1}, 54.6 kJ mol^{-1} and -248 J $K^{-1}\ mol^{-1}$, respectively. The second-order rate coefficients for HOCl reaction with Nile blue and Meldola's blue at 25 °C were (5.14 ± 0.01) x $10^3\ M^{-1}\ s^{-1}$ and (1.25 ± 0.03) x $10^2\ M^{-1}\ s^{-1}$, respectively.

KEY WORDS: Oxidation of phenoxazine dyes, Nile blue, Meldola's blue, Acidic chlorite, Hypochlorous acid

INTRODUCTION

The bright colours associated with organic dyes is attributed to the presence of conjugated π-bonds [1]. The characteristic spectral properties of the chromophoric compounds are ascribed to the magnitude of the energy gap between the highest occupied molecular π-orbital (HOMOs) and the lowest-unoccupied molecular π*-orbitals (LUMOs). The energy gap becomes narrower as the number of conjugated π-bonds and allylic non-bonding electrons increases. In organic dyes where the conjugation is extensive, the energy needed to effect electron promotion from the HOMOs to LUMOs falls within the UV-visible electromagnetic radiation range, hence the bright colours [2]. Nile blue (3-amino-7-diethylamino-8,9-benzo phenoxazine chloride, NB^+) and Meldola's blue (7-dimethylamino-8,9-benzo phenoxazine chloride, MB^+) belong to phenoxazine class of dyes. The phenoxazine back bone has no colour, but both the dyes are coloured and the difference in λ_{max} values is due to the number and nature of auxochrome (functional groups that have no chromophoric properties, but can affect spectral qualities of chromophores by induction or mesomeric shift of electrons) substituents on the backbone. Nile blue has λ_{max} at 633 nm {ϵ_{633} = (5.22 ± 0.05) x $10^4\ M^{-1}\ cm^{-1}$}, while Meldola's blue has λ_{max} at 570 nm {ϵ_{570} = 3.26 ± 0.04) x $10^4\ M^{-1}\ cm^{-1}$}. The π-bonds of phenoxozine backbone and nonbonding electron pairs on the auxochromes are quite sensitive to changes in the chemical environment and can lead to disruption of the π-conjugation. The spectral properties of the chromophores can be dramatically altered by this disruption.

The reactivities Nile blue and Meldola's blue with chlorite in acidic solution, as expressed by their kinetics are investigated. Nile blue is known for its staining ability. It stains tumours selectively and has some growth-retarding action. This staining property is achieved by boiling

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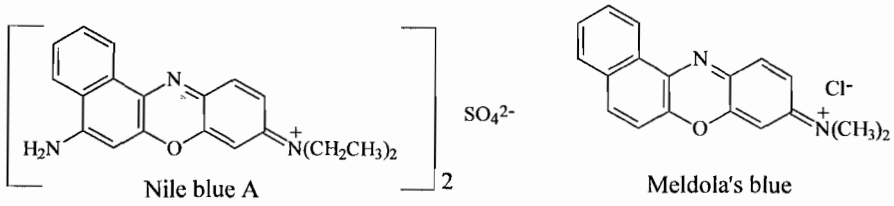
Nile blue with sulphuric acid and forming a red staining lysochrome called Nile red. It has been found to prolong the survival time of mice infected with bovine tuberculosis [3]. Meldola's blue is known as one of the electron carriers in the determination of dehydrogenase using tetrazolium salt. It can also be used for detecting metal halide anions [4]. The structures of Nile blue and Meldola's blue are shown below:



Nile blue A Meldola's blue

The amino group at 3rd position has diethyl- and dimethyl- substitutions in Nile blue and Meldola's blue, respectively, the amino group at 7th position in the Nile blue is of curiosity. It is of interest to investigate, how these differences affect the reaction rates of these dyes with acidic chlorite.

EXPERIMENTAL

Materials

Nile blue (Aldrich), Meldola's blue (Sigma), sodium sulphate, ruthenium(III) trichloride trihydrate (Aldrich) and sulphuric acid (Merck) were used as supplied. Sodium chlorite (Fluka) was recrystallised from water-ethanol-acetone mixture [5]. Solutions were prepared with double distilled and deionised water.

Methods

All kinetic experiments were conducted at constant ionic strength with excess concentrations of acid and chlorite and low concentration of the substrates and monitoring the depletion kinetics of the substrates for at least two half lives, either using a Cary 50 - UV Visible spectrometer or a Hi-Tech SF-61 DX2 micro volume double mixing stopped flow apparatus.

