Degradation of Tolonium Chloride Dye by Phosphate Ion in Aqueous Acidic Solution: Kinetic Approach

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Abstract: The degradation of tolonium chloride (TC^+) dye by phosphate ion (PO_4^{3-}) in an aqueous acidic solution was studied using spectrophotometric analysis at 301 K, I= 1.0 M, $[TC^+]$= 1.5×10^{-5} M, $[H^+]$= 1.0×10^{-3} M, and λ_{max} 600 nm. To determine the potency and rate of the reactant species, an aqueous acidic medium was employed. The reaction's direction and tendency were predicted using a thermodynamic analysis at an interval of 5.0 K and a temperature range of 301-321 K. Without the presence of intermediate complex/free atoms formation, a reaction that produced phenyl sulphoxide, phenylamine, and HPO_3^{2-} as products of the reaction was obtained with a molar ratio of 1:1 for both reactants. First-order tolonium chloride reactivity was found in the reaction and first-order for the phosphate ion, resulting in a second-order reaction overall. The reaction process accelerated as the concentration of hydrochloric acid rose. The response time decreased with an increase in ionic strength concentration and added Ca^{2+} and Cl^- did catalyze the reaction positively. A straight line that went through the origin was produced by plotting $1/k_o$ vs PO_4^{3-} concentration. The spectroscopic analysis showed no discernible shift from λ_{max} of 600 nm. Additionally, an increase in temperature accelerated the reaction process. The reaction has a negative free energy change, G (-3.13–1.12 KJ/mol) which indicates that it is spontaneous and that the reactants have more free energy than that of the products. While the enthalpy of activation, H is positive and indicates that the reaction was endothermic and followed an associative path, the entropy of activation, S, is also negative (-7.45–1.10 KJ/mol), indicating that the reaction is less disordered. Due to the added ions catalysis and absence of free atoms during the course of the reaction, an outer-sphere mechanism was suggested for the reaction.

Keywords: Degradation, Tolonium chloride, Phosphate ion, Acidic Medium.

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1. INTRODUCTION

An acidophilic metachromatic dye called tolonium chloride, hereafter referred to as TC^+ only stains acidic tissues (1). Since TC^+ has a strong affinity for nucleic acids, it can bind to tissues rich in DNA and RNA as well as nuclear material (2,3). Water and alcohol only partially dissolve TC^+ (2-4), which exits as the 3 isoforms ortho-, para-, and meta-toluidine (3). To do a quick microscopic inspection of a specimen, frozen sections are routinely stained with it. With TC+, frozen sections can be quickly dyed and examined (3) as well the identification of mast cells (5). It is utilized in forensic examination (6) and renal pathology (7). In premalignant lesions, the surgeons utilize the dye to help highlight areas of mucosal dysplasia (8).

Tolonium chloride is used to stain cellular nuclei, making it beneficial for detecting cancerous tissue that has a greater nuclear-to-cytoplasmic ratio and increased DNA concentration (9). It can be an aid in the routine screening of people at risk and is useful in the assessment and representation of subtle oral mucosal changes. It is possible to utilize it to guide a brush biopsy without interfering with the computer-assisted cytological analysis of these tissues (10). It was reported that for many years, tolonium chloride has been successfully used as a key stain and a tool in the early diagnosis of oral cancer, cancer of the oral mucosa, and cancer of the upper aerodigestive tract (11). It is advised to use TC^+ in addition to

chemiluminescence or other methods as a diagnostic tool (12). The ability to detect early oral squamous cell carcinoma (SCC) and high-grade dysplasias using TC$^+$ is also well documented (13–16).

