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Kinetic Analysis of the Oxidation of Nitrate Ion by Methylthioninium Chloride in Acid Medium

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ABSTRACT: The kinetic analysis of the oxidation of nitrate ion by methylthioninium chloride in acid medium was studied using large excess of the reductant. The progress of the reactions was investigated by monitoring the decrease in absorbance. The results showed a stoichiometry of 1:1, first-order dependence in both oxidant and reductant. A second order rate equation at constant temperature was obtained ©JASEM.

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An increase in the concentration of hydrogen ion increases the rate of reaction while a negative salt effect was observed as the rate of reaction decreased with increase in the concentration of ionic strength and acetone. Added Mg^{2+} ions did not show any effect on the rate of reaction while the presence of Ca^{2+} increased the rate of reaction. The result of spectroscopic and kinetic investigation did not indicate intermediate complex formation in the course of the reaction. A plausible mechanism for the reaction has been proposed in line with an outer-sphere reaction pathway.

Nitrates are mainly used as fertilizers in agriculture because of their high solubility and biodegradability (Laue, 2006). Nitrate is an oxidizing agent, most notably in explosives where the rapid oxidation of carbon compounds liberates large volumes of gases. Nitrate is used to remove air bubbles from molten glass and some ceramics. Humans are subject to nitrate toxicity, with infants being especially vulnerable to methemoglobinemia (methemoglobinemia in infants is known as the blue baby syndrome) due to nitrate metabolizing triglycerides present at higher concentrations than at other stages of development. Although nitrates in drinking water were once thought to be a contributing factor, there are now significant scientific doubts as to whether there is a causal link as reported by Addiscott and Benjamin (2004); Avery, (1999). Lettuce may contain elevated nitrate under growth conditions such as reduced sunlight, undersupply of the essential micronutrients such as molybdenum (Mo) and iron (Fe), or high concentrations of nitrate due to reduced assimilation of nitrate in the plant. High levels of nitrate fertilization also contribute to elevated levels of nitrate in the harvested plant (Marschner, 1999). In freshwater systems close to land, nitrate can reach high levels that can potentially cause the death of fish. Furthermore, nitrate is much less toxic than ammonia according to Romano and Zeng (2007) but levels more than 30 ppm of nitrate can inhibit growth, impair the immune system and cause stress in some aquatic species (Sharpe, 2013).

Methylene blue has been described as "the first fully synthetic drug used in medicine." Its use in the treatment of malaria was pioneered by Guttman and Ehrlich (1891). During this period before the first World War, researchers like Ehrlich believed that drugs and dyes worked in the same way, by preferentially staining pathogens and possibly harming them. Anti-malarial use of the drug has recently been revived. The blue urine after taking methlene blue was used to monitor psychiatric patients' compliance with medication regimes and as such became the lead compound in research leading to the discovery of chlorpromazine (Schirmer et al., 2003). Methemoglobinemia can be treated with methylene blue, which reduces ferric iron (3^+) in affected blood cells back to ferrous iron (2^+) (NYT, 2013). Due to the numerous advantages of methylene blue and nitrate ion has prompt the investigation of kinetic analysis of the oxidation of nitrate ion by methylthioninium chloride in acidic medium in order to add to knowledge.

MATERIAL AND REAGENTS

Methylthioninium chloride also known as methylene blue (MB) was obtained and used without further purification. All solutions were prepared with distilled water. The prepared solutions were wrapped with aluminum foil to avoid photochemical decomposition. Perchloric acid was used to study the effect of hydrogen ion on the rate while sodium perchlorate was used to maintain a constant ionic strength.