Product analysis and stoichiometry

The major oxidation products of both reactions were characterised. A 250 mg of dye sample was dissolved in 250 mL of water, acidified with 100 mL of 5.0 M sulphuric acid and reacted with 250 mg of sodium chlorite dissolved in 150 mL of water. The reaction was allowed to stand for 24 h. The precipitated organic component was filtered and the precipitate was dried over the silica gel before further analysis. The filtrate was neutralised with sodium bicarbonate and a precipitate was obtained. The TLC of this precipitate had the same R_f value and colour to that of the first precipitate mentioned earlier. The two precipitates were then combined. The precipitate was dissolved in minimum amount of acetone before separation. A silica gel column was used for the separation of these compounds using 8:2, hexane:ethyl acetate as a solvent. One major product was separated. The product was analysed using both ^1H and ^{13}C NMR spectroscopy. The ^{13}C NMR spectra of the starting material and major products showed that in both reactions heterocyclic ring structure is intact and reactions were restricted to dealkylation only. Further,

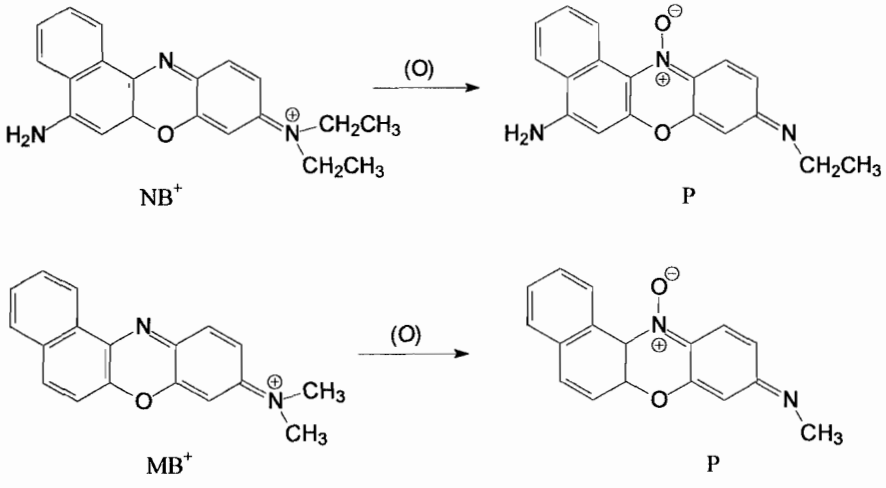
the recrystallised reaction products for both the reactions were colourless and showed an intense absorption peak between 1300-1000 cm^{-1}, suggesting N-O stretching and probably the N-oxide as the product [6, 7]. The high-resolution mass spectrum of one product gave m/z 305 (M^+, 10%). Significant peaks were observed at m/z 262, 246, 140 and 43, suggesting that the major oxidative product is the de-ethylated N-oxide derivative of Nile blue [8]. The major product of the Meldola's blue reaction with acidic chlorite had m/z 276 (M^+, 15%). Significant peaks were observed at m/z 247, 166 and 99, suggesting that the major oxidative product is the de-methylated N-oxide derivative of Meldola's blue. N-oxide formation from oxidation of pyridines by hydrogen peroxide was reported in the literature [9], as is formation of de-methylated products upon oxidation of phenothiazine dyes [10, 11]. Further, the TLC investigation of the reaction products, using benzene and ethyl acetate in 6:3 ratio as eluent, with a reference standard showed the presence of acetic acid as an oxidation product in the Nile blue reaction and formic acid as product in the Meldola's blue reaction.

The stiochiometry of both reactions were established based on the reactions of dyes with 1:10 and 1:20 molar ratio of chlorite and excess acid. After 6 h reaction, while the residual concentrations of the dyes and the amount of chlorine dioxide formed were measured photometrically, the residual chlorite was estimated by iodimetric titration.

It is observed that both dyes reacted with chlorite in 2:3 ratio resulting in 6 electron oxidation of the reaction products, but due to the disproportionation of chlorite to chlorine dioxide and chloride during the process, the basic stoichiometry results in 1:9 ratio of dye to chlorite. Further, the stoichiometry was observed variable and the amount of chlorine dioxide depended significantly on the initial concentration of chlorite and marginally on the initial acid concentration. Based on the ratios of the reacted reactants and the major products identified, the stoichiometric equations may be represented as follows:

$$NB^+ + 9ClO_2^- + 5H^+ \rightarrow P + CH_3COOH + 6ClO_2 + 3H_2O + 3Cl^-$$

$$MB^+ + 9ClO_2^- + 5H^+ \rightarrow P + HCOOH + 6ClO_2 + 3H_2O + 3Cl^-$$



NB$^+$ P

MB$^+$ P

In both the equations, P represents the dealkylated N-oxides of the dyes, namely (7-amino-3-ethylamino-8,9-benzo phenoxazinium 10-N-oxide) for Nile blue and (3-methylamino-8,9-benzo phenoxazinium 10-N-oxide) for the Meldola's blue reaction.

RESULTS AND DISCUSSION

In view of the ease and advantage in analysing the data, all the kinetic experiments were done under pseudo first-order conditions using $\{[H^+] > [ClO_2^-]\} >> [NB^+]$ or $[MB^+]$. The Nile blue-acidic chlorite reaction was studied at (25.0 ±0.1) °C. At that temperature, even with very high acid concentration, the reaction of Meldola's blue with chlorite was very slow. Hence, all reactions involving MB^+ were studied at (40.0±0.1) °C. Both the reactions showed exponential decay and first-order dependence on the dyes. Figures 1 and 2 show respectively the typical absorbance versus time plots of the depletion of Meldola's blue and Nile blue at varied chlorite concentrations. The plots of ln absorbance *versus* time were good straight lines with correlation coefficient, $R^2 = 0.99$, confirming that the reactions follow pseudo first-order kinetics with respect to dyes and the order with respect to both Nile blue and Meldola's blue are unity each.