The naturally occurring form of the element phosphorus, known as phosphates (PO$_4$$^{3-}$), is present in numerous phosphate minerals. A rock or mineral that contains phosphate ions is referred to as phosphate. Phosphorus is extracted from inorganic phosphates for use in industrial and agricultural purposes (17). They are phosphorus medicinal salts, and the hygroscopic anhydrous form of phosphate is easily soluble in water but insoluble in alcohol (18). Phosphate makes up the majority of the phosphorus in living things (19). Phosphates are often made by neutralizing phosphoric acid, either completely or partially, with sodium carbonate or sodium hydroxide (20). The resulting solution within the container can then be evaporated to produce crystals of the particular hydrate. The hydrate is stable over a wide temperature range (21). Usually, inorganic phosphate is produced during the synthesis of a substance like sucrose (22). PO$_4$$^{3-}$ plays fundamental functions in biochemistry, biogeochemistry, and ecology, making them very useful (17). Some phosphates are effective in treating urinary tract infections and preventing the formation of calcium stones in the urinary system. For patients who cannot acquire enough phosphorus in their diet daily, they utilize phosphates as dietary supplements (23). PO$_4$$^{3-}$ is used in a wide variety of food and water treatment processes (24). For instance, sodium phosphates are frequently employed as leavening agents, thickeners, and emulsifiers in baked goods. They are additionally utilized to regulate the pH of processed foods (25). It is known that phosphate can help with constipation and can get the bowels ready for surgery (25). They are effective anti-rust agents and are used in detergents to soften water (25). They serve a variety of purposes, including those related to antimicrobials (26), pH control agents (buffers), boiling water additions, cleansers, coagulants, leaving agents, stabilizers, emulsifiers, texturizers, minerals, and dietary supplements. In meals, mono- and tri-sodium phosphate is employed as a buffering salt. The main application is to maintain the pH of food systems for fruit and vegetable products (27). They are employed as an acidulent (18), as an excipient in pharmaceuticals, and saline solutions (18). The shelf life of soy products is said to be extended by the addition of phosphates (28). Despite not affecting gram-negative bacteria like Salmonella typhimurium, phosphorus is bacteriostatic in that it suppresses the growth of some gram-positive bacteria (29–31). To increase the stability, water holding capacity, and sausage emulsion of emulsified sausages, phosphate mixtures are used (31,32). When used in compliance with GMP, the U.S. Food and Drug Administration has designated food phosphates as generally safe (33). Phosphates may be included in meat products, minced meat, and meat preparations (34). Phosphorus peroxide (P$_2$O$_5$), alone or in combination, is allowed in final products at a rate of 5 g/kg according to European law (35). Phosphates are frequently utilized in the meat processing industry according to (36). Utilizing the right quantity and combination of phosphates can increase the finished products' ability to hold moisture, hold water, protect color, delay oxidation, extend shelf life, stabilize and improve structure, and more. Phosphates aid in the restoration of periodontal deficiencies, enhancement of alveolar bone, sinus lifts, tooth substitutes, and restoration of significant bone defects brought on by cancers (37–43). In tissue engineering, they are also utilized as scaffolds for the regeneration of bone or dentin (43–47). To reduce the bioactivity of the phosphates and the metal's strength, calcium phosphates in particular are utilized as injectable cements (48,49) and as coatings on titanium and titanium alloy implants (50,51).

Due to its uses in inorganic chemistry and living beings, phosphate reaction is one of the most cutting-edge fields of study. It is advantageous for plant development and has various potential uses in outstanding chemical processes in the pharmaceutical, food, and detergent sectors. In addition, the potential applications of tolonium chloride as a basic stain in the early detection of oral cancer (9) and the widespread use of phosphate in manufacturing and agriculture sparked our interest in the study of the degradation of tolonium chloride with phosphate ion. Literature has reported works done on TC$^+$ but little is known about its degradation by phosphate ion which prompted this research. The purpose therefore of this research is to see how phosphate ions can degrade tolonium chloride in an acidic medium. It is expected that TC$^+$ will be degraded to other forms to prevent pollution of water bodies by TC$^+$ when used as a dye in textile industries. We are confident that the information gleaned from this reaction will contribute to a better understanding of the bioactivities of the phosphate ion and the redox behavior of tolonium chloride.

2. EXPERIMENTAL SECTION

2.1. Materials/Chemicals
Chemicals from A.J. Chemical Company Kaduna were used throughout. All chemicals are of analytical grade and were used throughout the research without further purification. Distilled water was used to prepare all solutions. The chemicals used are: Tolonium chloride (CAS Number= 92-31-9), potassium phosphate(CAS Number= 7778-77-0), calcium nitrate (CAS Number= 10124-37-5), sodium chloride (CAS Number= 7647-15-5), hydrochloric acid (CAS Number= 7647-01-0) and barium chloride (CAS Number= 10361-37-2).

2.2. Methods

2.2.1. Stoichiometric and product scrutiny
Molar ratio calculations were used to determine the reaction's stoichiometry. The concentration of the TC$^+$ was at 1.0×10^{-6} M, the concentration of the hydrogen ion was at 1.0×10^{-3} M, the ionic strength was at 1.0 M, and the concentration of PO$_4$$^{3-}$ was changed between $(1.0$-$7.5) \times 10^{-6}$ M within an interval of 0.5×10^{-6} M. After 24 hours, when the reaction mixture had become colorless (52), the ultimate absorbance of each reacting mixture was measured,

and an absorbance versus concentration plot was made. Two drops of dilute lead acetate solution and two drops of acetic acid were added to the 2 mL of resultant colorless mixture to examine the presence of HPO_3^{2-} (53). Bromine water was further added to another 2 mL of the reaction solution to determine the presence of phenylamine as another reaction product.

