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KINETIC STUDIES ON THE OXIDATION OF NINHYDRIN –AMINO ACID SYSTEMS USING ALKALINE PERMANGANATE

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Symbols and abbreviations used.

Uv-Vis ⇒ Ultraviolet-Visible

RP ⇒ Ruhemann's purple

k_{obs} ⇒ observed rate constant

L-amino acid ⇒ levorotatory amino acid

μM ⇒ micro molar

R ⇒ Carbon side chain meaty or, Universal gas constant

α-amino acids ⇒ organic acids carrying one amino group at α position of carbonyl functional group

2, 4-DNP ⇒ 2, 4-Dintro Phenyl hadrazine.

DMSO ⇒ Dimethylsulphoxide

RE ⇒ Rare earth

Nm ⇒ nanometer

Ea ⇒ Energy of activation

k ⇒ rate constant

K ⇒ Equilibrium constant

A ⇒Absorbance

C ⇒ Concentration

l ⇒ optical path length

ε ⇒ Extinction constant

I ⇒Intensity of light transmitted

I_0 ⇒ Intensity of light entering the medium

r ⇒ Regression coefficient

S=Standard deviation

Abstract

The kinetics of oxidation of Ruheman's Purple by aqueous alkaline permanganate in alkaline medium at a constant ionic strength has been studied spectrophotometrically. The oxidation process in alkaline medium under the conditions employed in the present investigation proceeds initially through the formation of an RP-permanganate complex, which again rearranges it self to form an other complex. The latter complex decomposes into products up on reacting with alkali in fast stage of the reaction. The reaction constants involved in the mechanism were derived. There is good agreement between the experimental and calculated rate constants under different experimental condition. The reaction between permanganate and Ruheman's Purple in alkaline medium exhibits 1:2 stoichiometry (Ruheman's Purple: KMnO4). The reaction shows first order dependence on [permanganate] and apparent less than unit order dependence each on Ruhemann's Purple and alkali concentrations. Reaction rate increases with increase in ionic strength and decrease in solvent polarity of the media. Initial addition of reaction products did not affect the rate significantly. A mechanism of reaction involving the oxidant and substrate has been proposed. The activation parameters were computed with respect to the slow step of the mechanism and discussed.

1. Introduction

1.1 Application of chemical kinetics

1.1.1 Drug stability

Applications of kinetics in pharmacy results in the production of more stable drug preparations, the dosage and rationale of which may be established on sound scientific principles.

The experimental investigation of the possible breakdown of new drugs is an important aspect of kinetic study. A small expenditure of time and energy in this direction can yield results that may save the pharmaceutical industry both money and reputation [1].

Although the manufacturer is primarily responsible for assuring the stability of marketed products, the community pharmacist also must have some understanding of the stability characteristics to handle and store products under proper conditions. He or she must also recognize the alterations that may occur when a drug is combined with other ingredients [2]

For example, if thiamine hydrochloride, a vitamin most stable at a pH of 2 to 3 and is unstable above pH 6, is combined with buffered vehicle of say pH 8 or 9, the vitamin is rapidly inactivated. Knowing the rate at which a drug deteriorates at various PH conditions allows one to choose a vehicle that will retard or prevent the degradation. Thus, as a result of current research involving the kinetics of drug systems, the pharmacist is able to assist the physician and patient regarding the

proper storage and use of medicines. This introduction will highlight a number of factors that bear upon the formulation, stabilization, and administration of drugs. Concentration, temperature, light, and catalyst are considered important in relation to the speed and mechanism of reactions.

1.1.2 Estimating shelf-life based on Arrhenius plot

In the past, the practice of many pharmaceutical manufacturers to evaluate drug stability was to observe the formulated drug for a year or more, the normal time they remain in stock and in use. Such method was time-consuming and uneconomical. The method of accelerated stability testing of pharmaceutical products is based on the principles of chemical kinetics, more specifically on the Arrhenius plot [3]. According to this technique, the k values for the decomposition of the drug in solution at various elevated temperatures are obtained, and the logarithms of these k values are plotted against the reciprocals of the absolute temperatures (in Kelvins). The resulting line is then extrapolated to room temperature. The k at 25^{o}C is used to obtain a measure of the stability of the drug under ordinary shelf condition [4].

1.1.3 Factors that affect rate of chemical reaction

1.1.3.1 Nature of the Reactants

Depending upon which substances are reacting, the time varies. The acid reactions, the formation of salts, and ion exchange are fast reactions. When large molecules are formed the reactions tend to be very slow.

1.1.3.2 Physical State

The physical state (solid, liquid or gas) of a reactant is also an important factor of the rate of change. When reactants are in the same phase, as in aqueous solution, thermal motion brings them into contact. However, when they are in different phases, the reaction is limited to the interface between the reactants [5]. Reaction can only occur at their area of contact, in the case of a liquid and a gas, at the surface of the liquid. Vigorous shaking and stirring may be needed to bring the reaction to completion. This means that the more finely divided a solid or liquid reactant, the greater its surface area per unit volume, and the more contact it makes with the other reactant, thus the faster the reaction. To make an analogy, for example, when you start a fire, first you use wood chips and small branches - you don't start with big logs right away. In organic chemistry on water reactions are the exception to the rule that homogeneous reactions take place faster than heterogeneous reactions.

1.1.3.3 Concentration

Concentration plays a very important role in reactions. According to the collision theory of chemical reactions, this is due to the fact that molecules must collide in order to react together. As the concentration of the reactants increases, the frequency of the molecules colliding increases, striking each other faster by being in closer contact at any given point in time. Imagine two reactants being in a closed container. All the molecules contained within are colliding constantly. By increasing the amount of one or more of the reactants you cause these collisions to happen more often, increasing the reaction rate.

1.1.3.4 Temperature

A number of factors other than concentration may affect the reaction rate. Among these are temperature, solvents, catalysts, and light. The reaction velocity increases about two to three times with each 10^0C rise in temperature. The effect of temperature on reaction rate is given by the following equation, [6].

$$k = s\, e^{-E_a/RT} \quad \dashrightarrow \quad \log k = \log s - \frac{E_a}{2.303\,RT}$$

Where k is the specific reaction rate, s is a constant known as frequency factor, E_a is the energy of activation, R is the gas constant (1.987 cal/deg mole), and T is the absolute temperature. This relationship was first suggested by Arrhenius; hence it is often called the Arrhenius equation.

E_a and s may be evaluated by determining k at several temperatures and plotting 1/T against log k. The slope of the line will be $-E_a/2.303R$, and the intercept on the vertical axis is log s, from which E_a and s may be obtained. Temperature usually has a major effect on the speed of a reaction. Molecules at a higher temperature have more thermal energy [7]. When reactants in a chemical reaction are heated, the more energetic atoms or molecules have a greater probability to collide with one another. Thus, more collisions occur at a higher temperature, making a product in a chemical reaction [8]. It is the fact that at higher temperatures molecules have more vibrational energy, that is, atoms are vibrating much more violently, so raising the temperature not only increases the number of collisions but also collisions that can result in rearrangement of atoms within the reactant molecules.

1.1.3.5 Ionic strength

If reacting molecules are negatively charged or positively charged the effect of ionic strength on the rate of the reaction is more pronounced, but if the reacting molecules are neutral ones there should be no effect of the ionic strength on the reaction rate [9]

1.1.3.6 Catalysts

A catalyst is a substance that accelerates the rate of a chemical reaction but remains chemically unchanged afterwards. The catalyst increases rate of reaction by providing a different reaction mechanism to occur with a lower activation. In autocatalysis a reaction product is itself a catalyst for that reaction leading to positive feedback. Proteins that act as catalysts in biochemical reactions are called enzymes. Michaelis-Menten kinetics describes these types of reactions. In certain organic molecules specific substituents can have an influence on reaction rate in neighboring group participation.

1.1.3.7 Pressure

Increasing the pressure in a gaseous reaction will increase the number of collisions between reactants, increasing the rate of reaction. This is because the activity of a gas is directly proportional to the partial pressure of the gas. This is similar to the effect of increasing the concentration of a solution [10]**.**

1.1.3.8 Solvent effect

Solvent effects on the rates of some well-known nucleophilic substitution reactions have been analysed in terms of initial-state and transition-state contributions. Where possible, the latter have been further analysed by the method of model solutes, on which solvent effects on a transition state are compared to solvent effects on a solute that might be a suitable model for the transition state [11].

Solvent effects on reaction rates have also been analysed using the multiple linear regression procedure of Kamlet and Taft. It is shown that results from the two quite-different approaches are in good agreement with each other [12].

1.1.4 Importance of rate laws

(1). If we know the rate law and the constants in it we can use this to predict the rate for any set of conditions (concentrations). The rate law is thus a very succinct and practical way of expressing the rate. [13]. One might use this, for example, in a model of the atmosphere or in predicting the rate of an enzyme catalyzed reaction.

(2).The form of the rate law can tell us something about the mechanism of the reaction.

(3) Knowing the rate law enables us to separate the concentration dependence from the underlying, fundamental effect, which is the size of the rate constant [14].

1.1.5 Light absorbance

Molecules absorb light at characteristic frequencies. These frequencies are associated with transitions between energy levels, for example, those associated with vibrations of electrons. For kinetic studies, the commonest kinds of transitions to use are those in the visible or ultra-violet (UV) part of the spectrum (1000 – 200 nm).These transitions come about when the absorbed photon causes the molecule to move to an excited electronic energy level. All molecules absorb in the UV to some extent, but it takes the presence of special groups to cause strong absorptions in the visible region. Many species with characteristic function groups can be monitored by UV/vis absorption in kinetic experiments. The extent to which light is absorbed is related directly to the concentration of the absorbing species by the Beer-Lambert Law [15].