*Stoichiometry***:** Spectrophotometric titration using the mole ratio method was employed in determining the stoichiometries of the reactants. The concentration of the methylene blue was kept constant while that of nitrate ion was varied as follows; $[MB^+] = 1.0 \times 10^{-5}$ mol dm^{-3}; $[NO_3^-] = (1 - 8) \times 10^{-5}$ mol dm^{-3}; $[H^+] = 1.0 \times 10^{-3}$ mol dm^{-3}, $\mu = 1.0$ mol dm^{-3} ($NaClO_4$), T = 29 ± 1.0 ^oC. The reaction mixtures went into completion as a result of constancy in repeated measurements of absorbance at 663 nm. The absorbance (A_∞) was plotted against the concentration of the reductants and the point of sharp inflection in the plot gave the stoichiometry of the reaction.

Kinetics and Order of Reactions: The progress of the reactions was monitored by following the decrease in absorbance of the MB^+ at λ_{max} 663 nm. All kinetic runs were made with $[NO_3^-]$ in large excess over $[MB^+]$, while the ionic strength was maintained constant at 1.0 mol dm^{-3} ($NaClO_4$) and $[H^+] = 1.0 \times 10^{-3}$ at 29 ± 1^{o}C. Plot of log ($A_t - A_\infty$) against time were made from which pseudo-first order rate constants (k^1) were determined (fig 1). Second order rate constants (k_2) were obtained as $k_2 = k^1/[NO_3^-]^n$ where n is the order of the reaction. A plot of log k^1 vs log $[NO_3^-]$ was also made (fig 2).

Effect of Acid Dependence: The effect of hydrogen ion on the rate of the reaction was investigated in the range of $(0.5 - 4.0) \times 10^{-3}$ mol dm^{-3} ($HClO_4$) while the concentrations of other reactants were kept constant. The results are presented in Table 1. A plot of k_2 vs $[H^+]$ was also made (fig 3).

*Effect of Ionic Strength***:** The effect of ionic strength on the rate of the reaction was investigated in the range of 0.5 -4.0 mol dm^{-3} ($NaClO_4$) while the concentrations of other reactants were kept constant. The results are presented in Table 1. A plot of log k^1 vs $\sqrt{\mu}$ was also made (fig 4).

*Effect of Dielectric Constant***:** The effect of medium dielectric constant, D, on the rate of reaction was investigated by using 5 % mixture of water and acetone.

*Effect of Added Cations***:** At constant concentration of $[MB^+] = 1 \times 10^{-5}$ mol dm^{-3}, $[NO_3^-] = 20 \times 10^{-4}$ mol dm^{-3}, $[H^+] = 1.0 \times 10^{-3}$ mol dm^{-3}, $\mu = 1.0$ mol dm^{-3} ($NaClO_4$), the effect of added ions (Mg^{2+} and Ca^{2+}) on the rates of the reactions was investigated in the range $(0.5 - 3.0) \times 10^{-3}$ mol dm^{-3} (Table 2).

*Test for Intermediate Complex Formation***:** The electronic spectra of the partially reacted reaction mixtures were recorded at five minutes intervals after the commencement of the reaction were taken over the range 550-700 nm. A similar run was made for the reactant separately in each case.

Test for Free Radical: About 2 g of acrylamide was added to the partially oxidized reaction mixture of methylthioninium chloride and nitrate ions in excess methanol and to each of the reactant separately at stated condition in Table 1.

Product Analysis: At completion of the reaction, the reaction mixture was analyzed for the type of product(s) found. Nitrite ion was tested for as product. NO_2^- was tested qualitatively by mixtures $PbNO_3/AgNO_3$ respectively.

RESULTS AND DISCUSSION

The stoichiometry studies indicated that 1 moles of MB^+ was consumed by 1 mole of NO_3^- . This gives the overall equation of the reaction to be

$$MB^+ + NO_3^- \rightarrow \text{Product} \qquad 1$$

Similar stoichiometries has been reported for reactions of methylthioninium chloride. A stoiciometry of 1: 1 was reported for MB^+/thiourea by Osunlaja et al., 2012, $MB^+/[Fe_2(bpy)_4O]Cl_4$ by Idris et al., 2005.