Figure 3 illustrates the typical repetitive spectra of the reaction between Nile blue and acidic chlorite, which shows exponential decay of the substrate and accumulation of chlorine dioxide with characteristic wave like structure and an absorption maxima at 325 nm. Chlorine dioxide is relatively unreactive and stable at low pH.

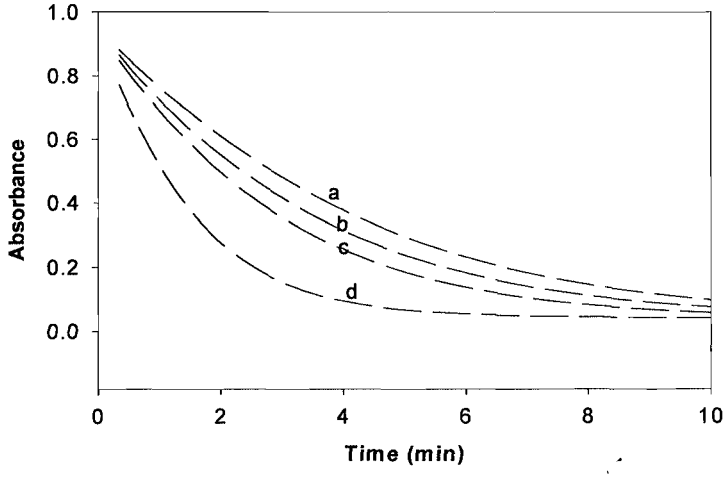


Figure 1. Typical kinetic curves showing effect of chlorite on Meldola's blue depletion at 633 nm. $[MB^+] = 2.0 \times 10^{-5}$ M, $[H^+] = 2.0$ M and $[ClO_2^-]$/M: a = 0.07, b = 0.10, c = 0.16 and d = 0.22.

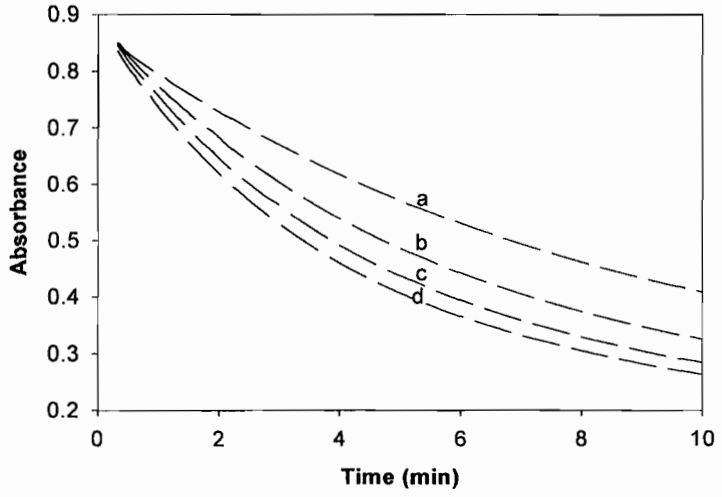


Figure 2. Typical kinetic curves showing effect of chlorite on Nile blue depletion at 633 nm. $[NB^+] = 2.0 \times 10^{-5}$ M, $[H^+] = 0.10$ M and $[ClO_2^-]$/M: a = 0.02, b = 0.03, c = 0.04 and d = 0.05.

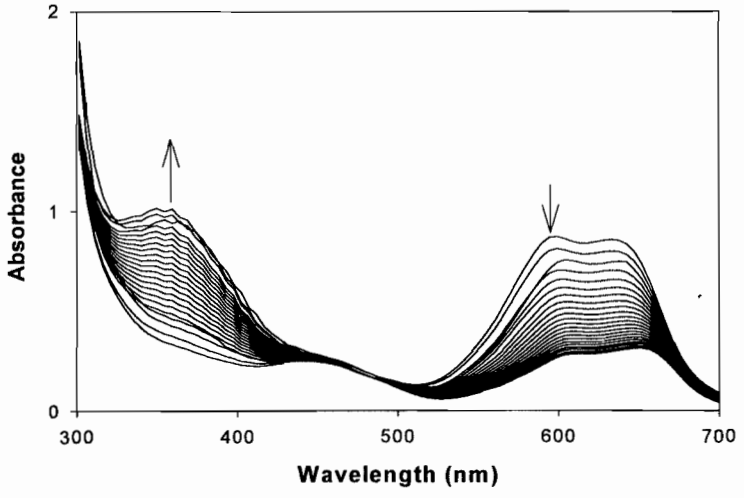


Figure 3. Repetitive scan spectra illustrating the depletion of Nile blue at 633 nm and rapid formation of ClO_2 at 360 nm. $[NB^+]=2.0 \times 10^{-5}$ M, $[ClO_2^-]= 0.02$ M and $[H^+] = 0.10$ M.