$I = I_0 \exp(-\varepsilon c l)$

Where I_0 is the intensity of the light entering the medium, I is the intensity of light exciting the medium, l is the path length through which the light passes, *c* is the concentration and ε is the extinction coefficient. The value of the extinction coefficient depends on the species absorbing the light and the wavelength. The path length is related to the physical dimensions of the container holding the sample. Typically, a glass or quartz "cuvette" of known dimensions (a 1 cm path length is common) is used for solutions.

To turn measured absorbance into absolute concentrations, we need to know Beer's Law i.e. $A = c\varepsilon l$.

The above equation can be rearranged to give the concentration as $c = A/\varepsilon l$

Usually this is done by measuring the absorbance of a series of solutions of known

concentration and then simply plotting *A* against *c*; the slope is εl. If we are studying first order processes then only a relative measure of concentration is needed, so it is sufficient just to know the absorbance [16].

Absorbance measurements are very convenient; they are non-invasive, rapid and can easily be automated. As different species absorb at different wavelengths it is possible to study different molecules in a reaction mixture. However, UV/Vis absorptions tend to be rather broad (especially in solution), so it is possible that more than one species will absorb at a given wavelength.

1.2 Review of Literature

1.2.1 Permanganate

The reactions of substrate with permanganate are governed by pH of the media. Among six oxidation states of manganese from 2+ to 7+, permanganate, Mn(VII) is the most potent oxidation state in acid as well as in alkaline media [17]. Oxidation by permanganate ion has extensive applications in organic syntheses, especially in the advent of phase-transfer catalysis, [18] which permits the use of solvents such as methylene chloride and benzene. Kinetic studies are important sources of mechanistic information on the reactions, as demonstrated by results referring to unsaturated acids in both aqueous [19] and non-aqueous media. The manganese chemistry involved in these multi-step redox reactions is an important source of information as the manganese intermediates are relatively easy to identify as they have sufficiently long lifetimes and the oxidation states of the intermediates permit useful conclusions as to the possible reaction mechanisms, including the nature of intermediate [20,21].

In strongly alkaline medium, the stable reduction product of permanganate ion is manganate ion, MnO^{-2}_4. No mechanistic information is available which would permit one to distinguish between a direct one electron reduction to Mn (VI) (Scheme 1), or prior formation of hypomanganate in a two-electron step followed by a fast reaction (Scheme2).

$$Mn^{VII} + S \xrightarrow{k'_1} Mn^{VI} + S\cdot$$

$$Mn^{VII} + S\cdot \xrightarrow{k'_2} Mn^{VI} + \text{product}$$

$$s = \text{substrate} \quad ; \quad k'_2 >> k'_1$$

Scheme1

$$Mn^{VII} + S \xrightarrow{k'_3} Mn^{V} + \text{products}$$

$$Mn^{VII} + Mn^{V} \xrightarrow{k'_4} 2Mn^{VI}$$

$$s = \text{substrate} \quad ; \quad k'_4 >> k'_3$$

Scheme2

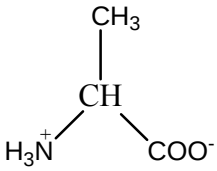
The permanganate oxidation of amino acids has been widely studied in strong acid media [22] as well as in neutral and weakly alkaline media. About the kinetics of permanganate oxidation of amino acids and L-α-amino -n-butyric acid [23] were widely studied. It is not only concentration of reductant and that of oxidant but also the carbon chain length of the substrate affects rate of chemical reaction [24]. In aqueous alkaline medium the permanganate ion oxidizes a number of organic compounds, which are not, or only very slowly, attacked in acidic or neutral media.

If [OH-] >0.1 mol dm^{-3}, permanganate is reduced in most case to manganate only, the reaction of which further proceeds at a much lower rate. Nevertheless ,since the process is very fast, it cannot be decided, in general, whether permangate is reduced in one-electron steps or hypomanganate is first formed in a two-electron step followed by a fast reaction.[25]. Different species of manganese have different properties. A Mn^{2+} has its own oxidizing properties. Recent time observation shows that manganese prevents oxidative brain injury in the iron-induced parkinsonian animal model. It has also been suggested that manganese retards while copper promotes the development of atherosclerosis. Manganese is a typical anti oxidant. Among transition metals, Cu^{2+} and Fe^{2+} (0.1 to 125 μM), but not Mn^{2+}, converted hydrogen peroxide to reactive hydroxyl radicals via the Fenton reaction at pH 7.4. Iron's pro-oxidative rate is relatively slow, but it is accelerated further by ascorbate (50 μM) in 37 degrees C Dulbecco's phosphate buffered saline. Moreover, Mn^{2+} (0-80 μM) concentration dependently retarded diene conjugation of human low density lipoproteins stimulated by 5 μM Cu^{2+}. Related recent research result finding shows that Mn^{2+} (0 to 20 μM) does not initiate brain lipid peroxidation while it inhibits iron-induced peroxidation of polyunsaturated fatty acids. These unexpected manganese results are somewhat at odds with a prominent theory that manganese is a prooxidative transition metal.

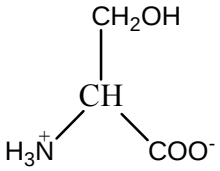
1.2.2 Amino acid

Amino acids are building block s in protein synthesis in organisms. Proteins are the basic structural and functional compounds of the cell [26]; and they are made up of amino acids .These amino acids are naturally occurring ,α-amino acids (except

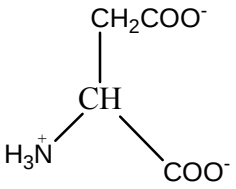
proline), and they are 20 in number and exist in the L-configuration (except glycine). About 18 of the 20 commonly occurring, genetically encoded amino acids involved in protein structure have a primary alpha-amino group and an optically active alpha-carbon center. All also have an alpha hydrogen atom. Any α-amino acids is characterized by a unique side chain, which distinguishes one amino acids from the other in addition to one amino and one carboxyl group [27]. The general structure of α-amino acids is shown in scheme 3 below.



L-Alanine



L-Serine



L-Aspartate

scheme3

The function of protein is determined primarily by its structure, which in turn is determined by the sequence of α-amino acids with which it is constituted.The amino acid sequence is generally determined and is responsible for the shape, physical characteristics and biological activity. Amino acids contain the same acidic (-COOH) and basic ($-NH_2$) groups but differ in the side chain (R). There fore classification of amino acids depends on the side chain (R). Amino acids under go reactions characteristics of both their amine and carboxylic acid functional group, e.g.acylation. A well known reaction of α-amino acids that is used in their identification and estimation is the formation of purple color known as Ruhemann's purple on treatment with ninhydrin.

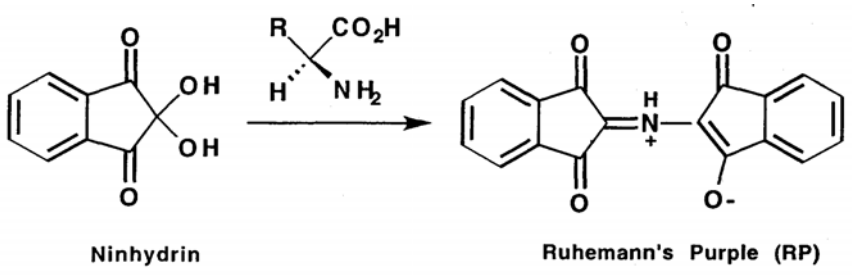
1.2.3 Ninhydin and Ruhemann’s purple

The reaction of ninhydrin with amines, amino acids, peptides, and proteins is used extensively in qualitative and quantitative biochemical investigations. Although the chemistry of this reaction has been widely studied and reviewed [28] a number of features associated with it appear anomalous. For example, no mechanistic explanations have been offered as to why primary aromatic amines do not give the expected color reaction with ninhydrin or why diamino and aminothiol acids do not yield stoichiometric amounts of Ruhemann’s purple .The mechanism of Ruhemann’s purple formation has been extensively investigated [29]. Starting with condensation between the primary amine and carbonyl functions groups (Schiff base formation), the reaction proceeds in several sequential steps and ends with Ruhemann’s purple. Several intermediates appearing during the formation of Ruhemann’s purple have potential metal binding centers. Of these, the Schiff base initially formed and the Ruhemann’s purple terminally obtained have bee noted for stabilizing metal complexes.

Ruhemann's purple was used in the form of its salt for its kinetic study purpose. It can to be oxidized with permanganate in aqueous alkaline media. Although some work on the oxidation of organic [30] and inorganic [31] substrates by permanganate in aqueous alkaline medium has been carried out, there is no report in the literature on the oxidation of Ruhemann's purple at all. This work was carried out on such a reaction in order to elucidate the redox chemistry of permanganate in alkaline media. And oxidizing nature of RP on the given condition.

The reaction of amines with ninhydrin to form the colored reaction product known as Ruhemann's purple was discovered by Siegfried Ruhemann in 1910 [32].He first detected its reaction with ammonia and extended his experiments to α-amino acids, peptides and proteins [33]. However, the value of ninhydrin for the development of latent fingerprints was not realized until 1954 when Odén and von Hofsten suggested its use in criminal investigations. Ninhydrin is now the most widely used method for developing latent finger marks on paper surfaces.

Ninhydrin, as well as its analogues, reacts with the amino acid compound of the latent fingerprint deposit (eccrine secretion) to give the dark purple product known as Ruhemann's purple (RP)[34]. The chemical reactions involved are complex and, as a result, the development conditions need to be controlled if optimum results are to be obtained. The method is very effective for the development of fingerprints on porous surface such as paper. However, some paper surfaces (certain bank notes, for example) react strongly [35].



