

Oxidation of Indole by Peroxomonosulfate in Acetic Acid Medium: A Kinetic and Antibacterial Study

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Kinetics of oxidation of indole by peroxomonosulfate (PMS) has been studied at 283 to 308 K in aqueous acetic acid medium. The reaction follows a total second order, first order each with respect to [Indole] and [PMS]. The rate of the reaction was not affected by added $[H^+]$. Variation of ionic strength (μ) had no influence on the rate. There was no solvent effect when increase the percentage of acetic acid. There was no polymerization occur in reaction pathway. Thermodynamic parameters such as free energy of activation ($\Delta E^\ddagger$), enthalpy of activation ($\Delta H^\ddagger$) and entropy of activation ($\Delta S^\ddagger$) have been evaluated. The most plausible mechanism scheme proposed by these kinetic observations and the antibacterial activity was carried out by agar diffusion method.

Keywords: Kinetics, Mechanism, Oxidation, Indole, Acetic acid, Peroxomonosulfate.

INTRODUCTION

Indole, an electron-rich heteroaromatic nitrogen containing compounds appears various plants, alkaloids and fungal metabolites. Indole-3-acetic acid (IAA) oxidized by peroxomonosulphate (PMS) [1], peroxodisulphate (PDS) [2], dioxygen [3], Ce(IV) [3], 1,4-phenanthroline-manganese(II) complexes [4], hydrogen peroxide, persulphate, N-chloro-succinimide and sodium hypochlorite were reported [5]. 3-Methylindole oxidized by PMS and PDS in ethanol medium have been studied.

Indole-3-acetic acid (IAA) and indole derivatives have anti-inflammatory [6], tumor growth inhibitor [6], antiviral, antitubercular, antibacterial, antiallergic and psychotropic activities [7], endogenous antioxidants [8] and growth regulatory functions [9]. The indole based pharmaceutical constitute very important class of therapeutic molecules and medicinal chemistry such as anticancer [10,11], antioxidant [12] and anti HIV [13,14].

The composition of the oxidizing agent peroxomonosulphate (PMS) is $(2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4)$. The active component potassium monosulphate ($KHSO_5$) is a salt from Caro's acid (H_2SO_5). Organic and inorganic complexes [15] oxidized by PMS and the oxidation of 2, 3-dialkyl indoles by peroxodisulfate [16], PMS [16], peroxomonophosphoric acid [17], peroxodiphosphoric acid [17,18] has been already reported in the literature. The lack of kinetic and antibacterial study on the oxidation of indole by PMS in acetic acid medium was investigated in this work.

EXPERIMENTAL

Indole (Qualigens, India) and PMS (E.I. Du Pont de Nemours Company, U.S.A.) were used as such. Solutions of this salt were assayed iodometrically and by cerimetry [19]. Other chemicals and reagents such as sulphuric acid, acetic acid, sodium bisulphate used were of analytical grade with 99.9 % purity. Water distilled from Kilburn Manesty still was again distilled over alkaline permanganate in an all-glass Pyrex vessel. All reagents and solutions were prepared using this doubly distilled water.

Kinetic measurements: Kinetic studies were carried out in 50 % (v/v) aqueous acetic acid medium under pseudo first-order conditions. The reactions were performed by maintaining a large excess of [Indole] over [PMS] in the temperature range of 283-308 K. The reaction was followed by estimating the unreacted PMS as a function of time by using the iodometric method. The liberated iodine was titrated against standard sodium thiosulfate solution by using starch as indicator. From the titre values, plots of $\log [PMS]$ vs. time were made and from the slope of such plots, the pseudo first order rate constants, k' (s^{-1}) were obtained. It was checked that the results were reproducible within ± 5 % error.

Stoichiometry: The stoichiometry of the reaction, [Indole]:[PMS] was determined by taking an excess of PMS over the indole and allowing the reaction to completion. Since the concentration of the substrate (indole) taken is less compared to that of PMS, all the substrate taken would have completely

reacted leaving behind the unreacted PMS, the concentration of which was determined iodometrically. Based on the amount of substrate and PMS consumed, the stoichiometry, [Indole]:[PMS] was determined to be 1:2.