2.2.2. Kinetic inquiry
The concentration of PO_4^{3-} was produced to surpass that of $[TC^+]$ by at least 15.33 times at $[TC^+]$= 1.5×10^{-5} M, $[PO_4^{3-}]$= $(2.3-3.6)\times10^{-4}$ M in an interval of 0.3×10^{-4} M, $[H^+]$= 1.0×10^{-3} M, I= 1.0 M, T= 301 K, and λ_{max}= 600 nm. Using a UV/visible spectrophotometer spectrum lab 725s, the reduction in the absorbance of the reaction with time was detected due to the alteration in the concentration of the reacting species. It was possible to get kinetic plots of log (A_t-A_∞) where A_t is Absorbance at the time "t" and A_∞, Absorbance at infinity vs time (s) and log k_o (observed rate constant) versus log $[PO_4^{3-}]$ which was used to evaluate the order of the reaction (54,55).

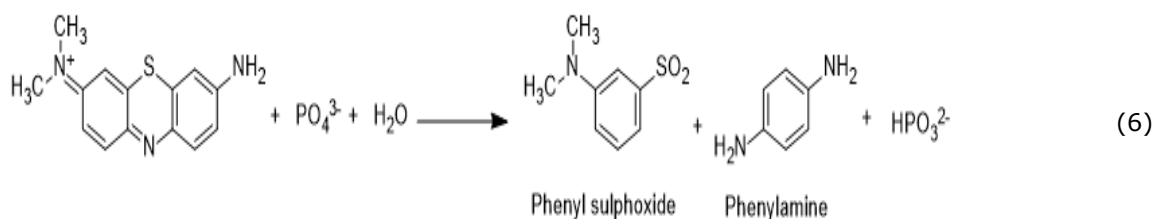### 2.2.3. Change in the concentration of hydrochloric acid
Change in the concentration of hydrochloric acid was investigated between $(0.4-1.0)\times10^{-3}$ M within a range of 0.2×10^{-3} M intervals with TC^+= 1.5×10^{-5} M, $[PO_4^{3-}]$= 2.9×10^{-4} M, I= 1.0 M, T= 301 K, and λ_{max}= 600 nm. A log of k_2 vs $[H^+]$ plot was produced(56).

2.2.4. Study of ionic strength
The study of ionic strength for the reaction was observed by making the concentration of TC^+= 1.5×10^{-5} M, $[PO_4^{3-}]$= 2.9×10^{-4} M, $[H^+]$= 1.0×10^{-3} M, I= 1.0 M, T= 301 K and λ_{max}= 600 nm while that of ionic strength was varied between $(0.4-2.0)$ M (56,57) within an interval of 0.2 M after which plot of log of k_2 versus $\sqrt{I}$ was attained.

2.2.5. Effect of added Ca^{2+} and Cl^- on the reaction rate
The effect of added Ca^{2+} and Cl^- on the reaction rate was investigated by adding $(2.0-3.5)\times10^{-2}$ M of Ca^{2+} and Cl^- respectively. The concentration of tolunuim chloride, hydrogen ion, ionic strength, and PO_4^{3-} were kept constant (57).

2.2.6. Consequence of temperature variation on reaction speed
At intervals of 5.0 K, the effect of temperature fluctuation on reaction speed was examined between 301 and 321 K. Other parameters such as $[TC^+]$= 1.5×10^{-5} M, $[PO_4^{3-}]$= 2.9×10^{-4} M, $[H^+]$= 1.0×10^{-3} M, I= 1.0 M, and λ_{max}= 600 nm had constant concentrations. It was possible to plot the reciprocal of temperature $(1/T$ $K^{-1})$ against the log computed speed constant (log k_{cal}). Equation 1 was used to compute the speed constant from the measured speed constant k_o, and Equation 2 was used to determine the activation energy (58):

$$\mathbf{k_{cal}} = \frac{k_0}{[PO_4^{3-}][H^+][I]} \qquad (1)$$

$$\mathbf{E_a}; \quad Slope = \frac{-E_a}{2.303\times R} \qquad (2)$$

Where R = gas constant

2.2.7. Thermodynamic exploration
The equations employed for the investigation of the common changes in free energy (G),8enthalpy (H), and entropy (S) are given in (Equations 3, 4, and 5) respectively (58).