Scheme 4

Identification and quantification of constituent amino acids in a mixture is required in the biochemical investigations of proteins and peptides. The most extensively used

method is ninhydrin reaction, in which ninhydrin reacts with amino acids to give a characteristically blue /purple colored compound popularly known as RP, after Sigfried Ruhemann who first observed the color reaction [36] .The blue compound maximally absorbs at 570nm [37] and this forms the basis for the spectrophotometric quantitative determination of amino acids that can detect as little as one micro gram of material. Ninhydrin shows three bands in the C=O stretching region: 1768, 1754and 1720cm^{-1}. The 1754and 1720cm^{-1} band are characteristic of its 1, 3-dicarbonyl functional group [38] and 1768 cm^{-1} is characteristic of the intermediate carbonyl in the tricarbonyl species. As the solution of pH plays a significant role toward the yield and stability of Ruhemann's purple [39], the rate of α-amino acid–ninhydrin reaction was studied in the pH range 3.5–6.0. The rate constant increased up to pH 5.0 and thereafter became constant In order to confirm whether the, RP, colored product is formed in the absence and presence of organic solvents, a series of UV–visible spectra were recorded after completion of the reaction at 80^0C [40]. These results are shown graphically as absorbance–wavelength profiles in figure1. The absorption spectra of mixtures containing the reactants in different solvents exhibited the same maxima as that of a solution of Ruhemann's purple in aqueous micellar medium (Fig.1). The spectra consist of two broad bands with λ_{max} =400 and 570 nm. No variation in λ_{max} in the presence of different solvents clearly indicates the product of valine– ninhydrin reaction to be the same as in aqueous micellar media, i.e,diketohydrindylidenediketohydrindamine or Ruhemann's purple [41].

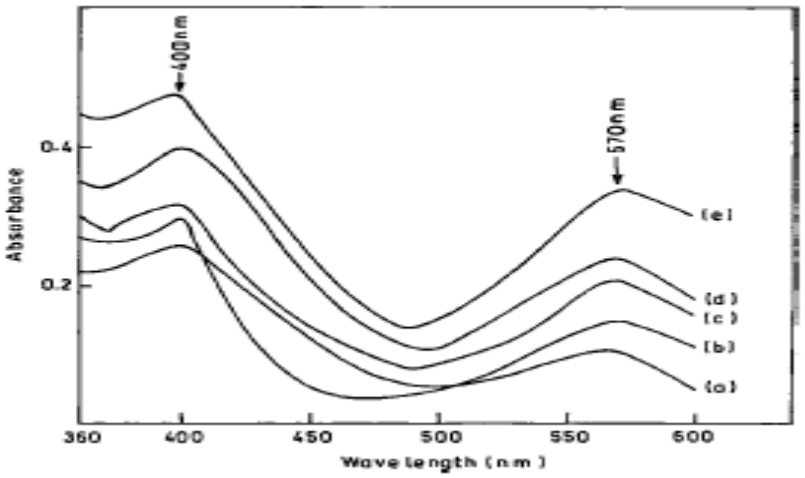
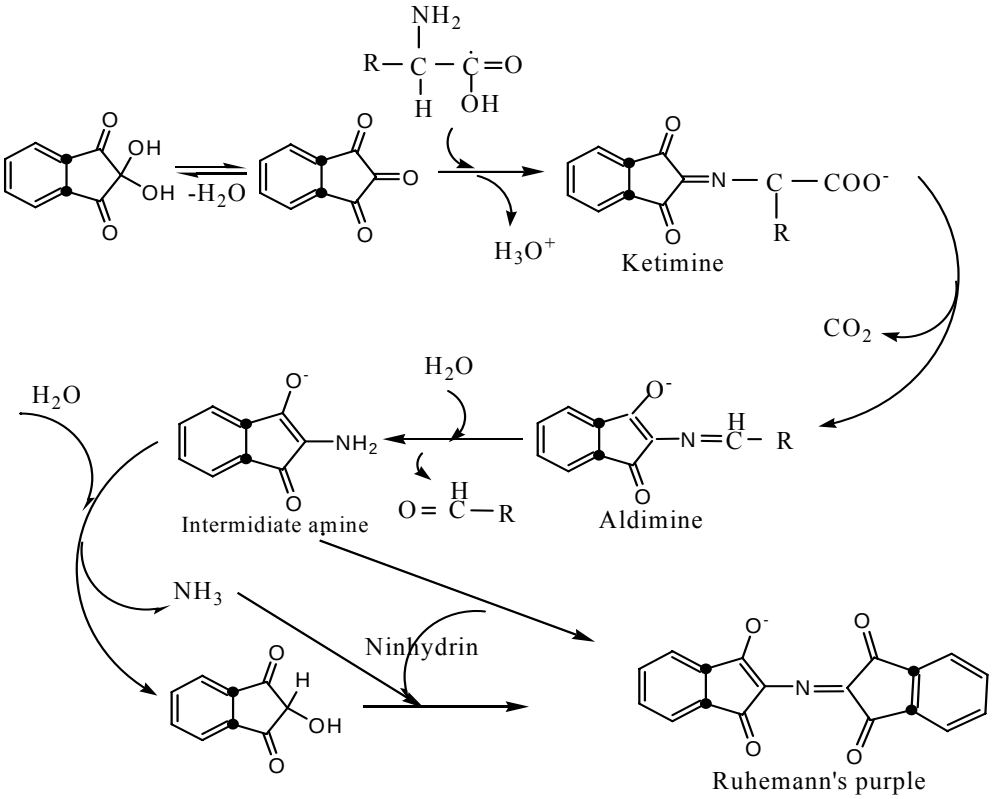


Fig.1. Increase in the absorbance of RP during synthesizing RP from amino acid and ninhydrin.

Graph shows gradual increase in concentration of Rp during its preparation from ninhydrin and α-amino acids inside the cell of spectrophotometer.

Ninhydrin (the fingerprint-developing agent), a well-known compound with interesting forensic, biochemical, pharmacological, and biomedical properties, is widely used for the estimation of amino acids, peptides, amines, and amino sugars in analytical chemistry [42]. To enhance the usefulness of the ninhydrin method in forensic science, several studies have been made including the use of aqueous–organic mixed solvents as well as addition of surfactants[43]. As the ninhydrin reaction results in decarboxylative deamination of amino acids to an aldehyde, the DMSO–water mixed solvent system enhanced the rate of oxidative deamination and favored specific staining of tissues [44]. Although some qualitative information is available on the role of organic solvents, kinetic evidence to distinguish their role is limited. Introduction of organic solvents may cause the use of low [reactants] as well as maximize the rate, thus, enhance the sensitivity of the technique/reaction.



Scheme 5.

Step wise mechanism of RP formation

Schiff bases are good ligands for metal ions [45]. The Ketimine, is therefore a potential ligand to metal ions, that can act as a tridentate ligand forming two stable five membered rings on complexation [46]. Ninhydrin has been shown to exhibit strong antibacterial, antiviral, etc.activities [47]. Chemicals which develop latent fingerprints include no fluorescent amino acid reagents. Ninhydrin and ninhydrin analogues, considered here, is among these chemicals. Several types of chemicals and procedures are available. Ninhydrin and ninhydrin analogues develop colored fingerprints which do not fluoresce visibly. If developed colored fingerprints require secondary treatments to render them

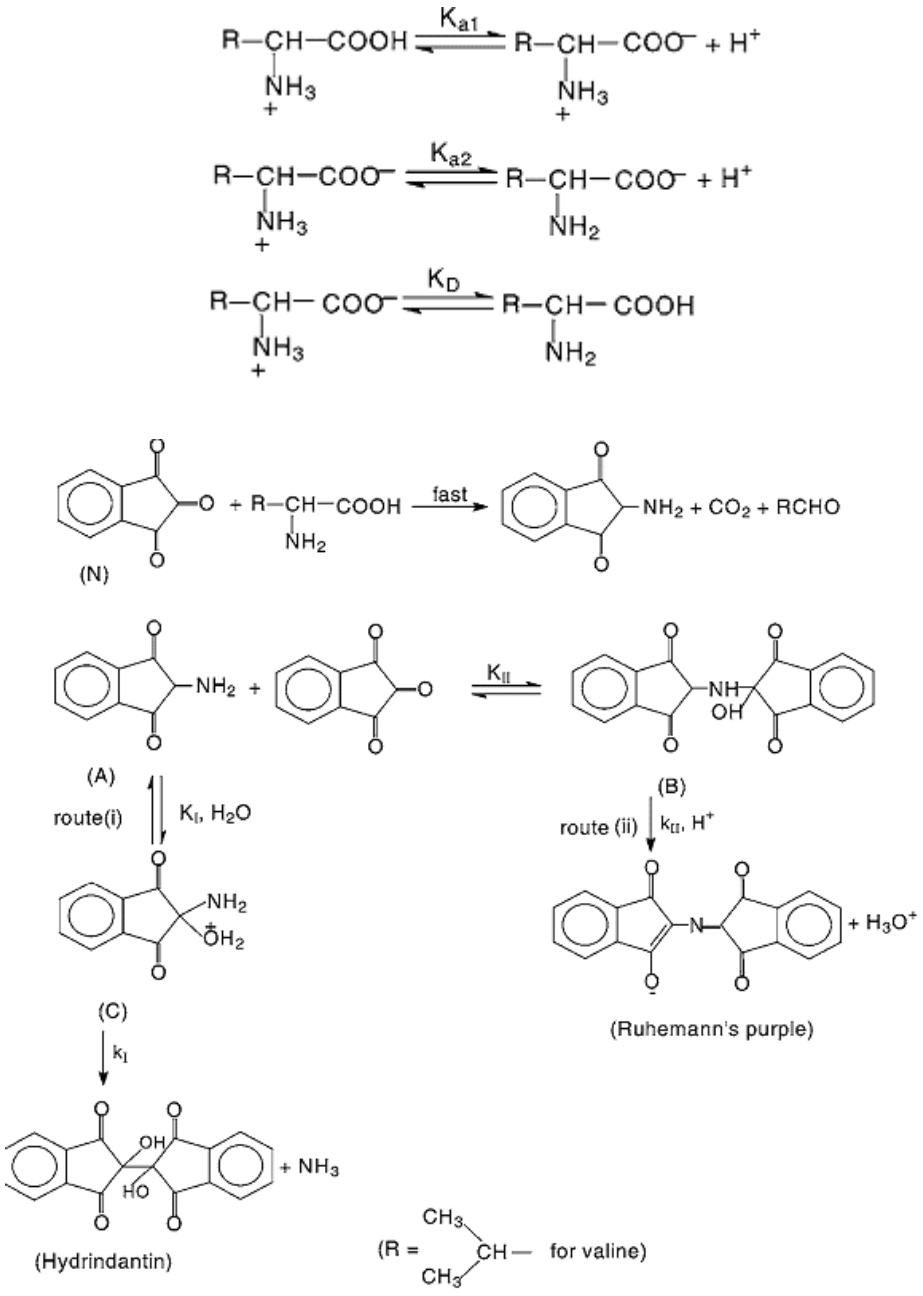
visibly fluorescent, then complexation with $ZnCl_2$ is routine procedure [48]. Fluorescamine, o-phthaladehyde, etc. develop invisible fingerprints which fluoresce visibly without any secondary treatment. Excitation in this case is usually in the ultraviolet (UV) region since the developed fingerprints are invisible and thus absorb only nonvisible radiation. [49].