Absence of free radical intermediates: The reaction mixture failed to induce the polymerization of acrylonitrile monomer was added. These rules out the possibility of free radicals as intermediates during the course of the reaction.

Rate law: Based on kinetic observations made such as dependence of rate on concentration, $[H^+]$, μ and solvent effect, following rate equations are suggested:

$$\text{Rate} = \frac{-d[\text{PMS}]}{dt} = k_2[\text{PMS}][\text{Indole}]$$

$$\frac{\text{Rate}}{[\text{PMS}]} = k' = k_2[\text{Indole}]$$

$$k' = k_2[\text{Indole}]$$

k' = Pseudo first order rate constant and k_2 = Second order rate constant.

RESULTS AND DISCUSSION

A number of factors affect the oxidation of indole by PMS in acetic acid medium, (i) [Indole], (ii) [PMS], (iii) ionic strength (μ), (iv) $[H^+]$ and (v) dielectric constant. Rate and activation parameters were evaluated.

Effect of [Indole]: The dependence of reaction rate on [Indole] was studied with fixed [PMS], $[H^+]$, μ and solvent percentage but using various initial concentrations of indoles. It was found that the pseudofirst-order rate constants k' (s^{-1}) increases with increase in [Indole] (Table-1 and Fig. 1) over a range of [Indole] used (2.0×10^{-2} to 4.0×10^{-2} mol dm^{-3}). This indicates the first order dependence of rate on [Indole]. Such a kinetic behaviour was commonly observed the first

order with respect to [Indole] is confirmed by the straight lines passing through origin in the plots of $\log k'$ (s^{-1}) vs. $\log [\text{Indole}]$ with unit slope ($r = 0.999$) (Fig. 2) and also the absence of any self-decomposition [18] of oxidant peroxomonosulphate. The observation of total second order kinetics that is first order each with respect to [PMS] and [Indole]. The value of k_2 ($\text{mol}^{-1} \text{dm}^3 \text{s}^{-1}$) was obtained from the slope of k' (s^{-1}) vs. [Indole] plots. The k_2 ($\text{mol}^{-1} \text{dm}^3 \text{s}^{-1}$) values ($r = 0.999$) calculated from the factor $k' (s^{-1})/[\text{Indole}]$.

Effect of [PMS]: While varying [PMS], the other parameters like [Indole], $[H^+]$, percentage of aqueous acetic acid

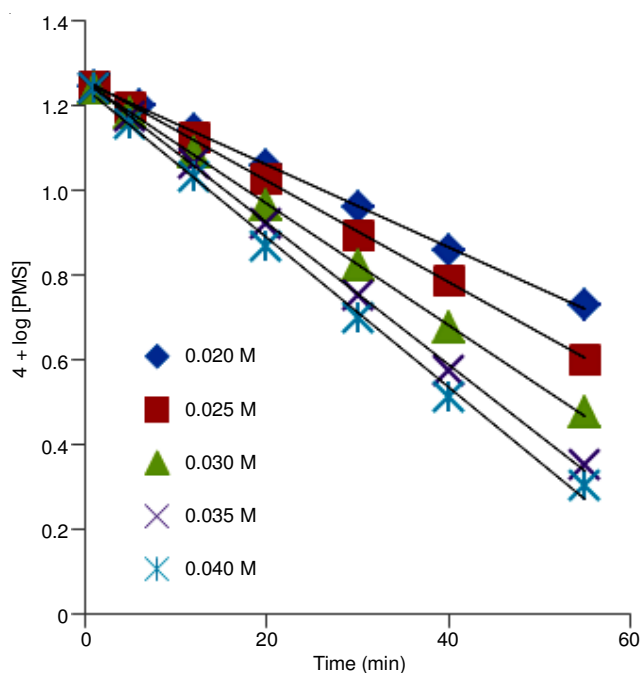
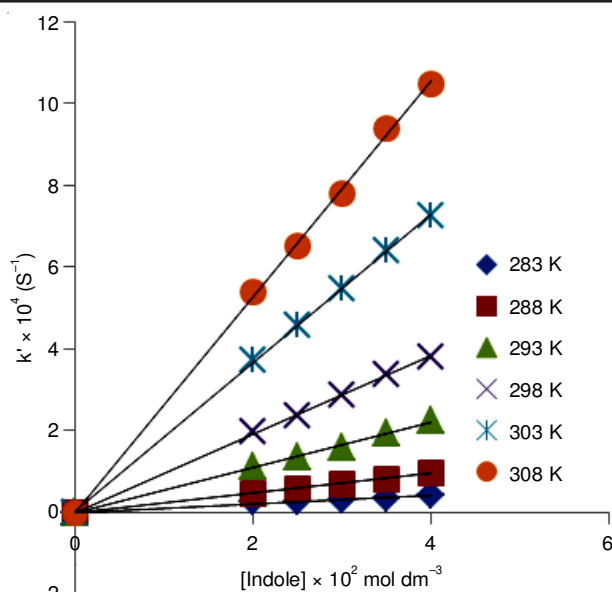