$$\mathbf{\Delta G}; = -2.303 \times R \times T \times \log k_{cal} \qquad (3)$$

$$\mathbf{\Delta H}; \quad Slope = \frac{-\Delta H}{R\times T} \qquad (4)$$

$$\mathbf{\Delta S}; \quad \Delta G - \Delta H \qquad (5)$$

2.2.8. Intermediate complex investigation and data analysis
The intermediate complex was examined using the formula; $1/k_o$ versus $1/[PO_4^{3-}]$ plot. The data collected underwent statistical analysis to determine their mean and standard deviation.

3. RESULTS AND DISCUSSION

3.1. Stoichiometry and Product Scrutiny
As demonstrated in (Equation 6), one mole of PO_4^{3-} was consumed by one mole of TC^+ Equation 6 is a representation of the degradation of TC^+ to phenyl sulphoxide and phenylamine.

Phenyl sulphoxide   Phenylamine

This result is not in agreement with the reports of (59-61) for the autocatalyzed reaction between phenyl hydrazine and toluidine blue in aqueous solution, Redox reaction of bis-(2-pyridinealdoximato)dioxomolybdate(IV) complex with thiosulphate ion in aqueous acidic and surfactant media, the study of Piszkiewicz's and Berezin's models on the redox reaction of allylthiourea and bis-(2-pyridinealdoximato)dioxomolybdate(IV) complex in an aqueous acidic medium respectively. When lead acetate solution was added to the colorless reaction mixture, a white precipitate resulted, which became soluble in two drops of acetic acid to reveal HPO_3^{2-} as one of the reaction's end products (53). In addition to two drops of bromine

water to the reaction mixture, the bromine water was decolorized and a white precipitate was formed confirming the presence of phenylamine as another product of the reaction.

3.2. Kinetic Inquiry

The graph of log ($A_t - A_\infty$) versus time (s) has an R^2 of 0.995 Figure 1, indicating that it is linear and is first-order about [TC^+]. The kinetic plot of log k_o vs log [PO_4^{3-}) in Figure 2 was also linear with a slope of

0.9972 which is almost unity, indicating also a first-order reaction concerning [PO_4^{3-}]. The second-order speed constants k_2 were computed by dividing the values of k_o with the concentration of PO_4^{3-}, and the values were discovered to be fairly constant (Table 1). The rate law for the reaction is displayed In (Equation 7).

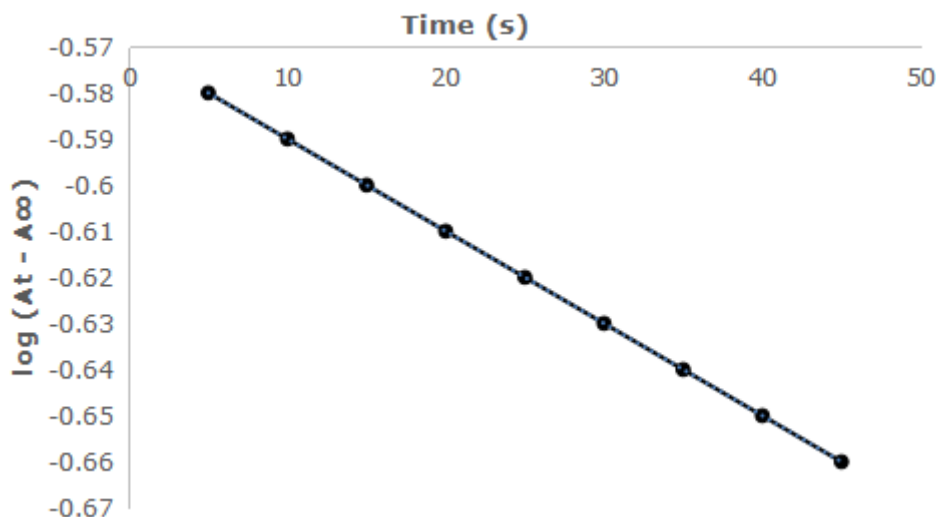$$\frac{-d[TC^+]}{dt} = k_2[TC^+][PO_4^{3-}] \qquad (7)$$