Order with respect to chlorite and acid

To establish the order with respect to chlorite and acid, the effect of initial concentration of chlorite and acid on the kinetics was studied at fixed initial concentration of other reagents. Table 1 shows the values of *pseudo* first-order rate constants at different initial concentrations of the two organic substrates, which remained unvaried within the limits of experimental errors. Table 2 summarises the values of *pseudo* first-order and third-order rate coefficients for both NB^+ and MB^+ reactions at varied initial chlorite and acid conditions for duplicate experiments. The experiments were done at constant ionic strength and sodium sulphate solution was employed for adjusting the ionic strength. For Nile blue, the plot of ln k' versus ln $[ClO_2^-]$ gave a straight line with the slope of 1.04 (R^2 = 0 99) and the ln - ln plot of k' versus $[ClO_2^-]$ for Meldola's blue gave a slope of 1.01 (R^2 = 0.98) indicating that the order of the reaction with respect to ClO_2^- is unity in both cases.

Table 1. Pseudo first-order rate constants for Nile and Meldola's blue reactions.

$[ClO_2^-]$ = 0.05 M, $[H^+]$ = 0.10 M, Temperature = 25 °C and μ = 0.20 M		$[ClO_2^-]$ = 0.20 M, $[H^+]$ = 2.0 M, Temperature = 40 °C and μ = 3.1 M	
$[NB^+]/10^{-5}$ M	k'/ 10^{-3} s^{-1}	$[MB^+]/10^{-5}$ M	k'/ 10^{-3} s^{-1}
1.40	1.97	1.50	1.45
1.60	2.00	1.70	1.30
1.80	1.96	1.90	1.21
2.00	2.02	2.00	1.20
2.2	2.05	2.20	1.25
Mean k = (2.00 ± 0.04) x 10^{-3} s^{-1}		Mean k' = (1.28 ± 0.10) x 10^{-3} s^{-1}	

Table 2. Order with chlorite and acid.

$[NB^+]$ = 2.0 x 10^{-5} M and Temp. 25 °C				$[MB^+]$ = 2.0 x 10^{-5} M and Temp. 40 °C			
$[ClO_2^-]$/M	$[H^+]$/M	k'/10^{-3} s^{-1}	k_3/M^{-2} s^{-1}	$[ClO_2^-]$/M	$[H^+]$/M	k'/10^{-3} s^{-1}	$k_3/10^{-3}$ M^{-2} s^{-1}
μ = 0.38				**μ = 3.1**			
0.050	0.1	1.78	0.356	0.07	2	0.07	3.14
0.055	0.1	1.98	0.360	0.1	2	0.608	3.04
0.060	0.1	2.19	0.365	0.13	2	0.78	3.00
0.065	0.1	2.38	0.366	0.16	2	0.98	3.06
0.070	0.1	2.55	0.369	0.19	2	1.16	3.05
0.075	0.1	2.78	0.360	0.22	2	1.41	3.21
0.080	0.1	2.89	0.361	0.25	2	1.59	3.18
	Mean = (0.363 ± 0.005) M^{-2} s^{-1}				Mean = (3.09 ± 0.08)$10^{-3}M^{-2}s^{-1}$		
μ = 1.55				**μ = 3.55**			
0.05	0.1	1.32	0.264	0.2	1.6	0.97	3.03
0.05	0.2	2.75	0.275	0.2	1.7	1.01	2.97
0.05	0.3	4.22	0.281	0.2	1.8	1.1	3.06
0.05	0.4	5.58	0.279	0.2	1.9	1.14	3.00
0.05	0.5	7.02	0.281	0.2	2.0	1.18	2.95
				0.2	2.1	1.28	3.05
				0.2	2.2	1.34	3.04
	Mean = (0.28 ± 0.01) M^{-2} s^{-1}				Mean = (3.00 ± 0.04)$10^{-3}M^{-2}s^{-1}$		

High acid concentration facilitates the disproportionation of chlorite ion to chlorine dioxide and chloride. Although the initial acid concentrations were different, both the reactions exhibited first-order dependence on the acid concentration. The ln k'- ln $[H^+]$ plots for the Nile

blue and Meldola's blue reactions gave slope of 1.04 (R^2 = 0.99) and 1.01 (R^2 = 0.98), respectively. The overall third order rate constant for the acidic chlorite reaction with NB^+ at 25 °C and ionic strength, μ = 0.38 M was found to be 0.36 ± 0.01 $M^{-2} s^{-1}$. For MB^+, even at higher temperature reaction was evidently slow and the third-order rate constant for the MB^+ reaction at 40 °C and μ = 3.55 M was (3.01 ± 0.08) x 10^{-3} $M^{-2} s^{-1}$.

Effect of the ionic strength on the reactions

For the Nile blue - acidic chlorite reaction, the primary salt effect was studied with initial concentrations of $[H^+]$ 0.10 M, $[ClO_2^-]$ 0.05 and $[NB^+]$ 2.0 x 10^{-5} M. Using sodium sulphate as the neutral salt, when the ionic strength was increased from 0.2 to 1.82 M, the *pseudo* first-order rate constant decreased from 2.0 x 10^{-3} s^{-1} to 1.26 x 10^{-3} s^{-1}. The log k' *versus* square root of ionic strength plot gave a slope of -0.66 with (R^2 = 0.99). Obviously due to high ionic strength of the reaction, it is difficult to quantify the kinetic salt effect exactly, but the salt effect is negative, suggesting the reaction between oppositely charged species, i.e. probably between NB^+ and ClO_2^-. Due to very high ionic strength resulting from huge acid concentrations of the Meldola's blue reaction, the ionic strength is excessive and could only be varied marginally between 3.1 and 3.55 M. The log k' *versus* $\sqrt{\mu}$ plot gave a slope of -0.38 with (R^2 = 0.98) showing a negative salt effect and rate limiting reaction is between oppositely charged species.