Fig. 1. Variation of [Indole] at 303 K

TABLE-1
PSEUDO-FIRST ORDER RATE CONSTANT FOR THE OXIDATION OF INDOLE BY PMS

[Indole] $\times 10^2$ (mol dm^{-3})	[PMS] $\times 10^3$ (mol dm^{-3})	$[H^+]$ (mol dm^{-3})	μ (mol dm^{-3})	EtOH (%, v/v)	$k' (10^4 s^{-1})$					
					283 K	288 K	293 K	298 K	303 K	308 K
2.0	2.0	0.02	0.3	50	0.255	0.476	1.105	1.963	3.720	5.377
2.5	2.0	0.02	0.3	50	0.276	0.585	1.365	2.373	4.587	6.808
3.0	2.0	0.02	0.3	50	0.308	0.702	1.602	2.866	5.481	7.805
3.5	2.0	0.02	0.3	50	0.337	0.824	1.942	3.377	6.420	9.377
4.0	2.0	0.02	0.3	50	0.424	0.968	2.248	3.818	7.250	10.48
3.0	1.0	0.02	0.3	50	—	—	—	—	5.560	—
3.0	1.5	0.02	0.3	50	—	—	—	—	5.264	—
3.0	2.0	0.02	0.3	50	—	—	—	—	5.481	—
3.0	2.5	0.02	0.3	50	—	—	—	—	5.446	—
3.0	3.0	0.02	0.3	50	—	—	—	—	5.472	—
3.0	2.0	0.005	0.3	50	—	—	—	—	5.381	—
3.0	2.0	0.01	0.3	50	—	—	—	—	5.506	—
3.0	2.0	0.02	0.3	50	—	—	—	—	5.481	—
3.0	2.0	0.05	0.3	50	—	—	—	—	5.468	—
3.0	2.0	0.09	0.3	50	—	—	—	—	5.400	—
3.0	2.0	0.02	0.1	50	—	—	—	—	5.439	—
3.0	2.0	0.02	0.2	50	—	—	—	—	5.375	—
3.0	2.0	0.02	0.3	50	—	—	—	—	5.481	—
3.0	2.0	0.02	0.4	50	—	—	—	—	5.375	—
3.0	2.0	0.02	0.3	30	—	—	1.898	2.994	5.811	—
3.0	2.0	0.02	0.3	40	—	—	1.774	2.924	5.653	—
3.0	2.0	0.02	0.3	50	—	—	1.602	2.866	5.481	—
3.0	2.0	0.02	0.3	60	—	—	1.542	2.658	5.224	—
3.0	2.0	0.02	0.3	70	—	—	1.354	2.541	5.124	—