*Kinetics and Order of Reaction***:** The plots of log ($A_t - A_\infty$) versus time were linear to about 95 % of the reaction suggesting that the reaction is first order in $[MB^+]$ (fig.1). Plot of log k^1 versus log $[NO_3^-]$ was linear with a slope of one indicating that the reaction is first order with respect to $[NO_3^-]$ (fig. 2). A similar order has been reported by Jonnalagadda et al., 1996, Babatunde et al., 2013 and Babatunde and Umoru, 2014 for the reactions of $MB^+/[S_2O_5^{2-}]$ and $MB^+/[SO_4^{2-}]$ respectively. The overall order is therefore second order for both MB^+ and$[NO_3^-]$.Thus, the rate equation for the reaction is:

$$-d[MB^+]/dt = k_2[MB^+][NO_3^-] \qquad 2$$

at $[H^+] = 1.0 \times 10^{-3}$ mol dm^{-3} and T = 29 ± 1°C, and $k_2 = 3.02$ dm^3 mol^{-1} s^{-1}

Table 1: Pseudo-First Rate Constant and Second Order Rate Constant for the Reaction of Methylene Blue and Nitrate ion at 29 ± 1ºC, λ_{max} = 663 nm, $[NO_3^-]$ = 20 x 10^{-4} mol dm^{-3} , $[MB^+]$ = 1 x 10^{-5} mol dm^{-3}, $[H^+]$ = 1 x 10^{-3} mol dm^{-3}

10^4 $[NO_3^-]$ mol dm^{-3}	μ ($NaClO_4$) mol dm^{-3}	10^3 $[H^+]$ mol dm^{-3}	10^3 k^1 s^{-1}	k_2 dm^3 mol^{-1} s^{-1}
20	1.0	1.0	6.01	**3.01**
30	1.0	1.0	9.07	**3.02**
40	1.0	1.0	12.15	**3.04**
50	1.0	1.0	15.22	**3.04**
60	1.0	1.0	18.08	**3.01**
70	1.0	1.0	20.98	**3.00**
80	1.0	1.0	24.07	**3.01**
9.0	1.0	1.0	27.11	**3.01**
20	1.0	0.5	5.54	**2.77**
20	1.0	1.0	6.02	**3.01**
20	1.0	1.5	6.72	**3.36**
20	1.0	2.0	6.94	**3.47**
20	1.0	2.5	7.34	**3.67**
20	1.0	3.0	8.54	**4.27**
20	1.0	3.5	9.33	**4.67**
20	1.0	4.0	10.12	**5.06**
20	0.5	1.0	6.59	**3.30**
20	1.0	1.0	6.01	**3.01**
20	1.5	1.0	5.64	**2.82**
20	2.0	1.0	5.30	**2.65**
20	2.5	1.0	4.88	**2.44**
20	3.0	1.0	4.61	**2.31**
20	3.5	1.0	4.15	**2.08**
20	4.0	1.0	3.64	**1.82**

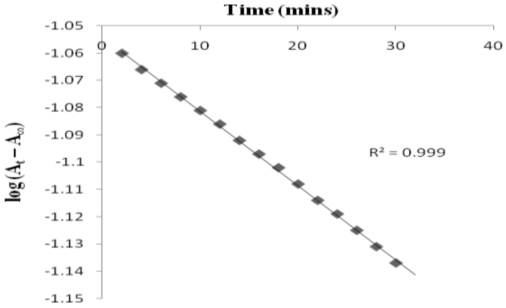

Fig 1: Plots for the Reaction of Methylthioninum Chloride with Nitrate Ion. $[MB^+]$ = 1.0 x 10^{-5} mol dm^-, $[NO_3^-]$ = 20 x 10^{-4} mol dm^{-3}, $[H^+]$ = 1.0 x 10^{-3} mol dm^{-3}, μ = 1.0 mol dm^{-3} and T = 29 ± 1°C and λ_{max} = 663 nm.

The order of one in the oxidant concentration in this reaction is consistent with what was reported for similar reactions of MB and ferrocyanide, sulphide ion and ascorbic acid (Sutter and Spencer (1990); Resch et al., (1989) and Khan and Swan (2001).