Figure 1: Graph for the Redox Reaction of TC^+ and PO_4^{3-} at [TC^+]= 1.5×10^{-5} M, [H^+]= 1×10^{-3} M, I= 1.0 M, λ_{max}= 600 nm and T= 301 K.
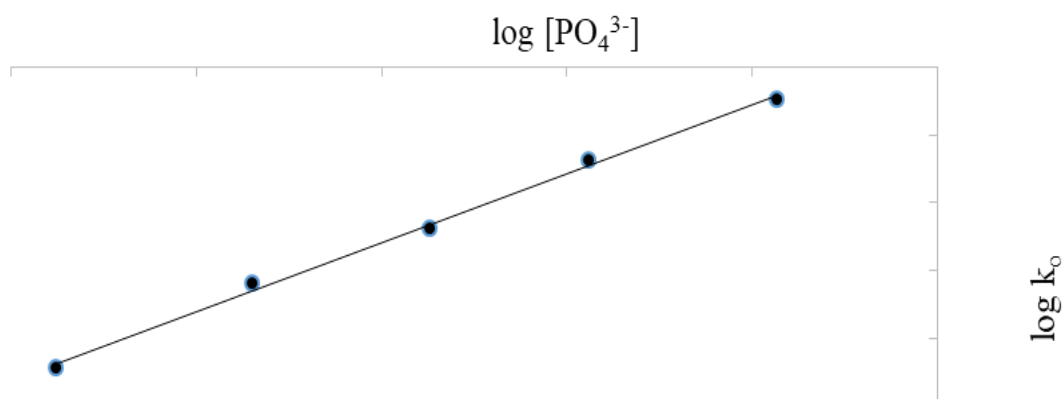

Figure 2: Plot of log k_o versus log [PO_4^{3-}], [TC^+] = 1.5×10^{-5} M [H^+] = 1×10^{-3} M I = 1.0 M λ_{max} = 600 nm and T= 301 K

Table 1: Speed constant for the reduction of TC^+ by PO_4^{3-} at 301 k, I= 1.0 M, [TC^+]= 1.5×10^{-5}, [H^+]= 1.0×10^{-3} M, [PO_4^{3-}]= $(2.3–3.6)\times10^{-4}$ and λ_{max}= 600 nm.

10^4 [PO_4^{3-}] M	I = 1.0 M	[H^+]= 1.0×10^{-3} M	10^3 k_o/s	k_2 dm^3/mol.s
2.30	1.00	1.00	5.99	26.04
2.60	1.00	1.00	6.80	26.15
2.90	1.00	1.00	7.59	26.17
3.20	1.00	1.00	8.40	26.25
3.60	1.00	1.00	9.44	26.22
2.90	0.40	1.00	7.60	26.21
2.90	0.60	1.00	5.99	20.66
2.90	0.80	1.00	4.84	16.69
2.90	1.00	1.00	3.76	12.97
2.90	1.00	0.40	4.14	14.28
2.90	1.00	0.60	5.07	17.48
2.90	1.00	0.80	6.22	21.45
2.90	1.00	1.00	7.60	26.21

3.3. Change in The Concentration of Hydrochloric Acid

It was discovered that by increasing the concentration of hydrochloric acid in the reaction, the rate of reaction was observed to increase (Figure. 3). The relationship between k_2 and $[H^+]$ was linear, with a 5.955 dm^6/mol^2.s intercept on the k_2 axis. This suggests that the protonated and non-protonated forms are in a quick state of pre-equilibrium, this also suggests that both forms are reactive (62). This is in contrast with the finding reported by (63), which showed that the kinetics and mechanism of the redox interaction between toluidine blue and nitrite ions in an aqueous acidic medium did not exhibit any intercept. The rate laws are depicted in Equations 8 and 9:

$$k_2 = (a + b[H^+]) \qquad (8)$$

$$\frac{-d[TC^+]}{dt} = (a + b[H^+])[TC^+][PO_4^{3-}] \qquad (9)$$

Where 'a' the intercept= 5.955 dm^6/mol^2.s and 'b' the slope= 1.985 dm^3/mol.s
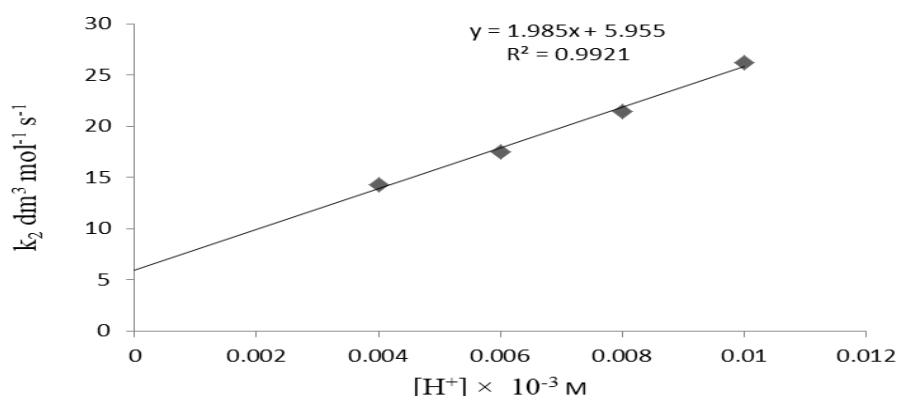
Figure 3: Plot of k_2 versus $[H^+]$ for the reduction of phosphate ion at $[TC^+]$= 1.5×10⁻⁵ M, $[PO_4^{3-}$= 2.9×10⁻⁴ M, $[H^+]$= (0.4–1.0×10⁻³ I= 1.0 M, λ_{max}= 600 nm and T= 301 K.

3.4. Study of Ionic Strength

As the ionic strength concentration was raised, the rate of reaction between TC^+ and PO_4^{3-} was observed to decrease. According to this, the transfer of electrons takes place when two dissimilar charges are in action. The findings are in contrast to that reported by (64) regarding the kinetics and mechanism of the oxidation of indigo carmine by permanganate ion in an aqueous acidic medium which showed that the reaction rate was unaffected as the ionic strength concentration was increased. The system plot was linear, as depicted in Figure 4's log k_2 versus $\sqrt{I}$ plot.
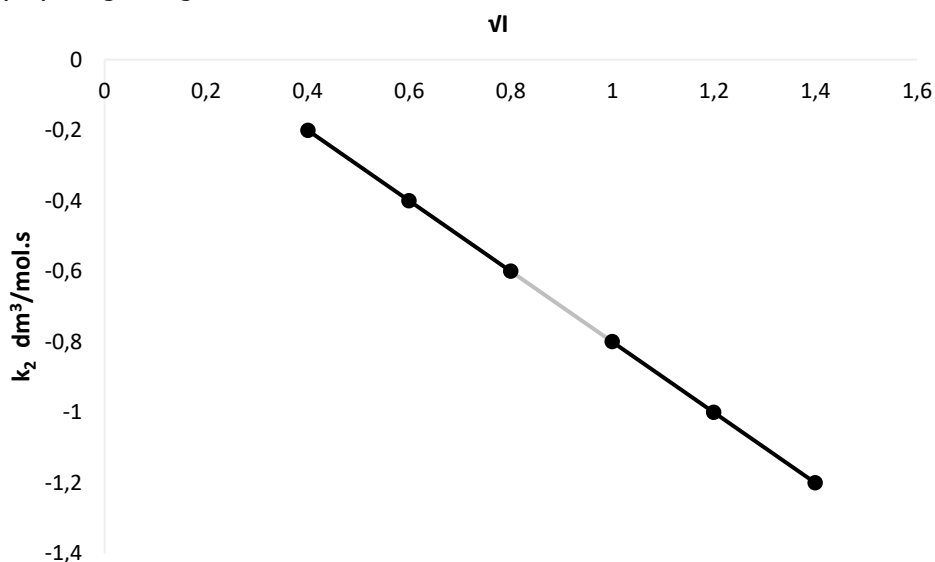

Figure 4: Plot of log k_2 versus $\sqrt{I}$ for the Reduction of TC^+ by PO_4^{3-}, $[PO_4^{3-}]$= 2.9×10⁻⁴ M, $[TC^+]$= 1.5×10⁻⁵ M, $[H^+]$= 1×10⁻³ M, I= 1.0 M, λ_{max}= 600 nm and T= 301 K.

3.5. Effect of Added Ca^{2+} and Cl^-

The addition of Ca^{2+} and Cl^- to the reaction mixture increased the rate of reaction (Table 2). This suggests that the reaction probably follows an outer-sphere mechanism.

3.6. Consequence of Temperature Variation on the Speed of Reaction

The results demonstrated that the speed constant, k_o, increased as the temperature rose (Table 3). It was possible to create a linear graph (Figure 5) with a slope of -1.671 by plotting log k_{cal} against the

temperature reciprocal (1/T K^{-1}). The activation energy (Ea) yielded a value of 31.99 KJ/mol.