Fig. 2. Evaluation of k_2

and ionic strength (μ) were all kept constant. The reaction was followed based on the disappearance of [PMS] at different time intervals iodometrically. The concentrations of [PMS] varied were in the range 1×10^{-3} mol dm^{-3} to 3×10^{-3} mol dm^{-3} (Table-1, Fig. 3). The values of pseudo-first order rate constant (k' , s^{-1}) obtained in experiments with different [PMS] are shown in Table-1 and the values of k (s^{-1}) remain constant for different [PMS]. This observation confirms the first order with respect to [PMS] from the linear plots of $\log \text{rate}$ vs. $\log [\text{PMS}]$ (Fig. 3) the slope almost very close to unity.

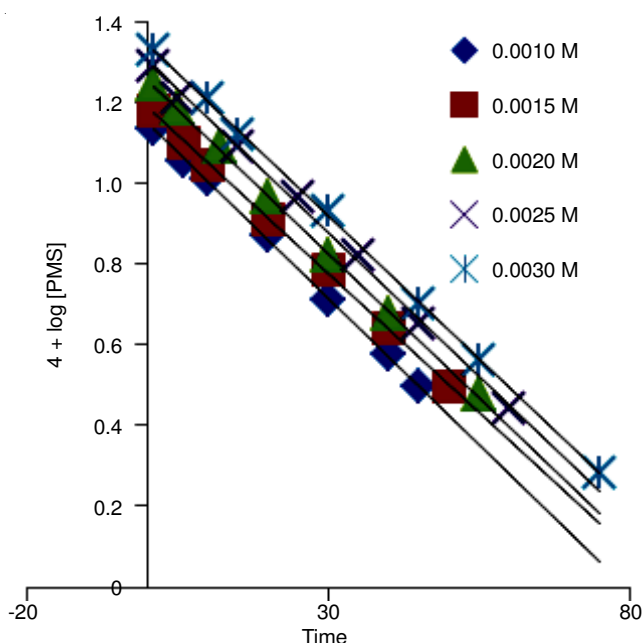


Fig. 3. Variation of [PMS] at 303 K

Effect of ionic strength (μ): In order to test if the reaction is ionic or not, reactions were conducted at different μ values by using different concentrations of the salt (1.0×10^{-1} – 4.0×10^{-1} mol dm^{-3}), NaHSO_4 , while the other additives were maintained constant. It was noticed that increase of μ did not alter

the reaction rate significantly (Table-1). This shows that the reaction occurs between a neutral species namely the indole molecule and mononegative ions HSO_5^- .

Effect of $[\text{H}^+]$: The reaction rates measured at constant [Indole], [PMS], μ and percentage of acetic acid, but with various $[\text{H}^+]$ (5×10^{-3} to 9×10^{-2} mol dm^{-3}) were found to be the same (Table-1). Such a kinetic behaviour indicates the non-existence of any protonation equilibrium with respect to both PMS and indole under the present experimental conditions employed.

Effect of dielectric constant: So as to determine the effect of dielectric constant (D) of the medium on rate, the oxidation of indole by PMS was studied in aqueous acetic acid mixtures of various compositions (30-70 % (v/v)). It was found that there was no effect of varying the percentage of aqueous acetic acid [solvent] on the rate of oxidation of indole by peroxomonosulphate (Table-1 and Fig. 6). This is due to the fact that the reaction of indole, an unsubstituted compound proceeds through an activated complex which is not probably significantly solvated. This is also supported by the less negative $\Delta S^\ddagger$ value obtained for indole. It may be noted that the indole (with no substituents) is relatively more basic in nature when compared to others like indole-3-butyric acid, indole-3-propionic acid, indole-3-acetic acid, indole-3-acetonitrile, indole-3-carboxylic acid and indole-2-carboxylic acid. Hence, the ground state of indole (more basic) in acetic acid medium (acidic) is probably more stabilized than its transition state [20]. Thus, there is no predominant role of solvent on its reaction.