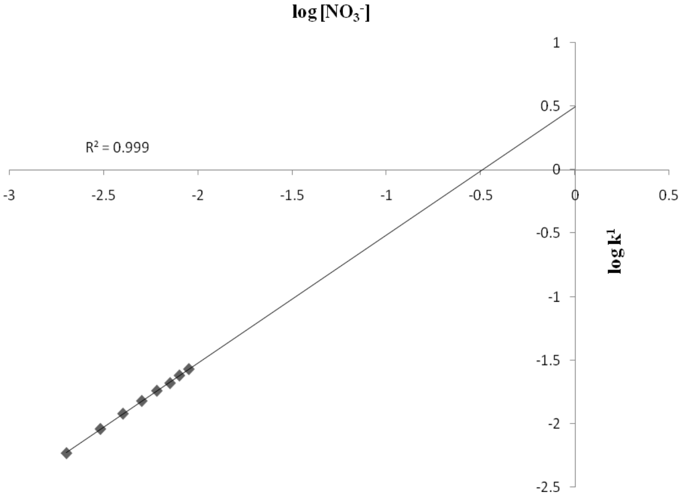

Fig 2: Plot of log k^1 vs log $[NO_3^-]$ for the Reaction of Methylthioninum chloride and Nitrate Ion. $[MB^+] = 1.0 \times 10^{-5}$ mol dm^{-3}, $[NO_3^-] = 20 \times 10^{-4}$ mol dm^{-3}, $[H^+] = 1.0 \times 10^{-3}$ mol dm^{-3}, $\mu = 1.0$ mol $dm^{-3}$and 29 ± 1ºC and $\lambda_{max} =$ 663 nm.

Effect of $[H^+]$ The result on Table 2 shows that the rate constant of the reaction increased with increase in hydrogen ion concentration in the range of (0.5-4.0) x 10^{-3} (Table 1). Plots of k_H^+ versus $[H^+]$ (Fig. 3) was linear with a slope of 0.649 and intercept of 2.32 x 10^{-3} The acid dependent rate equation for the reaction can be represented by equation 3,

$$k_H^+ = (a + b[H^+])[MB^+][NO_3^-] \qquad 3$$

where a = 2.32×10^{-3} dm^3 mol^{-1} s^{-1}, b = 0.649 dm^3 mol^{-1} s^{-1}

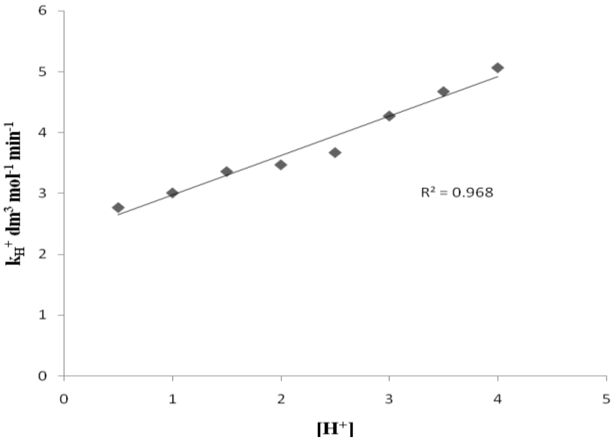

Fig 4: Plot of k_H^+ vs **[**H^+] for the Reaction of Methylthioninum chloride and Nitrate Ion. $[MB^+] = 1.0 \times 10^{-5}$ mol dm^{-3}, $[NO_3^-] = 20 \times 10^{-4}$ mol dm^{-3}, $[H^+] = 1.0 \times 10^{-3}$ mol dm^{-3} , $\mu = 1.0$ mol $dm^{-3}$and 29 ± 1ºC and $\lambda_{max} = 663$ nm.