1.2.4 The reaction of ninhydrin with α-amino acids

Reaction of ninhydrin is valuable for detection and qualitative determination of α-amino acids. Two moles of ninhydrin along with one moles of α-amino acids yield a product (Ruhemann's purple) that absorb visible light at ~410 nm and 570nm .The reaction mechanism of α-amino acids and ninhydrin been presented by different researchers. As can be seen from scheme 5, the mechanism of this reaction has four steps [50]. These are;

1. Condensation: results ketimine
2. Decarboxylation: leads aldimine
3. Hydrolysis: gives the intermediate amine
4. Further condensation: results the Ruhemann's purple (scheme3**)**

In the presence of transition metal ions, this reaction may form a stable intermediate Schiff base complex or Ruhemann's purple of amino acids by their specific colors **[**51**].** Reaction of ninhydin with simple amino acids to produce RP and other derivatives [52] are presented in scheme 6.



Scheme 6

Production of RP and other ninhydrin derivatives.

2. Experimental

2.1 Devices

Since the initial reaction was too fast to be monitored by the usual methods, kinetic measurements were performed on a GENESYS 2PC UV–Vis Spectrophotometer (to which temperature control and personal computer labeled as OMEGA are attached). Infrared spectra (KBr disks) were recorded using Perkin Elmer spectrum BX spectrophotometer in the range 400-4000$cm^{-1.}$ And BECKMAN DU-65 Spectrophotometer for measuring absorbance at selected wave length of 526nm.

2.2 Materials and chemicals

Reagent grade chemicals and doubly distilled water were used during the experiment. Stock solutions of Ruhemann's Purple (RP) was prepared from ninhydrin and L-serine (BDH, England). Potassium permanganate (Wagtech International, UK) was prepared by dissolving the appropriate amounts of samples in doubly distilled water. And the concentration of the sample was determined by titrating it with oxalic acid. The stock solution of permanganate was standardized against oxalic acid (BDH, England). A solution of potassium permanganate was heated to boiling above 120°C in 8.0 mol/dm^3 in potassium hydroxide solution until a green color was produced. The solid potassium manganate formed on cooling was recrystallized from the same solvent. Using the required amount of recrystallized sample, a stock solution of potassium manganate was prepared in aqueous potassium hydroxide. The manganate solution was standardized spectrophotometrically. The stock solution of potassium hydroxide was prepared by dissolving the solid sample of potassium hydroxide with doubly distilled water. Following the same procedure stock solution of sodium per chlorate was prepared by

dissolving the solid $NaClO_4$ in doubly distilled water. A 2, 4-DNP (BDH, England) was used for spot test. All colored solutions of the experiment were kept under the laboratory shelf. During the course of the experiment freshly prepared solutions were used. All other reagents were of analytical grade and their solutions were prepared by dissolving the requisite amounts of the samples in doubly distilled conductivity water. NaOH and $NaClO_4$ were used to provide the required alkalinity and to maintain the ionic strength, respectively.

All kinetic measurements were performed under pseudo-first-order conditions with RP at many-fold excess over permanganate ion at a constant ionic strength of 0.50 mol /dm^3. The reaction was initiated by mixing solutions of MnO^-_4 and RP which also contained the necessary quantities of NaOH and $NaClO_4$ to maintain the required alkalinity and ionic strength, respectively. The temperature was maintained at 25 ^{0}C.

UV-Vis spectrophotometer studies of the reaction in aqueous medium at room temperature were done in the range of 300-700nm using a SPECTRONIC GENESYS 2PC with 1cm cuvette or cell. Base line was collected for the universal solvent i.e. water. And spectra of each reacting species was run and saved .The reaction progression was followed using SPECTRONIC GENESYS 2PC spectrophotometer in quartz cell with path length 1cm. The course of the reaction was followed by monitoring the decrease in the absorbance of MnO^-_4 in a 1 cm cuuvet spectrophotometer at its absorption maximum of 526 nm as a function of time. It was verified that the interference from other reagents at this wavelength was negligible. The application of Beer's law to permanganate at

526nm had been verified, giving extinction coefficient, ε=2083±50 dm^3/mol (literature ε=2200 dm^3/mol). During the course of measurement the color of the solution was changed from violet to blue and then to green. The spectrum of green solution was identical to that of MnO^{-2}_4. It is probable that the blue color originated from violet of permanganate and the green color is due to manganate. The first-order rate constants k_{obs} was evaluated by plots of log (A_{t1}-A_{t2}) versus time. Where A_{t1} and A_{t2} are the absorbances of $KMnO_4$ of time t_1, and t_2 respectively. It is also evident from absorbance spectra that the absorbance of permanganate decreases at 526nm whereas the absorbance of manganate increases at 608. In almost all cases the reaction was followed up to 75% completion of the reaction. The observed initial and final absorbance values were average of two or more experiments and were calculated by (PENTIUM 4 DELL computer). In general, the rate constants obtained by observing the rate of formation of the manganate were comparable to those calculated from the rate of disappearance of permanganate ion. The rates were obtained from the slopes of log of concentration versus time graphs in the initial stages of the reactions. The rates were reproducible to within ±6% .

Infrared spectra (KBR disks) were recorded using Perkin Elmer spectrum BX spectrophotometer in the range 400-4000cm^{-1}.

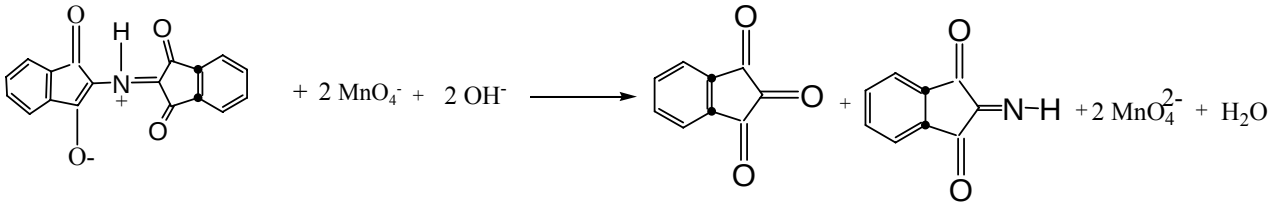
2.3 Objectives of the Study

Kinetic study on the oxidation of RP using permanganate in aqueous alkaline media at which oxidation process can be more powerful and oxidizing species is more stable. Identifying the species of manganese suitable to the type of media has significant importance in proposing mechanism of the reaction. Investigating the form of reductans that exists in the given media is important for indicating manner of oxidation.

Determining the rate of the reaction, computing the order of the reaction from the plot of log of concentration of oxidant versus time with respect to each reactant has great significance to indicate the degree of dependence of rate on the concentration of reactant. The factors that affect the rate of the reaction can be studied by varying one factor at a time while keeping the others constant. Calculating important thermodynamic parameters using Arrhenius plot enables to see the stability of the reaction.