Energy parameters

The values for energy, enthalpy and entropy of activation, and the frequency factors for both the reactions were studies by measuring the rate constants under similar conditions as function of temperature. Table 3 summarises the results obtained. High energy of activation values in the range of 50-60 kJ mol^{-1} indicates that reactions are slow. Relative to the Nile blue reaction, the activation enthalpy and energies were higher for the Meldola's blue reaction and the entropy charge was also greater and negative. The high energy of activation renders the reactions to be slow, which justifies the slower reaction of Meldola's blue with acidic chlorite in energy terms. The large negative entropy of activation values of -233 and -248 J K^{-1} mol^{-1} associated with Nile blue and Meldola's blue reactions, respectively, show that significant restriction in the freedom of reacting molecules occur during the reaction, which is clear indication of formation of compact activated complex.

Table 3. Temperature dependence of the rate coefficients.

$[NB^+]$ = 2.0 x 10^{-5} M, $[ClO_2^-]$ = 0.03 M, and $[H^+]$ = 0.1 M.			$[MB^+]$ = 2.0 x 10^{-5} M, $[ClO_2^-]$ = 0.2 M, and $[H^+]$ = 2.0 M		
Temp./K	k' / 10^{-3} s^{-1}	k/ $M^{-2} s^{-1}$	Temp./K	k' / 10^{-3} s^{-1}	k / 10^{-3} $M^{-2} s^{-1}$
298	1.08	0.360	333	0.93	2.33
303	1.46	0.487	338	1.26	3.15
308	1.88	0.627	343	1.65	4.13
'313	2.62	0.873	348	2.36	5.90
318	3.68	1.227	353	3.01	7.53
Energy of activation, E_a = 47.8 kJ mol^{-1} Frequency factor, A = 8.41 x 10^7 L $M^{-2} s^{-1}$ Enthalpy of activation, $\Delta H^{\neq}$ = 40.4 kJ mol^{-1} Entropy of activation, $\Delta S^{\neq}$ = -233 J K^{-1} mol^{-1}			Energy of activation, E_a = 62.4 kJ mol^{-1} Frequency factor, A = 1.46 x 10^7 L $M^{-2} s^{-1}$ Enthalpy of activation, $\Delta H^{\neq}$ = 54.6 kJ mol^{-1} Entropy of activation, $\Delta S^{\neq}$ = -248 J K^{-1} mol^{-1}		

Reactions of hypochlorite and hypochlorous acid with the dyes

The chlorite ion in acidic solutions is known to generate HOCl as the reaction intermediate, during the decomposition and oxidative processes. In presence of acid, chlorite reduces to HOCl and oxidises the reductant in a two-electron step. Further, HOCl acts as an auto catalyst for the ClO_2 formation [12]. Hence, the oxidation kinetics of hypochlorite ion with Nile blue and Meldola's blue were investigated. Some of the reactions involving HOCl were fast and studies were carried out using the stopped-flow apparatus. The reaction of NB^+-OCl^- at alkaline pH (8.5) was first-order with respect to OCl^-. Upon addition of acid the reaction was intricate and faster. For the reaction between hypochlorite and the two dyes, all the kinetic data followed first-order kinetics. From the initial analysis of the effect of the increase in acid on the pseudo first-order rate constants, the reaction order with respect to acid was found to be fractional value and it further dwindled at very low pH. After careful consideration of the data, it is observed that data fits well to a bi-exponential equation ($y = A_1 \exp(k_1 x) + A_2 \exp(k_2 x) + Mx + C$), suggesting that two pseudo-first order reactions occur simultaneously. In both the reactions, one rate coefficient was higher than the other. An increase in rate of the reaction with increase in acid concentration suggests that the slow path is initiated by hypochlorite ion and the faster one by hypochlorous acid. Hence, using the kinetic data, the two *pseudo* first-order rate coefficients in each case were estimated. Table 4.1 and 4.2 summarise the analysed results for the Nile blue and Meldola's blue reactions, respectively, using the experimental kinetic data for the variation of concentrations of hypochlorite and the calculated equilibrium concentrations of OCl^- and HOCl for different acid concentrations. The perusal of the *pseudo* first-order rate coefficients in Tables 4.1 and 4.2 show that at pH = 8.5, with the increase in initial concentration of hypochlorite, both the pseudo rate constants increased, which is obvious due to proportionate increase in the concentrations of both {OCl^-} and [HOCl]. This can be explained based on the $pK_a = 7.4$ of HOCl [13], which will be 75% dissociated at pH 8.5, thus the 25% of the total hypochlorite will be in the form of hypochlorous acid and 75% as hypochlorite ion. With the increase in initial acid, the rate coefficient of HOCl initiated oxidative reaction increased significantly, while the other constant decreased. The second-order rate coefficients calculated for the two reactions using the estimated concentrations of hypochlorite and hypochlorous acid are summarised in the table.