Equation 3 suggests that the reaction occurred via acid-dependent and acid-independent pathways. Similar report for the oxidation of bromate ion; ethylenediamine tetracetatocobaltate(II) ion, β-mercaptoethanol, sulphite ion, metabisulphite and sulphate ion by MB^+ have been observed by Iyun and Asala (1994); Bugaje (2006)**,** Ukoha (2005), Olajire and Olajide (2014) Babatunde et al., (2013) and Babatunde and Umoru (2014) respectively.

Effect of changes in Ionic Strength The result in Table 3 shows that the rate of the reaction decreased with increase in ionic strength suggesting a negative Bronsted – Debye salt effect which suggests that the activated complex is formed from two ions of unlike charges (Bronsted, 1922). A plot of k^1 vs √I gave a linear graph with a slope of -0.178 showing negative effect as shown in fig 4.

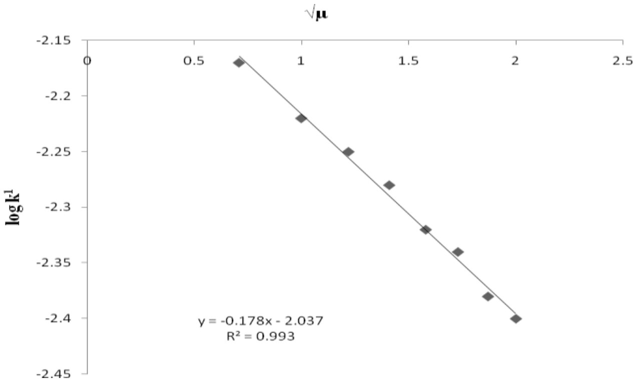

Fig 4: Plot of log k^1 vs √μ for the Reaction of Methylthioninum Chloride and Nitrate Ion. $[MB^+] = 1.0 \times 10^{-5}$ mol dm^{-3}, $[NO_3^-] = 20 \times 10^{-4}$ mol dm^{-3}, $[H^+] = 1.0 \times 10^{-3}$ mol dm^- , μ = 1.0 mol dm^{-3} and 29 ± 1ºC and $\lambda_{max} = 663$ nm.

Effect of Total Dielectric Constant The effect of changing dielectric constant was studied by using a binary solvent mixture of water and acetone (5 %). It was found that as the concentration of acetone increased, the reaction rate decreased. This is in line with observed effect on the rate of reaction on varying the concentration of ionic strength. Similar report was observed by Osunlaja et al., (2012a, 2012b) for the reaction between MB^+ /MnO4$^-$ between MB^+ /1-methyl-2-thiourea respectively.

Effect of Added Ions; The addition of cations (Ca^{2+} and Mg^{2+}) showed that the presence of Ca^{2+} increased the rate of reaction on increasing the concentration of Ca^{2+} while added Mg^{2+} did not show any effect as shown in Table 2. This is in agreement with an earlier report and is suggestive of the outer sphere pathway (Idris et al., 2004).

Table 2: Effect of Changes in Total Dielectric Constant (D) $[MB^+] = 1.0 \times 10^{-5}$ mol dm^{-3}, $[NO_3^-] = 20 \times 10^{-4}$ mol dm^{-3}, $[H^+] = 1 \times 10^{-3}$, μ = 1.0 mol dm^{-3}, T = 29 ± 1ºC and $\lambda_{max} = 663$ nm.

D	1/D	10^3 k^1 min^{-1}	k_2 dm^3 mol^{-1} min^{-1}
0.5	2.00	11.52	5.76
1.0	1.00	10.82	5.41
1.5	0.67	9.90	4.95
2.0	0.50	8.98	4.49
2.5	0.40	7.60	3.80
3.0	0.33	7.14	3.57

Table 3: Effect of Added Cations (Ca^{2+} and Mg^{2+}**)** $[MB^+] = 1.0 \times 10^{-5}$ mol dm^{-3}, $[NO_3^-] = 20 \times 10^{-4}$ mol dm^{-3}, $[H^+] = 1 \times 10^{-3}$, μ = 1.0 mol dm^{-3}, T = 29 ± 1ºC and $\lambda_{max} = 663$ nm.