3.7. Thermodynamic Exploration
According to Table 4, the reaction's free energy, or G, was negative (-3.13 1.12 KJ/mol), meaning that the reactants had greater free energy than the

products. Additionally, it indicates that the reaction was unplanned (65,66). The entropy of activation, or S, is also negative, showing that the reaction is less disordered, while the enthalpy of activation, or H, is positive (4.32 0.10 KJ/mol), suggesting that the reaction is endothermic and follows an associative path.

Table 2: Pseudo First Order and Second Order Rate Constants for the effect of added Ca^{2+} and Cl^- for TC^+ by PO_4^{3-} at $[TC^+]=1.5\times10^{-5}$ M, $[PO_4^{3-}]=2.9\times10^{-4}$ M, $[H^+]=1.0\times10^{-3}$ M, I=1.0 M, T=301 K and $\lambda_{max}=600$ nm.

Ion	10^2 [Ion] M	10^2 k_o/s	k_2 dm^3/mol.s
Ca^{2+}	2.0	2.810	1.405
	2.5	3.690	1.476
	3.0	5.200	1.733
	3.5	6.399	1.828
Cl$^-$	2.0	3.286	1.643
	2.5	4.368	1.747
	3.0	6.388	2.129
	3.5	7.531	2.152

Table 3: Consequence of temperature variation on the speed of reaction.

Temp. K	$10^3$1/T K^{-1}	10^4[PO$_4^{3-}$] M	10^3[H$^+$] M	$10^7$$k_o$	k_{cal} MS	log k_{cal}
301	3.32	2.9	1.00	5.97	2.06	0.31
306	3.27	2.9	1.00	7.91	2.73	0.44
311	3.22	2.9	1.00	9.59	3.31	0.52
316	3.16	2.9	1.00	10.52	3.63	0.56
321	3.12	2.9	1.00	13.94	4.81	0.68

$$y = -1.671x + 5.8793$$
$$R^2 = 0.9572$$

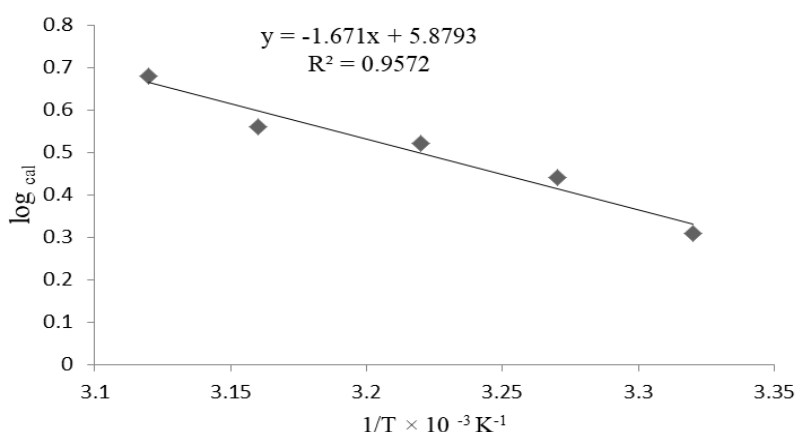**Figure 5:** Plot of $\log k_{cal}$ versus 1/T for the reduction of phosphate ion at $[TC^+]= 1.5\times10^{-5}$ M, $[PO_4^{3-}]= 2.9\times10^{-4}$ M, $[H^+]= (0.4–1.0)\times10^{-3}$ I= 1.0 M, $\lambda_{max}= 600$ nm and T= 301–321 K.

Table 4: Thermodynamic exploration.

Temp. K	log k_{cal}	ΔG kJ/mol	ΔH kJ/mol	ΔS kJ/mol
301	0.31	-1.79	4.18	-5.97
306	0.44	-2.58	4.25	-6.83
311	0.52	-3.10	4.32	-7.42
316	0.56	-3.39	4.39	-7.78
321	0.68	-4.81	4.46	-9.27

3.8. Intermediate Complex Investigation
The graph of $1/k_o$ versus $1/[PO_4^{3-}]$, which produced a straight line from the origin (Figure 6), indicates that there were no intermediate complex/free atoms