Rate and activation parameters: Experiments were conducted at varying temperatures (283–308 K) and corresponding rate k' (s^{-1}) data obtained and the values of activation parameters (E_a , $\Delta H^\ddagger$, $\Delta S^\ddagger$ and $\Delta G^\ddagger$) calculated based on Eyring equation. The Arrhenius plot of $\log k_2$ vs. $1/T$ and the Eyring's plot k_2/T vs. $1/T$ was linear. Energy of activation (E_a) was calculated from the above plot (Table-2, Figs. 4 and 5). The value of $\Delta S^\ddagger$ was computed from Eyring equation. The $\Delta S^\ddagger$ value are less negative (Table-3) showing that in this cases, the electrostriction is not more prominent hinting a less polar and loose type of transition state [21].

TABLE-2
EFFECT OF TEMPERATURE ON THE REACTION RATE

Temp. (K)	k_2 ($10^2 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$)	SD
283	0.1088	± 0.0012
288	0.2368	± 0.0061
293	0.5500	± 0.0014
298	0.9612	± 0.0007
303	1.8338	± 0.0012
308	2.6413	± 0.0032

TABLE-3
THERMODYNAMIC PARAMETERS

Thermodynamic parameters of oxidation of indole	Activation parameters
Energy of activation (E_a) (kJ mol^{-1})	94.03
Enthalpy ($\Delta H^\ddagger$) (kJ mol^{-1})	91.51
Entropy ($\Delta S^\ddagger$) ($\text{J K}^{-1} \text{ mol}^{-1}$)	23.69
Free energy ($\Delta G^\ddagger$) (kJ mol^{-1})	84.33

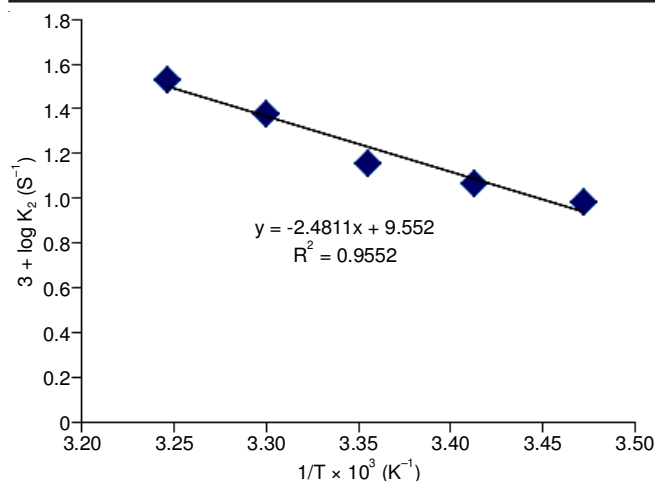
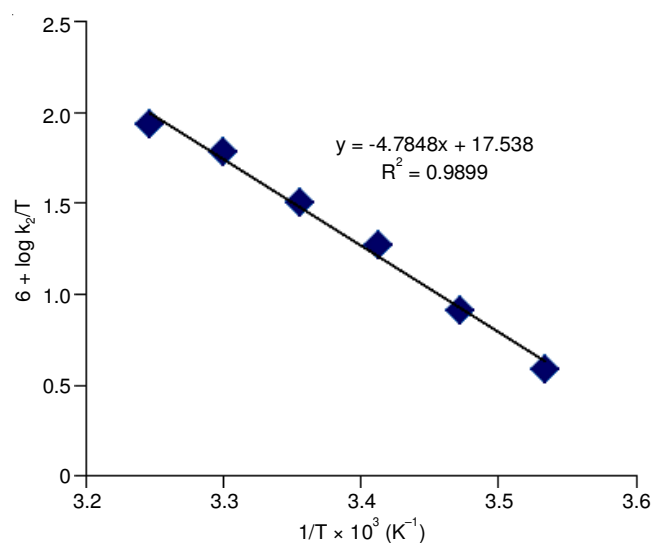
Fig. 4. Evaluation of energy of activation (E_a)