The results in the Table 4.1 show the k_1 and k_2 values which are the second-order rate constants respectively for the hypochlorite ion and hypochlorous acid initiated oxidations. The k'_1 and k_1 values were of lower magnitude than the k'_2 and k_2 values. At constant pH, both the *pseudo* first-order rate constant values k'_1 and k'_2 amplified with an increase in the hypochlorite concentration, but with an increase in acid concentration the hypochlorite concentration reduces and the trend is reflected in the corresponding pseudo first-order constants. The last two columns of the Table 4.1, clearly show that the second order rate constants for the hypochlorous reaction are much higher than with hypochlorite. Furthermore, the rate coefficients for the Meldola's blue reactions were again of much lower value than for the Nile blue reaction.

The effect of variation in the initial concentrations of chlorite and acid on the generation of chlorine dioxide was investigated. Figure 4 and 5 show kinetic profiles of ClO_2 formation at varied concentrations of chlorite and acid, respectively. The figures also indicate that increase in chlorite concentration has pronounced enhancement in ClO_2 production relative to acid variation.

Table 4. Rate constants for hypochlorite and hypochlorous acid with the dyes.

4.1 Nile blue oxidation with HOCl.

Total $[OCl^-]/10^{-3}$ M	$[H^+]/ x 10^{-4}$ M	$[OCl^-]/10^{-3}$M	$[HOCl]/10^{-3}$ M	k'_1/ s^{-1}	k'_2 / s^{-1}	$k_1/10^2 M^{-1}s^{-1} = k'_1/[OCl^-]$	$k_2/10^3 M^{-1}s^{-1} = k'_2/[HOCl]$
	pH = 8.5						
1.21		0.908	0.303	0.098	1.55	1.08	5.12
2.41		1.81	0.603	0.204	3.02	1.13	5.01
3.62		2.72	0.905	0.282	4.68	1.04	5.17
4.82		3.62	1.212	0.391	6.17	1.08	5.12
6.03		4.52	1.511	0.505	7.56	1.12	5.01
	pH = 7.0						
2.41	0.0	0.967	1.443	0.111	7.22	1.15	5.00
2.41	1.0	0.867	1.543	0.095	8.01	1.10	5.19
2.41	2.0	0.767	1.643	0.086	8.42	1.12	5.13
2.41	3.0	0.667	1.743	0.075	8.89	1.12	5.10
2.41	4.0	0.567	1.843	0.065	9.74	1.15	5.29
2.41	5.0	0.467	1.943	0.052	10.32	1.11	5.31
2.41	7.0	0.267	2.143	0.027	11.12	1.01	5.19
2.41	8.0	0.168	2.242	0.018	11.55	1.07	5.15
				Mean±SD		(1.10 ± 0.04) $10^2 M^{-1} s^{-1}$	(5.14 ± 0.01) $10^3 M^{-1} s^{-1}$

4.2. Meldola's blue oxidation with hypochlorous acid.

Total $[OCl^-]/10^{-3}$ M	$[H^+]/ x 10^{-4}$ M	$[OCl^-]/10^{-3}$ M	$[HOCl]/ 10^{-3}$ M	$k'_1/10^{-2}$ s^{-1}	$k'_2/10^{-1}$ s^{-1}	$k_1 / M^{-1} s^{-1} = k'_1/[OCl^-]$	$k_2/10^2 M^{-1} s^{-1} = k'_2 /[HOCl]$
	pH = 8.5						
2.41		1.81	0.60	1.08	0.78	5.98	1.29
3.62		2.72	0.91	1.65	1.15	6.08	1.27
4.82		3.62	1.21	2.20	1.45	6.09	1.20
6.03		4.52	1.51	2.68	1.85	5.93	1.23
7.24		5.43	1.81	3.31	2.24	6.10	1.24
	pH = 7.0						
3.62	0.0	1.45	2.17	0.882	2.68	6.07	1.24
3.62	1.0	1.35	2.27	0.809	2.88	5.98	1.27
3.62	2.0	1.25	2.37	0.761	2.97	6.07	1.25
3.62	3.0	1.15	2.47	0.689	3.11	5.98	1.26
3.62	4.0	1.05	2.57	0.648	3.17	6.15	1.23
3.62	5.0	0.95	2.67	0.552	3.23	5.79	1.21
3.62	7.0	0.75	2.87	0.467	3.68	6.20	1.28
3.62	8.0	0.65	2.97	0.391	3.79	5.99	1.28
				Mean± SD		(6.03±0.10) $M^{-1} s^{-1}$	(1.25±0.3) $10^2 M^{-1} s^{-1}$

k_1 and k_2 are the second-order rate constants respectively for the hypochlorite ion and hypochlorous acid initiated oxidations.