3. Result and Discussion

Reaction mixtures containing an excess permanganate concentration over RP and adjusted to constant ionic strength of 0·50 mol dm^{-3} was allowed to react for 24 hour at 25^0 C. The remaining permanganate was then analyzed spectrophotometrically. The results indicated that two moles of MnO_4^- are consumed by one mole of Ruhemann's Purple as given by the reaction:



Scheme 7

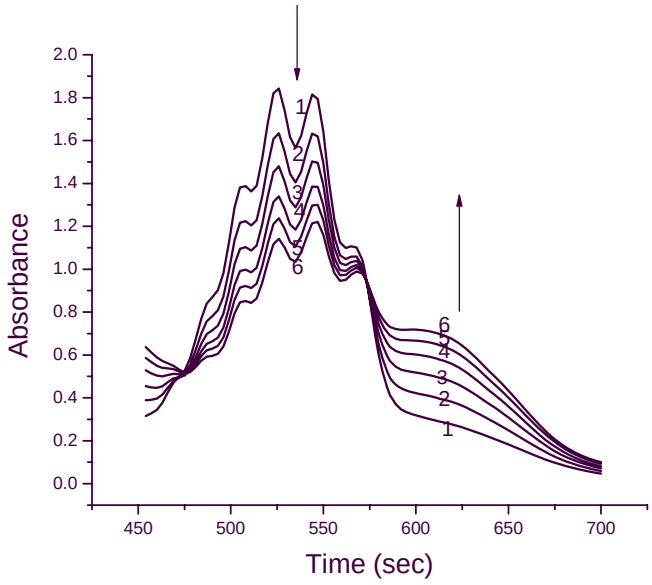


Fig.2. Uv-Vis spectral change during the oxidation of RP by aqueous alkaline permanganate at 25^0C with scanning time interval of 1min at $[MnO4^-] = 4x10^{-4}$M, [RP] = $2x10^{-3}$M and $[OH^-]$ =0.05M

In the above spectra, the numbers 1, 2, 3, 4, 5 and 6 shows the six different scans for the reaction mixture. As can be seen from the spectra, the decrease in absorption from 1-6 at 526nm indicates the decrease in concentration of permanganate and the increase in the absorption from 1-6 at 608nm indicates the concentration of manganate (product) increases as reaction proceeds.

3.1 Chemical identification test for product analysis

3.1.1 Ammonia gas test

To check whether N- containing functional group in reactant was removed in the form of NH_3 gas or not, the following test was done.

(i) Holding red litmus paper over /above the reaction mixture, red litmus paper was

observed not to change color from red to blue (because ammonia is the only common alkaline gas). This negative test for ammonia gas confirms absence of ammonia gas release from reaction mixture.

(ii) Bringing the reaction mixture near to fumes of concentrated hydrochloric acids, did not give white clouds with HCl fumes. This test again shows the absence of release of ammonia gas from the reaction mixture. If ammonia gas were present fumes of concentrated hydrochloric acids would forms fine ammonium chloride crystals with HCl.

3.1.2 Ketone test

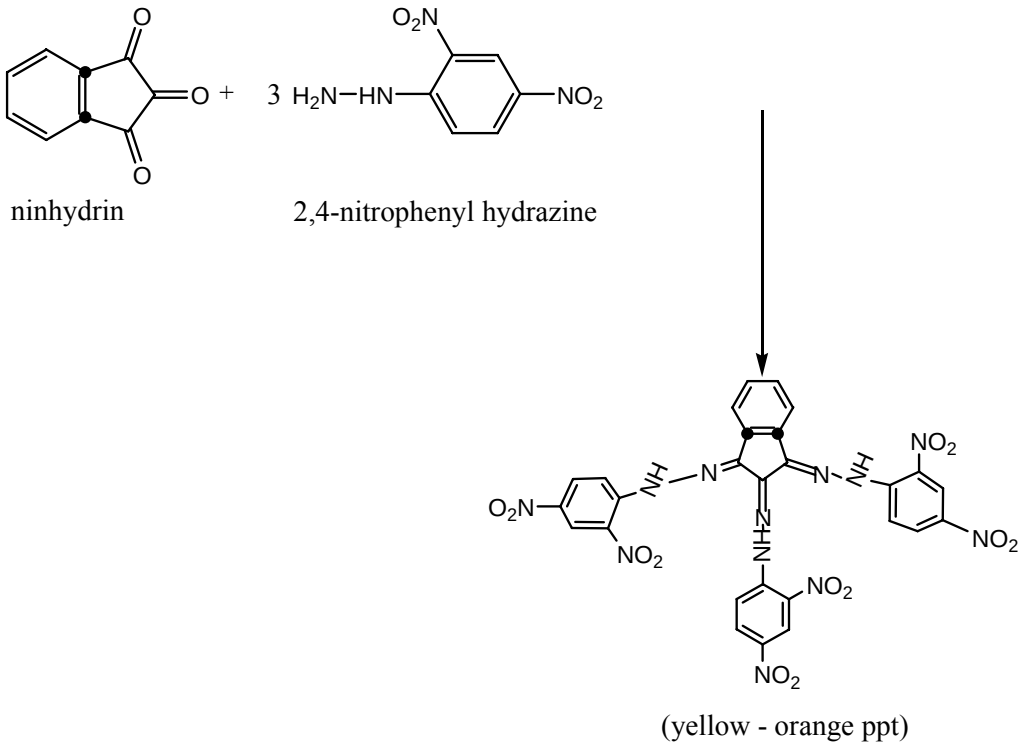
A few drops of the reaction mixture containing the suspected carbonyl compound (ketone) were added to the Brady's reagent (2, 4-dinitrophenyl hydrazine). The yellow-orange precipitate was formed. This confirms the presence of carbonyl functional group i.e. ketone.

3.1.3 Carbon dioxide test

The reaction mixture suspected to release CO_2 was brought to into limewater. It did not turn limewater into cloudy (fine milky white precipitate of calcium carbonate). This is negative test for the release of CO_2 from the reaction mixture.

The main reaction products containing carbonyl compound was identified by a spot test, and manganate by its visible spectrum. The chemical test for carbon dioxide was negative, by holding calcium hydroxide over the reaction mixture no observation of milky or cloud formation in forming calcium carbonate. Test for ammonia gas was negative ,red litmus paper over the reaction mixture was not changed to blue(be cause

ammonia is the only alkaline gas) and using fumes of concentrated hydrochloric acids there was no formation of ammonium chloride (i.e. can be known for the absence of formation of white crystal) . Tests for the corresponding acid were negative. It was also observed that the ketone does not undergo further oxidation under the present kinetic conditions.



(yellow - orange ppt)

Scheme 8

Reaction of tri ketone with 2, 4-dinitrophenylhydrazine for ketone test

3.2 Interpretation of spectra

Organic products, ketone, were also confirmed by its IR spectrum, which showed a band at 1700, 1696, 1684.87cm^{-1}, due C=O stretching. 1696 and 1684.87cm^{-1}are due to 1, 3 dicarbonyl functional groups and 1700cm^{-1} is due to intermediate carbonyl in the tri

carbonyl. The strong band at 2917.61 cm^{-1} is due to sp^2 C-H stretching. Strong band from 3340-3000 cm^{-1} due to N-H of imine and an intermediate OH stretching of ninhydin . The strong bands from 1653-1617.04 cm^{-1} due to aromatic C=C stretching. The strong band at 1559.98cm^{-1} due to N-H bending. A four step reaction between ninhydrin and amino acid gives colored compound, called Ruhemann's purple, which absorbs maximally at 570nm (17,544cm^{-1}) and 410nm (24,390 cm^{-1}). Absorbance of the reaction mixture containing tenfold excess of Ruhemann's purple over permanganate at constant concentration of, alkali and sodium perchlorate showed blue shift from λ_{max} of Rp i.e. from 570nm to around 350nm. This shift to the shorter wavelength from 570nm to 350nm confirms the formation of ninhydrin as a product. This confirms the proposed mechanism of the reaction. Maximum absorbances at 570nm and at 410nm are the pecular nature for identification of RP. The spectral changes during the reaction are shown in Figure2, from which it is evident that [Mn^{VII}] decreases at 526nm whereas [Mn^{VI}] increases at 608nm during the reaction.

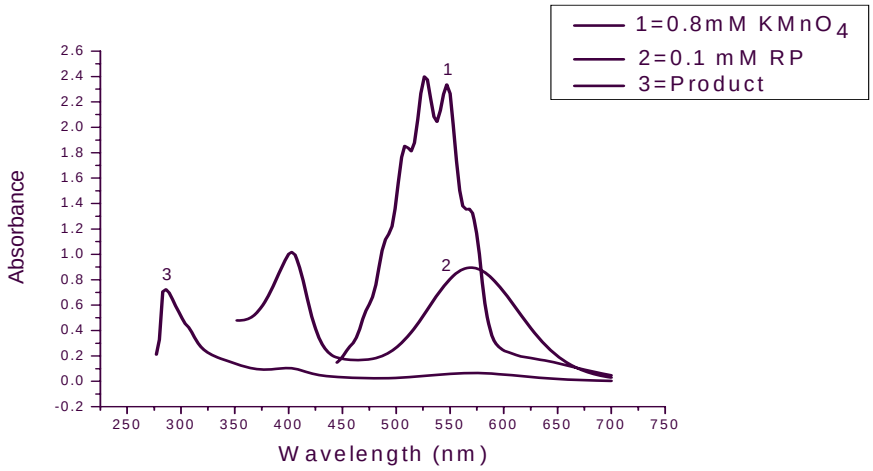


Fig.3 Spectras of reaction product (after 24 h of mixing reactants) and reactants before reacting plotted together.

The spectra in figure 3 shows the formation of product which has lower conjugation length than the reactant RP, and the formed product absorbs at around 350nm. This confirms IR spectra of the products, imine and ninhydrin

3.3 Effect of concentration of Reductant

The effect of concentration of substrate was studied by varying the concentration of RP from $1x10^{-3}$M to $6x10^{-3}$M keeping concentration of all reaction species constant. It was observed that the rate of the reaction increases with increase in concentration of reductant. As it can be seen from the figure below, the rate of reaction increases as concentration of RP increases from 2mM to 6mM.