Fig. 5. Eyring's plot

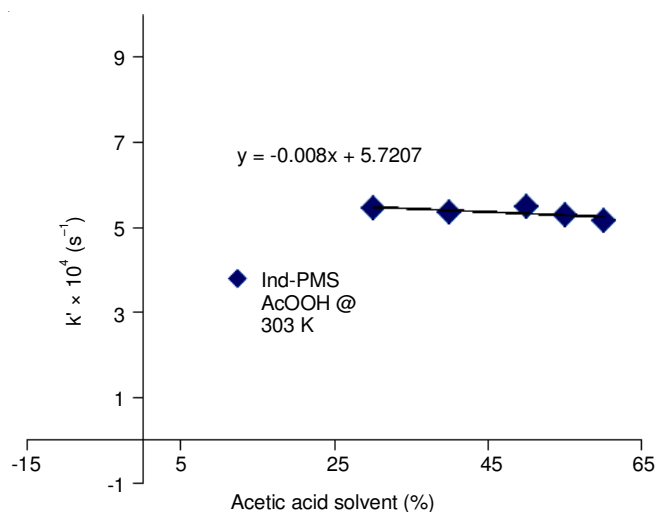


Fig. 6. Effect of dielectric constant

Product analysis: The reaction mixture containing a slight excess of PMS over the indole with other additives maintained as in the regular kinetic runs was kept aside at room temperature for about 24 h, for completion of the reaction. This was confirmed by TLC analysis of the reaction mixture. Then the

remaining of the reaction mixture was poured into doubly distilled water. A residual solid thus obtained was filtered, washed and dried. The solid mass was characterized to be the product by FT-IR and ^1H NMR spectral data. The above product was identified as isatin from IR frequencies (Fig. 7) at 1735 cm^{-1} for C=O (str), at 1628 cm^{-1} for C=O str (amide), at 3402 cm^{-1} for N-H (str).

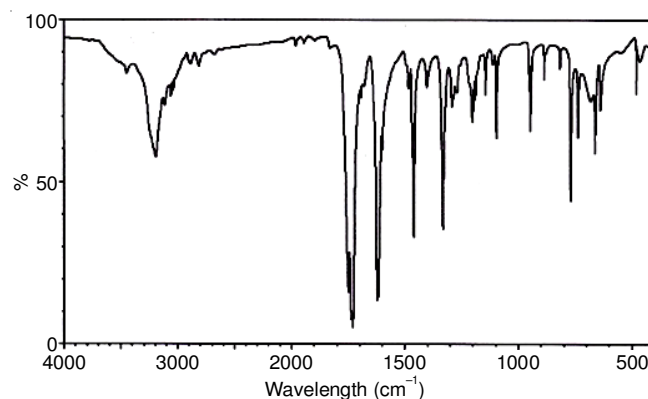


Fig. 7. FT-IR spectrum of product

The proton NMR spectrum of the product obtained in present experiment showed distinctly different features from that of the starting material, indole. The product spectrum exhibited signals at $\delta = 3.3$ corresponding to $>\text{NH}$, at $\delta = 6.79$ - 7.24 corresponding to benzene aromatic ring (Fig. 8).