Reaction mechanisms

Based on the reaction orders one each experimentally observed with respect to dye, chlorite and acid and the observed negative salt effect it can be suggested that the rate determining step is the reaction between two single oppositely charged species, possibly dye^+ and ClO_2^-. On the basis of the experimental results the rate-limiting step is the reaction between the reductant and oxidant

followed by protonation to give the reaction products. HOCl, which is an autocatalyst, controls rates of all the oxyhalogen reactions in solution, and is responsible for the formation of ClO_2 [12]. To propose the mechanism, the formation and accumulation of the chlorine dioxide during the reaction, the disproportionation chemistry of chlorite ion in acidic solution to chlorine dioxide and chloride and the existing equilibrium between chlorite, H^+ ions and HOCl plus the reactions involving the formation and decomposition of reactive oxychloro intermediates (HOCl and Cl_2O_2 and Cl_2) need to be considered [12, 14-16]. The oxidation reactions involving Cl_2 have been omitted because of the low amounts of Cl_2 that is produced in the reaction and it readily reacts with chlorite ion to give chlorine dioxide [15]. Furthermore, based on the major organic products identified, the reactions involving the dyes and other oxychloro species may be summarised as follows:

Mechanism:

Acid chlorite chemistry (equations C1 to C6)

C1 $H^+ + ClO_2^- \leftrightarrow HClO_2$

C2 $HOCl + Cl^- + H^+ \leftrightarrow Cl_2$

C3 $Cl_2 + 2ClO_2^- \rightarrow 2ClO_2 + 2Cl^-$

C4 $HOCl + ClO_2^- + H^+ \leftrightarrow Cl_2O_2$

C5 $Cl_2O_2 + 2ClO_2^- + 2H^+ \leftrightarrow 2ClO_2 + 2HOCl$

C6 $HClO_2 + Cl^- + H^+ \rightarrow 2HOCl$

Nile blue oxidation chemistry (equations N7 to N13)

N7 $NB^+ + ClO_2^- + H^+ \rightarrow$ [Act. Complex] Rate limiting step

N8 [Act. Complex]$\rightarrow I_1 + HOCl + H^+ + CH_3CHO$

N9 $NB^+ + HOCl \rightarrow I_1 + H^+ + Cl^- + CH_3CHO$

N10 $I_1 + HOCl \rightarrow P_1 + H^+ + Cl^-$

N11 $I_1 + ClO_2^- + H^+ \rightarrow P_1 + HOCl$

N12 $I_1 + Cl_2O_2 + H_2O \rightarrow P_1 + 2HOCl$

N13 $CH_3CHO + HOCl \rightarrow CH_3COOH + H^+ + Cl^-$

Meldola's blue oxidation chemistry (equations M7 to M13)

M7 $\mathbf{MB^+ + ClO_2^- + H^+ \rightarrow [Act.\ Complex]}$ **Rate limiting step**

M8 $[Act.\ Complex] \rightarrow I_1 + HOCl + H^+ + HCHO$

M9 $\mathbf{MB^+ + HOCl \rightarrow I_1 + H^+ + Cl^- + HCHO}$

M10 $I_1 + HOCl \rightarrow P_1 + H^+ + Cl^-$

M11 $I_1 + ClO_2^- + H^+ \rightarrow P_1 + HOCl$

M12 $I_1 + Cl_2O_2 + H_2O \rightarrow P_1 + 2HOCl$

M13 $HCHO + HOCl \rightarrow HCOOH + H^+ + Cl^-$

Rate laws

The rate equation for the catalysed NB^+ - acidic chlorite reaction can be represented by the equation,

$$-d[NB^+]/dt = k\,[NB^+]\,[ClO_2^-]\,[H^+] \quad (1)$$

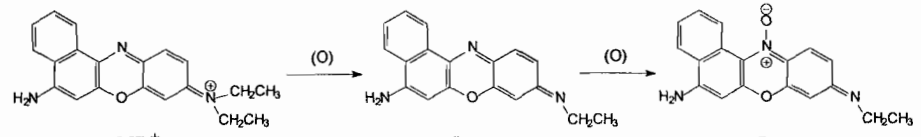
When $[ClO_2^-]$ and $[H^+]$ are in excess, the above equation (1) reduces to

$$\text{Rate} = k'[NB^+] \text{ where, } k' = k\,[ClO_2^-]\,[H^+] \quad (2)$$

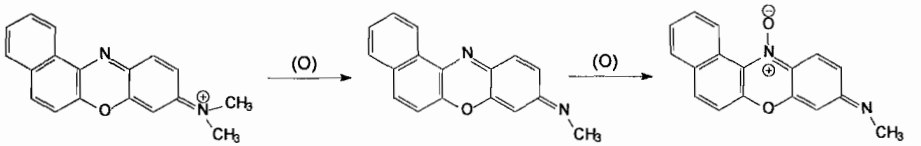
Similarly, the Meldola's blue reaction may be represented as

$$-d[MB^+]/dt = k\,[MB^+]\,[ClO_2^-]\,[H^+] = k'\,[NB^+], \text{ where, } k' = k\,[ClO_2^-]\,[H^+]$$

The third-order rate constants, k values obtained for different experimental runs for the two reactions support the proposed rate laws.



NB^+ I P



MB^+ I P

When the reactivities of NB^+ and MB^+ with acidic chlorite and HOCl are compared in both cases, the NB^+ reactions are much faster. The reactivity of Nile blue is higher due to the presence of amino substitution in the 3 position. The lone pair of electrons on the amino group are delocalized in the ring and increase the electron density and reactivity of $=N^+(C_2H_5)_2$ group. Due to the increase in electron density and relatively higher positive inductive effect of ethyl groups over the methyl groups, the $N\text{-}C_2H_5$ becomes more polar and reactive and facilitates the electrophillic attack by chlorite, hypochlorite or HOCl, thus require less acid for breaking of $N\text{-}C_2H_5$ bond. Initially, relative to nitrogen in the $=N^+(C_2H_5)_2$ group, the nitrogen in the ring is less reactive. After breaking of $N\text{-}C_2H_5$ bond, further oxidation of the ring nitrogen takes place to produce the N-oxide. Meldola's blue is resistive to oxidation due to lack of delocalised electrons and lower inductive effect from methyl groups, but the reaction follows a similar pathway as Nile blue but at slower pace.