formed during the course of TC^+ the degradation process. As a result, it supports an outer-sphere mechanism for the reaction by pointing to the lack of intermediate complex formation.
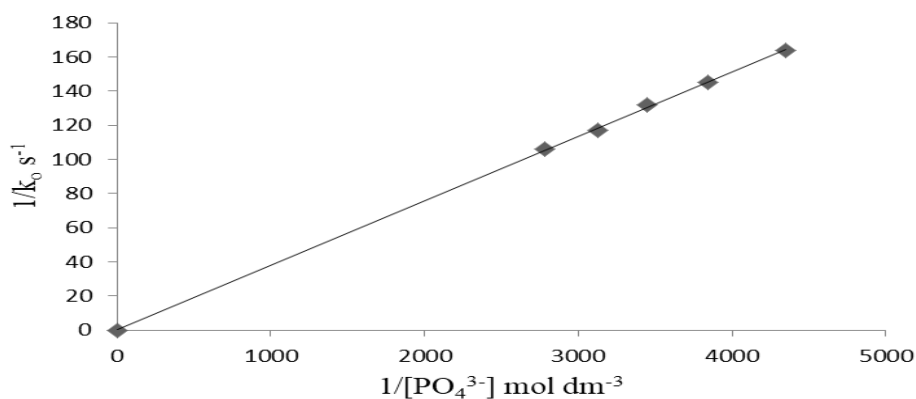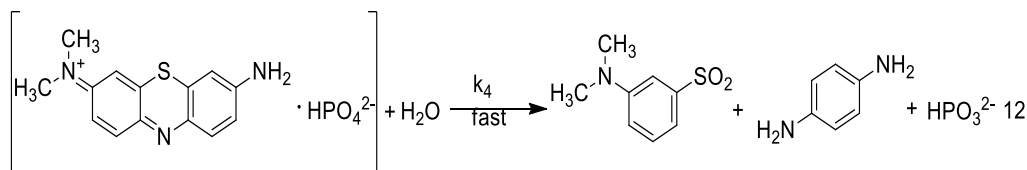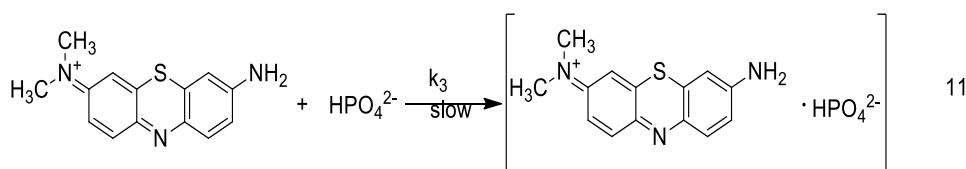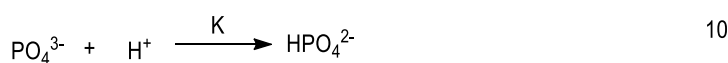
Figure 6: Plot of $1/k_1$ versus $1/[PO_4^{3-}]$, $[TC^+]= 1.5\times10^{-5}$ M, $[PO_4^{3-}]= (2.3–3.6)\times10^{-4}$ M, $[H^+]= 1\times10^{-3}$ M, $I= 1.0$ M, $\lambda_{max}= 600$ nm and T= 301 K.

Mechanism of Reaction for Totonium Chloride (TC) and Phosphate ion (PO_4^{3-})

$$PO_4^{3-} + H^+ \xrightarrow{K} HPO_4^{2-} \qquad 10$$

11

12

$$\text{Rate} = k_3[TC^+][HPO_4^{2-}] \qquad 13$$

from equation 10

$$[HPO_4^{2-}] = K[PO_4^{3-}][H^+] \qquad 14$$

Substituting equation 14 into 13;

$$\text{Rate} = Kk_3[TC^+][PO_4^{3-}][H^+] \qquad 15$$

Therefore; Rate = $k[TC^+][PO_4^{3-}]$ \qquad 16

Equation 16 is similar to equqtion 9

4. CONCLUSION

The degradation of tolonium chloride by phosphate ion (PO_4^{3-}) in an aqueous acidic solution was studied and the results revealed a molar ratio of 1:1. The reaction exhibited acid and ionic strength dependence which could account for TC^+'s quick detection of and attachment to biological systems. The results of the examination into intermediate complexes showed a straight line from the origin, indicating the lack of any intermediates. Alternatively, it's possible that the intermediate complex was created so quickly during the redox reaction that it was not visible. Thermodynamic analysis showed that a temperature rise sped up the reaction time. Free energy is spontaneous and has a negative sign, indicating that the reactants have more free energy than the products have.

Additionally, the activation entropy is negative, demonstrating that the reaction is less disordered, and the activation enthalpy is positive, demonstrating that the reaction was endothermic and continues via an associative pathway. The movement towards an outer-sphere mechanistic pathway has been influenced by the catalysis of added Ca^{2+} and Cl^- and the absence of an observable intermediate complex formation.

5. CONFLICT OF INTEREST

The authors affirm that they do not have any conflict of interest to disclose that is pertinent to the article's subject matter.

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