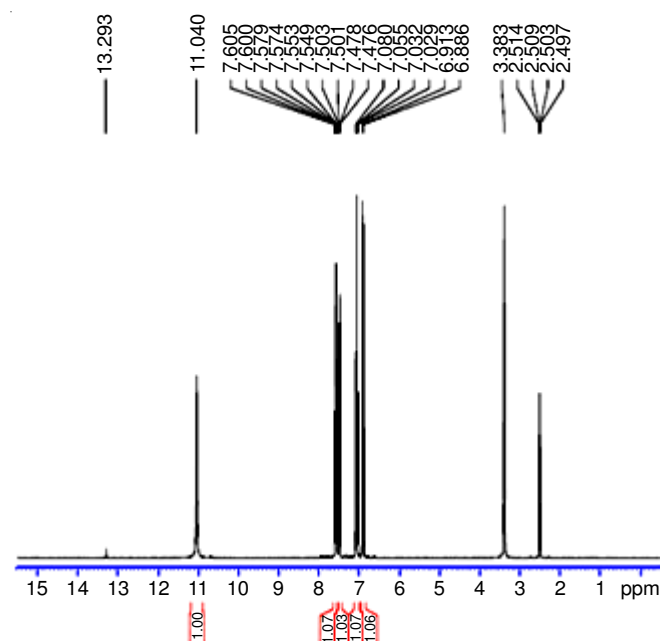
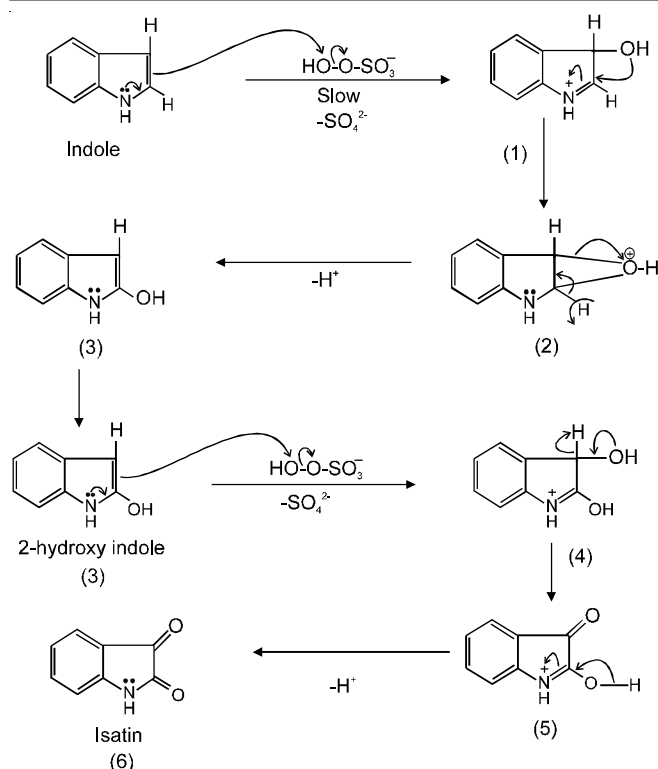


Fig. 8. NMR spectrum of product

Mechanism: Based on the kinetic observations such as first-order dependence of rate each on [Indole], [PMS], zero-order dependence on $[\text{H}^+]$, negligible effect of $[\mu]$ and the stoichiometry, the following mechanism is suggested in **Scheme-I**.

Peroxomonosulfate ion is known to be a mild electrophilic reagent capable of substituting activated aromatic compounds. From the experimental results, we suggest that the reaction proceeds through an electrophilic attack of the oxidant (PMS)



at C-3 by a mechanism involving nucleophilic displacement of peroxide oxygen [22] to form sulfuric acid mono-(3*H*-indol-3-yl) ester (**1**) as the rate determining step. Such a similar electrophilic attack on the C-3 of indoles is supported by earlier reports [21]. The compound (**1**) undergoes an intramolecular rearrangement to give 2-hydroxy-indole (**3**) through a cyclic intermediate (**2**). Infact, evidences for the involvement of a similar cyclic intermediate in the oxidations of *o*-benzoquinone [23], 3,5-dimethyl-2,6-diaryl 1-4-piperidones [24] and 2,6-diphenyl-4-piperidones [25] by PMS were obtained. The second PMS ion attacks the compound **3** to form 2,3-dihydroxy-indole (**4**) which gives isatin (**6**) has final products through the intermediate (**5**).

Antibacterial activity: In the present study antibacterial activity of the indole and isatin compound and the commercial antibiotic ampicillin were tested against the bacterial pathogens *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas* sp. and *Staphylococcus aureus*. Indole and isatin were tested at different concentrations of 500, 400, 300 and 100 mg/mL with

ethanol as solvent for indole and acetone as solvent for isatin using agar diffusion assay. The susceptibility zones were measured in diameter (mm) and were summarized in Table-4 and Fig. 9.