103 Ca2+	103 k1 Min-3	k_2 dm3 mol -1 Min-1
0.5	2.99	1.50
1.0	3.22	1.61
1.5	3.54	1.73
2.0	3.69	1.84
2.5	3.92	1.96
3.0	4.15	2.07
103 Mg2+		1.27
0.5	2.53	1.28
1.0	2.53	1.27
1.5	2.54	1.27
2.0	2.55	1.28
2.5	2.55	1.28
3.0	2.55	1.28

Product Analysis: At completion of the reaction the product was analyzed qualitatively. NO_2^- was tested qualitatively by mixtures $PbNO_3/AgNO_3$ respectively which gave white ppt. indicating the presence of NO_2^-. Furthermore, a UV visible spectrum of the colouless product showed no absorption peak at maximum wavelength of 663 nm. This indicates the destruction of the quinoid (chromophore) group.

Free Radicals: Addition of 2 ml solution of 10 % acrylamide to partially reacted mixture did not show the presence of a gel even in excess methanol, indicating the probable absence of free radicals in the reaction mechanism. (Bexendde, 1954).

Spectroscopic Test for Intermediate Complex Formation: The results of the spectroscopic studies indicate no significant shifts from the absorption maxima of λ_{max} = 663 nm. This suggests the absence of the formation of an intermediate complex in the reaction. Similar observation was reported for MB by MnO_4 and 1-methyl-2-thiourea by Osunlaja et al., (2012a and 2012b) respectively.

Reaction mechanism: Based on the results obtained from the experimental conditions, it is reasonable to postulate that NO_3^- is protonated in a fast step to give HNO_3 which then reacts with MB in a slow step to give the products. Also, the intercept obtained from the plot of k_2 versus $[H^+]$ indicates that the unprotonated NO_3^- also reacts with MB to form the products. Therefore, taking recourse to the experimental data, the following mechanistic steps have been postulated for the reaction:

$$NO_3^- + H^+ \overset{K_1}{\rightleftharpoons} HNO_3 \qquad 4$$

$$MB^+ + HNO_3 \xrightarrow{k2} \text{products} \qquad 5$$

$$MB^+ + NO_3^- \xrightarrow{k3} \text{products} \qquad 6$$

From equation 4

$$[HNO_3] = K_1[NO_3^-][H^+] \qquad 7$$

$$Rate = k_2[MB^+][HNO_3] + k_3[MB^+][NO_3^-] \qquad 8$$

Putting equation 7 into 8,

$$Rate = k_2K_1[MB^+][NO_3^-][H^+] + k_3[MB^+][NO_3^-] \qquad 9$$

$$Rate = k_3 + k_2K_1\ [MB^+][H^+][NO_3 \qquad 10$$

Equation 10 is similar to equation 3 where k_3 = ‘a’ = $2.32 \times 10^{-3}\ dm^3\ mol^{-1}\ s^{-1}$ and k_2K_1 = ‘b’ = $0.649\ dm^3\ mol^{-1}\ s^{-1}$

REFERENCES

Addiscott, TM and Benjamin, N (2004). "Nitrate and human health"

Avery, AA (1999). "Infant Methemoglobemia - reexamining the role of drinking water nitrates.

Babatunde, OA, Faruruwa, M.D. and Umoru, P. E. (2013). Mechanism of the reduction of 3,7bis(dimethylamino)phenothionium chloride by metabisulphite ion in acidic medium, *Der ChemicaSinica*, 4(3): 69-78.

Babatunde OA, and Umoru, Patricia Ese (2014). Kinetic Approach To The Mechanism Of P-Bis(Dimethylamino)-Phenothiazin-5-Ium Chloride Sulphate Ion In Acidic Medium, *International Journal of Scientific & Technology Research* 3(7):240-245.

Bexendde, JH and Hardy, HR (1954). Kinetics and equilibria in solution containing ferrous ions, ferric ions and some substituted hydroquinone and quinines. Trans Faraday Soc., 50: 808.