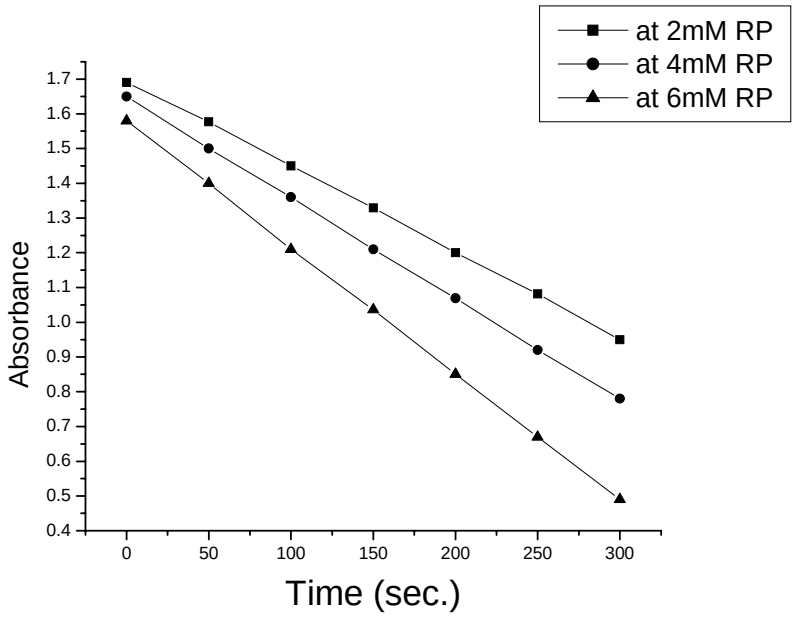


Fig.4 Plot of the spectra for the increase in concentration of RP from $2x10^{-3}$ to $6x10^{-3}$ M keeping other parameters constant taking linear part only

3.4 Effect of alkali

The effect of alkali on the reaction was studied at constant concentration of RP, potassium permanganate and at constant ionic strength of 0.5M at 25^0C. To study the effect of [alkali] on the rate, concentration was varied from 0.01M to 0.08M .As can be observed from figure5, the rate of the reaction increased with the increase in concentration of alkali.

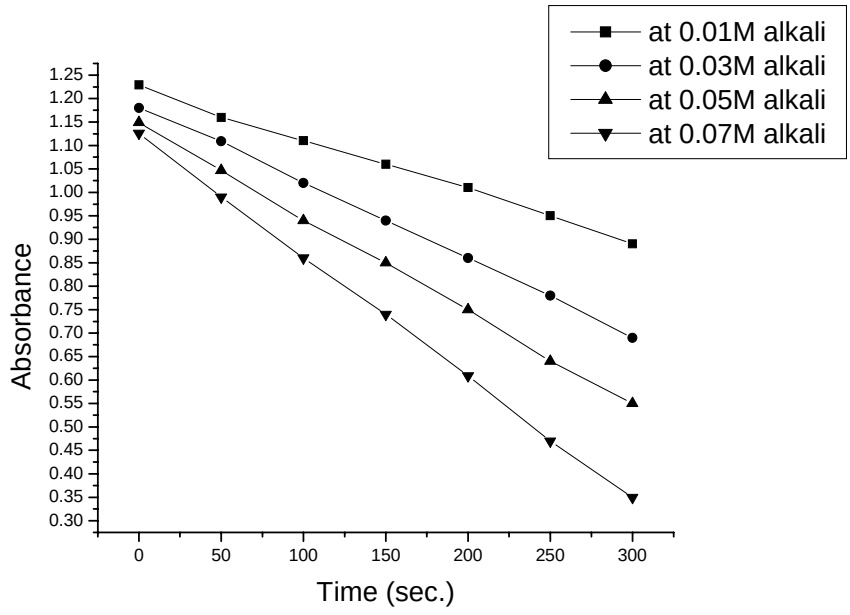


Fig .5 Effect of concentration of alkali by varying concentration from0.01M to0.07M

As can be observed from figure 5, the rate of the reaction increases with increasing concentration of alkali from 0.01M to 0.07M. As concentration of alkali increases the steepness of line increases. It shows the increase in rate as concentration of alkali increases.

3.5 Effect of ionic strength and solvent polarity

The effect of ionic strength was studied by varying the NaClO4 concentration from 0.20 to 2.0 mol dm^{-3} at constant concentration of permanganate, Ruheman's Purple and alkali.

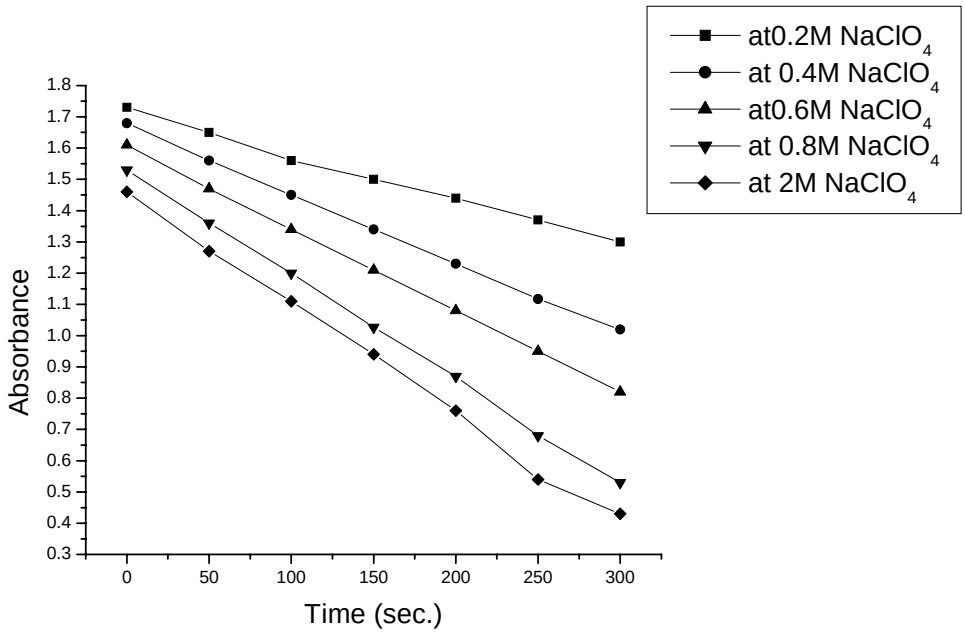


Fig .6 Effect of ionic strength by varying the concentration of NaClO4 from 0.2M to 2M

Table 1: The effect ionic strength on the rate constant of the reaction

k_{obs} x10^3 **(S^{-1})**	15	18.3	21.6	25	29.4
Log k_{obs}	-1.822	-1.737	-1.66	-1.602	-1.531
I	0.522	0.4522	0.6522	0.8522	1.0522
√I	0.5022	0.672	0.8075	0.923	1.025

Where I is ionic strength k_{obs} observed rate constant.

As can be seen from figure7 that the rate of the reaction increases with increase in the square root of ionic strength and decreases with decrease in square root of ionic strength. Since ionic strength is directly proportional to the concentration of $NaClO_4$, the rate also increases with increase in concentration of $NaClO_4$.

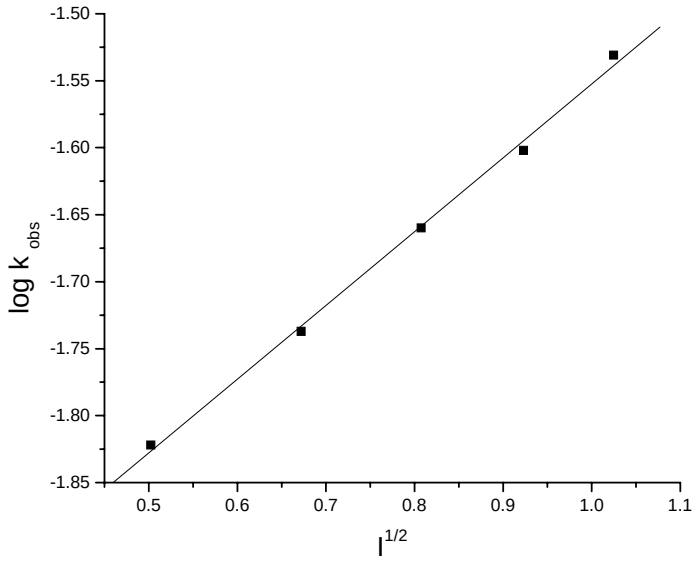


Fig .7 Plot of log of rate constant vs. square root of ionic strength

The plot of log k_{obs} versus $I^{1/2}$ is linear with a positive slope. The relative permitivity effect was evaluated by variation of the *t*-butanol-water content while keeping all other conditions constant. There was no reaction of the solvent with the oxidant. The rate of the reaction increases with decreasing dielectric constant of the media (figure8).

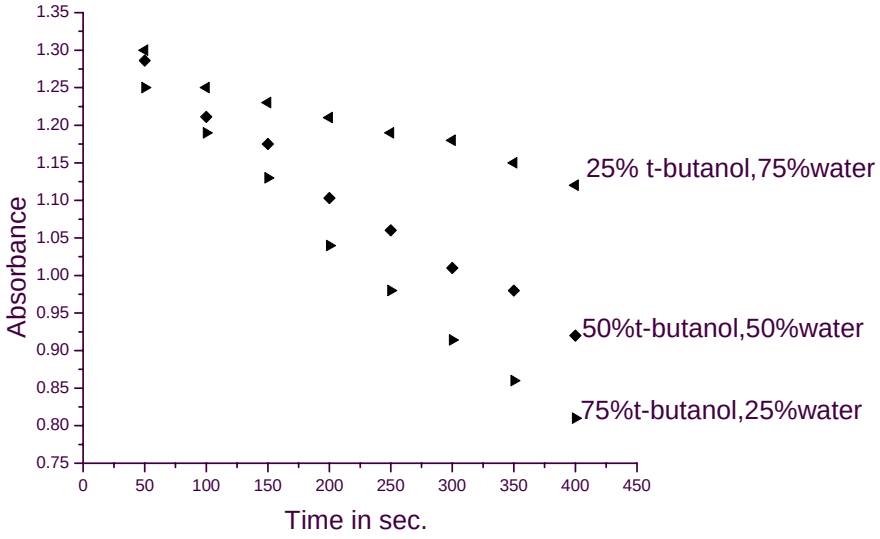


Fig.8 Absorbance versus time plot on the effect of relative permittivity.