Reaction profiles and simulations

Both the NB^+ and MB^+ reactions with acidic chlorite had similar profiles including the observed accumulation of ClO_2, except for the differences in the reactivities. The initial step of this oxidation is a $2e^-$ oxidation of the dye cation by ClO_2^- ion, yielding HOCl. HOCl in turn reacts with chlorite ion leading to formation of chlorine dioxide and with the reductant and corresponding intermediates generated to give the final products as indicated in equations, N10-N12 and M10-M12.

The simulations conducted were based on the proposed comprehensive mechanism involving C1-C6 and N7-N13 for Nile blue reaction using the Simkine software which uses semi-implicit extrapolation method, which uses the modified midpoint rule [17]. For the simulations, the rate constants for (C1-C6) were taken from literature, N7 and N9 are experimentally determined values and the others were estimated values (N8, N10-N13) (Table 5). The experimental values are indicated in bold. The simulated curves matched well with the experimental curves for the depletion of dye (Figure 2) and also for the formation of chlorine dioxide (Figure 4 and 5) for the Nile blue reaction under varied initial conditions of chlorite and acid, confirming the suggested mechanism to be probable.

Table 5. Forward and reverse rate constants used for simulations.

Reaction No.	Forward rate/ $M^{-1} s^{-1}$	Reverse rate/ $M^{-1} s^{-1}$
C1	1.0×10^9	3.16×10^7
C2	8.9×10^9	1.1×10^2
C3	9.8×10^6	
C4	5.0×10^7	6.6×10^2
C5	5.4×10^6	1.0×10^{-2}
C6	7.0×10^{-2}	
N7	**4.67×10^{-1}***	
N8	9.40×10^5	
N9	**5.14×10^{3}***	
N10	3.0×10^4	
N11	1.0×10^3	
N12	2.5×10^4	
N13	1.3×10^4	

*Experimental values derived from this study.

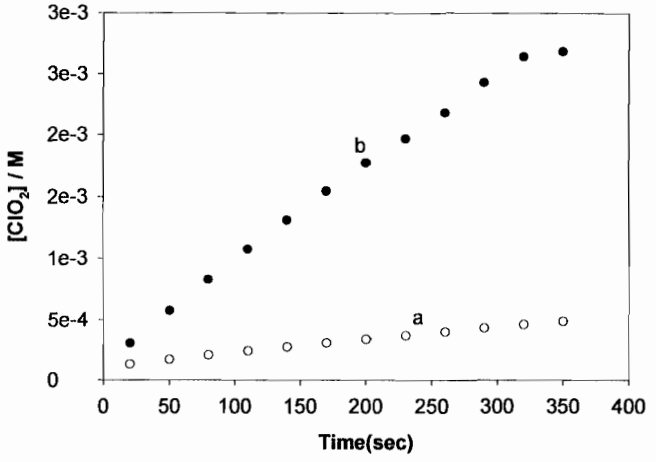


Figure 4. Effect of initial chlorite on formation of ClO_2. $[NB^+] = 2.0 \times 10^{-5}$ M, $[H^+] = 0.10$ M and $[ClO_2^-]$/M: a = 0.02 M and b = 0.06.

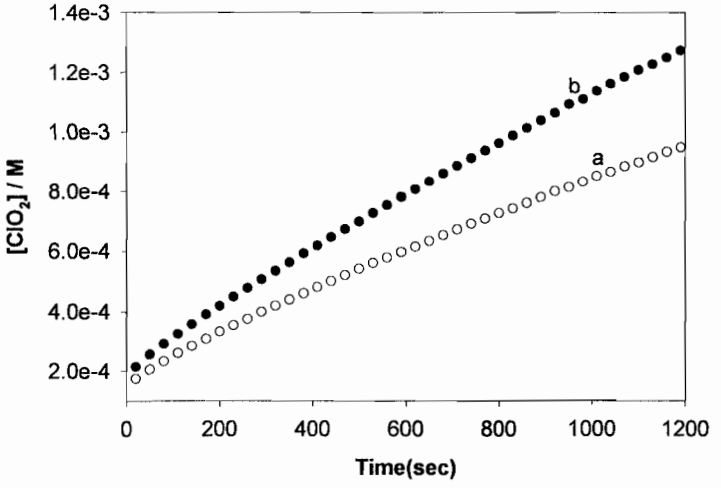


Figure 5. Effect of initial acid on formation of ClO_2. $[NB^+] = 2.0 \times 10^{-5}$ M, and $[ClO_2^-] = 0.02$ M and $[H^+]$/M: a = 0.05 M and b = 0.50.

ACKNOWLEDGEMENTS

Authors sincerely acknowledge the financial support received from the National Research Foundation, Pretoria in the form of bursary to Mr. Qwabe and postdoctoral fellowship to Dr. Pare.

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