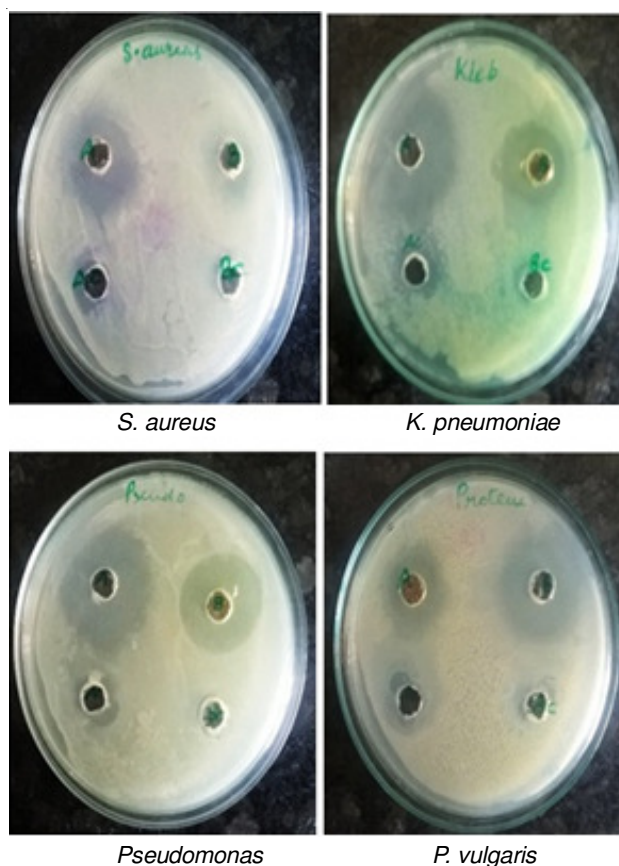


Fig. 9. Antibacterial activity of the indole and isatin at different concentration

The oxidized product individually exhibited varying degrees of inhibitory on the growth of tested pathogens and the solvent used does not show any inhibitory activity indicating antimicrobial activity of the indole. The pathogens like *Pseudomonas* sp, *Proteus vulgaris* and *Staphylococcus aureus* were found to be highly susceptible. Indole showed highest antibacterial activity in *Pseudomonas* sp and lowest activity recorded in *Proteus vulgaris* measured and also isatin was highest antibacterial activity in *Proteus vulgaris* and lowest activity recorded in *Staphylococcus aureus* measured.

TABLE-4
ANTIBACTERIAL ACTIVITY OF THE INDOLE AND ISATIN AT DIFFERENT
CONCENTRATION (mg/mL) AGAINST BACTERIAL PATHOGENS

Samples	Concentration (mg/mL)	Zone of inhibition (mm) for test bacteria in ethanol medium			
		<i>Klebsiella pneumoniae</i>	<i>Proteus vulgaris</i>	<i>Pseudomonas</i> sp.	<i>Staphylococcus aureus</i>
Indole	500	32	23	33	25
	400	26	19	29	22
	300	22	17	25	19
	100	17	14	18	16
Samples	Concentration (mg/mL)	Zone of inhibition (mm) for test bacteria in acetone medium			
		<i>Klebsiella pneumoniae</i>	<i>Proteus vulgaris</i>	<i>Pseudomonas</i> sp.	<i>Staphylococcus aureus</i>
Isatin	500	20	35	26	15
	400	16	31	22	12
	300	12	28	18	11
	100	10	25	21	12

Conclusion

Indole and PMS reaction involves of a peroxo linkage, follows first order with respect to [Indole] and [PMS] and overall follows second order reaction. The results indicate that there is no ionic strength and $[H^+]$. There was no effect of varying the percentage of aqueous acetic acid. Indole was highest antibacterial activity in *Pseudomonas* sp and lowest activity recorded in *Proteus vulgaris* measured and also isatin was highest antibacterial activity in *Proteus vulgaris* and lowest activity recorded in *Staphylococcus aureus* measured.

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