To study the effect of relative permittivity on the rate of reaction, the content of water and t-butanol was varied from 25% to 75%. By converting the absorbance into concentration and calculating the rate of the reaction, it was observed that the rate of the reaction increased with increase in percentage content of t-butanol. This effect was due to the difference in dielectric costant between water and t-butanol.

3.6 Effect of Initially added products

The effect of initially added products of the reaction, such as manganate, and ninhydrin on reaction rate were investigated. Addition of these products did not affect the reaction rate significantly.

3.7 Effect of dissolved oxygen

The effect of dissolved oxygen on the rate of reaction was checked by preparing the reaction mixture and following the reaction in an atmosphere of nitrogen. No significant difference between the results obtained under the nitrogen and in the presence of air was

observed. In view of the ubiquitous contamination of basic solutions by carbonate, the effect of carbonate on the reaction was also studied.

Added carbonate had no effect on the reaction rate. However, fresh solutions were used when conducting the experiments.

A regression analysis of experimental data in order to obtain the regression coefficient, r and standard deviation, s of plots from the regression line was performed with a Pentium - IV personal computer.

3.8 Effect of Temperature

The rate of reaction was measured at different temperatures under constant concentration of oxidant, reductant and ionic strength of 0.5M. The rate of reaction increased with the increase of temperature. The rate constants, for the four different temperature 25^0C, 30^0C, 35^0C, 40^0C were recorded as 3.16 x 10^{-3} s^{-1}, 3.43 x 10^{-3} s^{-1}, 4.03 x 10^{-3} s^{-1}, and 4.75 x 10^{-3} s^{-1} respectively.

The activation parameters corresponding to these constants were evaluated from the plot of log k versus 1/T ($r>0.9989$, $S<0.0135$) and are tabulated in Table 3.

Table 2: Data for effect of temperature.

Temperature(K^0)	298	303	308	313
1/T X10^3	3.35	3.3	3.24	3.19
Rate constant k 10^3 S^{-1})	3.16	3.43	4.03	4.75
logk	-2.5	-2.46	-2.39	-2.32

As can be seen from table 2 the rate constant of reaction increases with increase in temperature. Using the data in table 2 and by using Arrhenius equation given below, thermodynamic parameters can be calculated.

$$k=Ae^{-Ea/RT} \quad \text{.....1}$$

and $Ea=\Delta H^{\#}-T\Delta S^{\#}$ and $G= Ea + H$ 2

Where A = frequency factor, Ea = activation energy, R = universal gas costant = 1.987 cal/deg mole and T= temperature in Kelvin

By taking the logarithm of both side of the equation1 and by using table 2, Arrehenius plot can be obtained and from the slope of the plot, energy of activation (Ea) can be calculated. Making use of therodynamic equations (equation2), other thermodynamic parameters like enthalpy ($\Delta H^{\#}$), entropy ($\Delta S^{\#}$) and Gibb's free energy of the reaction also can be calculated. From the intercept of the Arrhenius plot frequency factor (A) can be obtained.

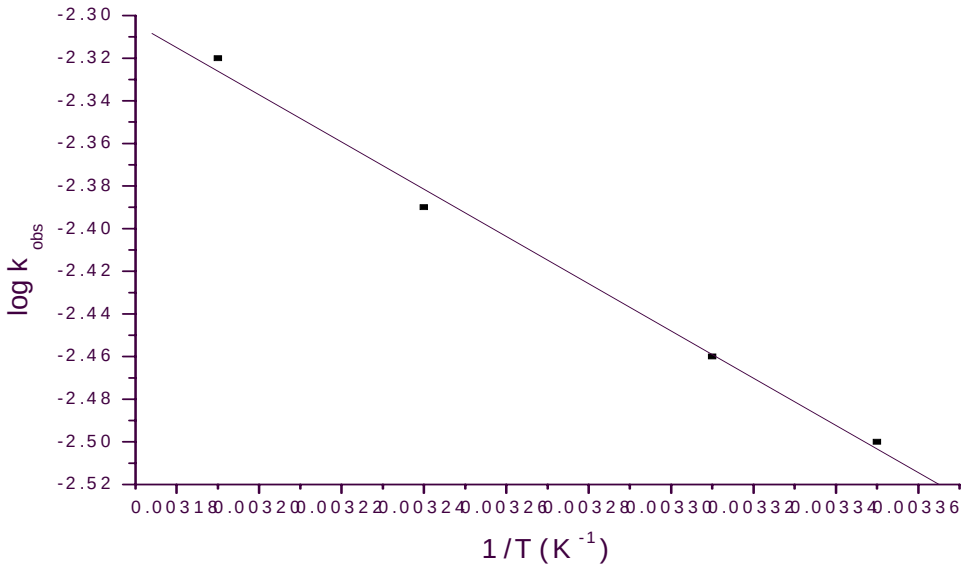


Fig.9 Plot of observed rate constant vs. temperature inverse in Kelvin

The plot in above figure is linear with regression constant (r) = - 0.998,

Slope = -1108.01964 and intercept = 1.20849

The activation parameters of the reaction were calculated by a plot of log k_{obs} versus1/T as given in table 3.

Table 3: Thermodynamic parameters and their values.

Symbol	Thermodynamic parameters	Values(Kj/mol)
Ea	Energy of activation	21.21
$\Delta H^{\#}$	Enthalpy	-14.148
$\Delta G^{\#}$	Gibbs free energy	7.09
$\Delta S^{\#}$	Entropy	-0.116.5
A	frequency factor	0.318

The experimental values of $\Delta H^{\#}$ and $\Delta S^{\#}$ were both favored for electron transfer process. The high negative value of $\Delta S^{\#}$ indicates that interaction of reacting ions of similar charges to form an activated complex and is more ordered than the reactants due to the loss of degree of freedom. This supported by a small value of frequency factor. The variation in the rate with in a reaction series may be caused by the change in enthalpy and entropy of activation. Changes in the rate are caused by changes both $\Delta H^{\#}$ and $\Delta S^{\#.}$

3.9 Reaction order

The reaction orders were evaluated from the slopes of log of rate versus log of concentration plots by varying the concentration of oxidant, reductant, and alkali while keeping the others constant. This shows that the order of reaction with respect to permanganate is first order. The order with respect to [RP] and [alkali] were found by of log rate versus log of concentration plots using the equation

log rate = log k + n log c; these orders were obtained by varying the concentration of Ruheman's Purple and alkali in turn while keeping others constant. Where n is order and

c is concentration and k is rate constant. And again the order can be confirmed by the plot log k_{obs} versus log concentration. The concentration of $MnO4^-$ was varied in the range, 1.0 x 10^{-4} to1.0 x 10^{-3} mol dm^{-3} at fixed [Ruheman's Purple], [OH^-] and at constant ionic strength of 0.5M. The non-variation in the pseudo-first order rate constants at various concentrations of MnO_4^- indicates the order in [MnO_4^-] as unity (Table 6). This was also confirmed from the linearity of plots of log of rate versus log[MnO_4^-] ($r>0.9984$, $SD<0.025$) up to 60% completion of the reaction (figure10). The substrate, [Ruheman's Purple] was varied in the range of 1.0 x 10^{-4} to1.0 x 10^{-3} mol dm^{-3} at 25 ºC keeping all other reactants concentrations constant. The k_{obs} values were increased with increase in concentration of Ruheman's Purple and it was found to be less than unit order dependence on [Ruheman's Purple] (Table 6). The effect of [alkali] on the rate of reaction was studied at constant concentrations of Ruheman's Purple, $MnO4^-$ and ionic strength at 0.5 mol dm^{-3}. The rate constants increased with increase in [alkali] and the order was also found to be less than unity (Table 6).

To calculate the order of the reaction with respect to each reacting species the measured absorbance for each spectrum was converted in to concentration using Beer's law.

For the variation of concentration of potassium permanganate and keeping other conditions constant, the following table was generated for calculation of order with respect to oxidant.

Table 4: The Data for calculating order with respect to variation of concentration of oxidant.

log rate	-6.30	-6.25	-6.209	-6.16	-6.12
log[MnO4]	-3.35	-3.40	-3.46	-3.53	-3.61
Time	0	100	200	300	400

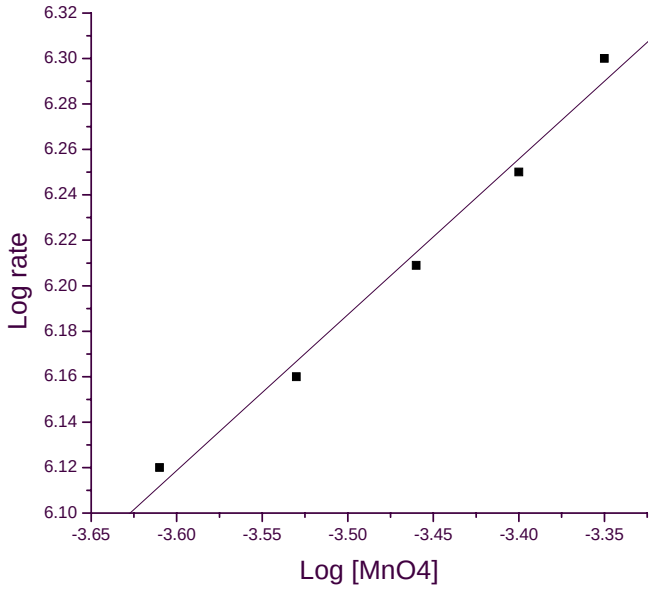


Fig .10 Plot of log of rate versus negative log of concentration of permanganate.