Bronsted, J N Z (1922). *Phys.Chem. K*, 102, 89.

Bugaje, BM (2006). Kinetics and mechanisms of redox reactions of methylene blue and ethylenediamine tetracetatocobaltate(II) with some oxy-anions in aqueous solution. M.Sc.thesis. Chemistry Department, Ahmadu Bello University, Zaria.

Guttmann, P and Ehrlich, P (1891). "Über die Wirkung des Methylenblau bei Malaria" (On the effect of methylene blue on malaria), *Berliner Klinische Wochenschrift*, **28** : 953-956.

Idris, SO, Iyun, J F and Agbaji, EB (2004). ChemClass Journal, , 27-30.

Idris, SO Iyun, JF and Agbaji, EB(2005). *ChemClass Journal.* 2**:** 85-87.

Iyun, J F and Asala, M (1994). Mechanism of bromate oxidation of 3,7-bis (dimethylamino) phenazothionium chloride. A paper presented at the 18th annual conference of the chemical society of Nigeria, Kano. 29th May – 2nd June.

Jonnalagadda, S B, Chinake, C R and Simoyi, R H (1996). J.Phys. Chem.100:13521-15530.

Khan, MN and Sarwar, A (2001). The influence of transition metal ions on the kinetics of ascorbic acid oxidation by methylene blue in strongly acidic media. Turk J. Chem., 25, 433-400.

Laue, W, Thiemann, M, Scheibler, E and Wiegand, K W (2006). "Nitrates and Nitrites" in Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH, Weinheim.

Marschner, H (1999). *Mineral nutrition of higher plants*. Academic Press, London. 889.

Olajire AA and Olajide, A (2014). Kinetic Study of Decolorization of Methylene Blue with Sodium Sulphite in Aqueous Media: Influence of Transition Metal Ions. J Phys Chem Biophys 4-2.

Osunlaja AA, Idris SO and Iyun JF (2012a).Mechanism of the Reduction of Methylene Blue by Thiourea in Aqueous Acidic Medium. Int. J. ChemTech Res, 4(2): 609- 617.

Osunlaja, A A, Idris, S O, Iyun, JF (2012b). Kinetics and mechanism of the methylene blue-permanganate ion reaction in acidic medium.*Archives of Applied Science Research,* 4 (2):772-780.

Osunlaja, AA, Idris, S O and Iyun, JF (2012c). Kinetic analysis of the oxidation of 1-methyl-2-thiourea by methylene blue in acid medium.*Der ChemicaSinica,* 3(6):1428-1437.

Resch, P, Field RJ, Schneider, FW and Burger, M (1989). Reduction of methylene blue by sulphide ion in the presence and absence of oxygen: simulation of the methylene blue-O2-HS-CSTR Oscillations. J Phys Chem 93: 8181 – 8186.

Romano, N and Zeng, C (2007). "Acute toxicity of sodium nitrate, potassium nitrate and potassium chloride and their effects on the hemolymph composition and gill structure of early juvenile blue swimmer crabs (*Portunus pelagicus*, Linnaeus 1758) (Decapoda, Brachyura, Portunidae)". Environmental Toxicology and Chemistry 26: 1955–1962.

Schirmer H., Coulibaly B. and Stich A. (2003). "Methylene blue as an antimalarial agent—past and future". Redox Rep 8 (5): 272–276.

Sharpe, S. (2013). Nitrate in Aquarium *About.com*. Retrieved October 30, 2013.

Sutter, JR and Spencer, W (1990). An Equilibrium and Kinetic Study of the Methylene Blue-ferrocyanide Reaction in Acid Medium.*J. Phys. Chem.* 94 (10): 4116–4119.

The New York Times. (2013). "Methemoglobinemia" Retrieved 31 October 2013.

Ukoha, PO (2005). Kinetics and mechanisms of the reduction of 3,7-bis(dimethylamino) phenazothionium chloride by β-mercaptoethanol in acid media. *.J. Chem. Soc. Nigeria.* 30(2): 165-170.