From the slope of the above graph order of the reaction with regard to variation of concentration of permanganate was calculated to unity.

Using the same analogy keeping concentration of oxidant, alkali and sodium perchlorate constant, the order of the reaction for variation of RP concentration can be calculated

Table 5:Data for calculating order with respect to reductant.

log rate	-4.096	-4.22	-4.398	-4.69	-4.83
log[RP]	-2.00	-2.24	-2.45	-2.80	-2.98
Time	0	100	200	300	400

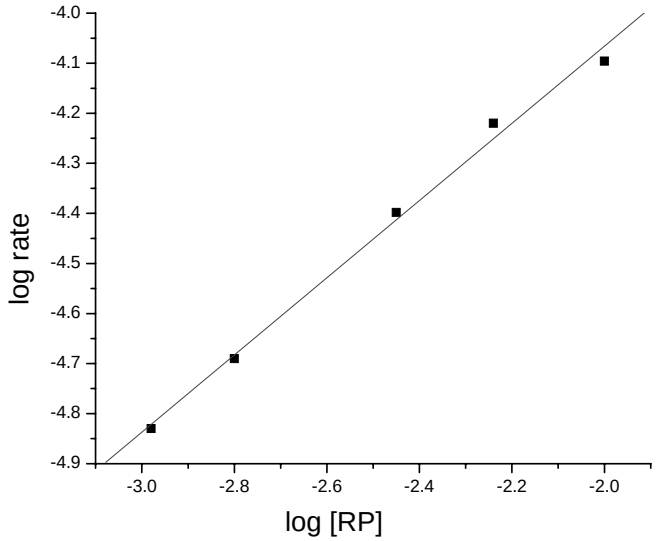


Fig.11. Plot of log rate versus log [RP] for calculating order of the reaction on [RP]

From the slope of above figure the order of reaction with respect to variation of [RP] from 1mM to 10mM was calculated to be 0.7

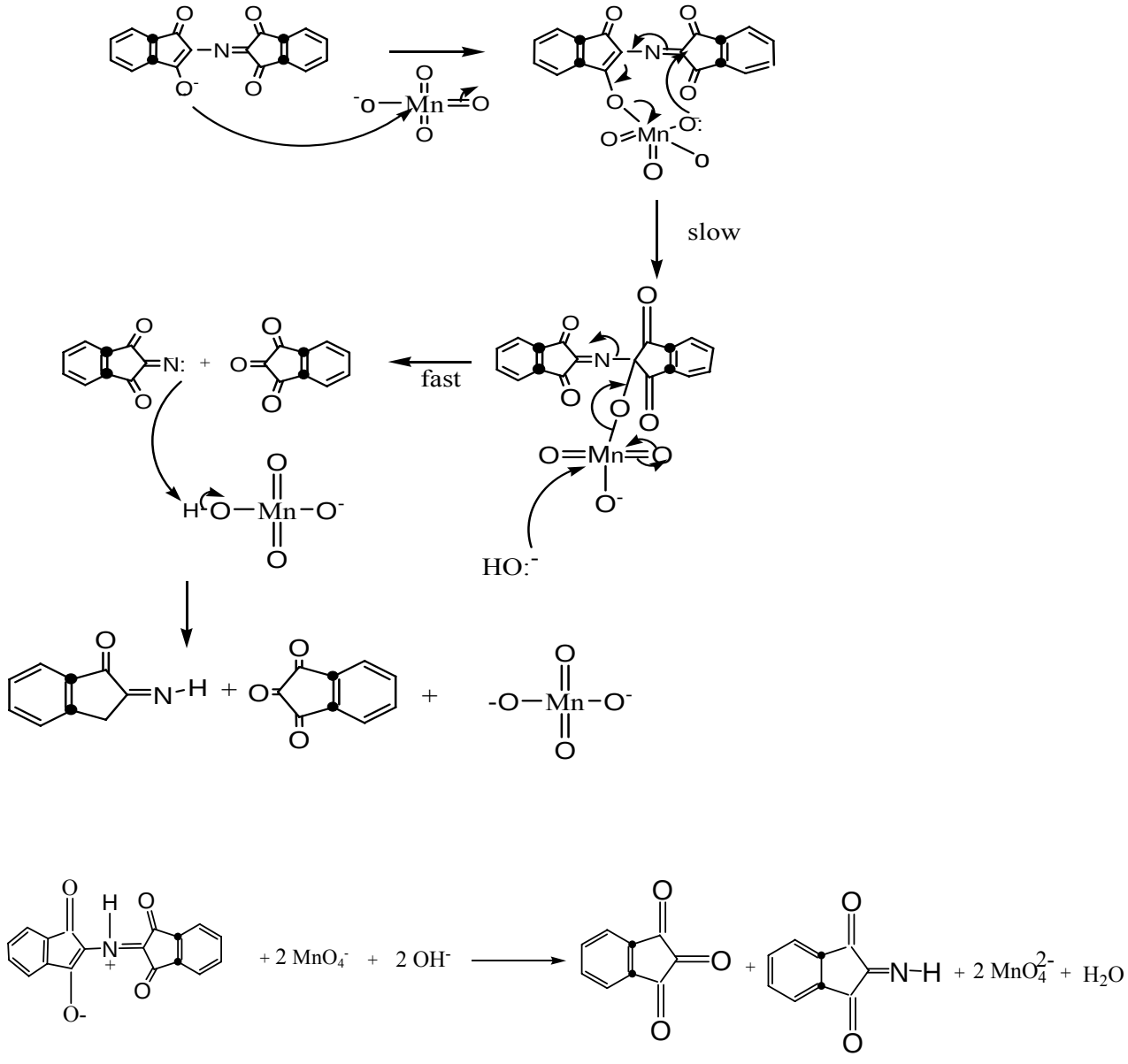
The order of alkali was also calculated to be 0.65 following the same method that was done for the variation of concentration of oxidant and reductant.

Table 6: Effect of concentration of oxidant, alkali and reductant

10^4 x[MnO4] (mol/dm3)	$10x^3$ [RP] (mol/dm3	[OH]	k_{obs} (s^{-1}) $x10^3$	k_{calc} (s^{-1})$x10^3$
1	2	0.05	2.48	2.50
2	2	0.05	2.44	2.47
4	2	0.05	2.47	2.45
6	2	0.05	2.48	2.51
8	2	0.05	2.51	2.50
2	1	0.05	1.39	1.42
2	2	0.05	1.43	1.51
2	4	0.05	1.47	1.53
2	6	0.05	1.52	1.55
2	8	0.05	1.55	1.57
2	2	0.01	0.05	0.0482
2	2	0.03	0.053	0.0566
2	2	0.05	0.055	0.0596
2	2	0.07	0.057	0.0610
2	2	0.09	0.057	0.06102

Mechanism of reaction

Since the media was basic, protonated form of RP was deprotonated and mechanism proceeded as follows:

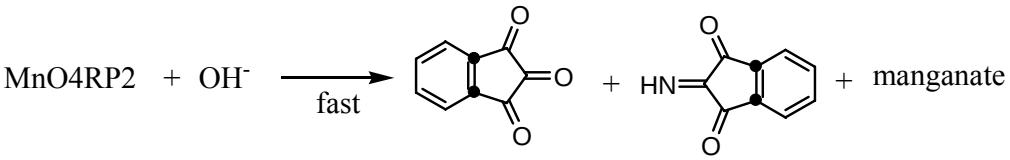


Scheme9

Mechanism for deducing rate law

$$MnO_4^- + RP \underset{}{\overset{K}{\rightleftharpoons}} MnO_4RP_1 \quad \text{.....1}$$

$$MnO_4RP_1 \xrightarrow[\text{slow}]{k} MnO_4RP_2 \quad \text{.....2}$$



According to equation 2 ,

$$\text{Rate} = -\frac{d[MnO_4^-]}{dt} = k[MnO_4RP_1]$$

From equation 1, $[MnO_4RP_1] = K[RP]\ [MnO_4^-]$

$$\text{Rate} = \frac{-d[MnO_4^-]}{dt} = kK[RP][MnO_4\text{-}]$$

$$k = \frac{\text{Rate}}{K[RP][MnO_4\text{-}]} \quad \text{(theoretical rate constant)}$$

4. Conclusions

Potassium permanganate is widely used as an oxidizing agent in synthetic as well as in analytical chemistry and also as a disinfectant. The reactions are governed by pH of the medium. Among six oxidation states of manganese from 2+ to 7+, permanganate, Mn (VII), is the most potent oxidation state in acid as well as in alkaline media. The most stable reduction product of permanganate in strong aqueous alkaline media is manganate, $MnO4^{-2.}$ The reaction between permanganate and Ruheman's Purple in alkaline medium has a stoichiometry of 1:2 reductant to oxidant with first order dependence on $[MnO4^{-}]$, apparent less than unit order dependent on $[OH^{-}]$ and [Ruheman's Purple]. The active species of permanganate in aqueous alkaline medium may be deduced from the dependence of the rate on $[OH^{-}]$ in the reaction media. The reaction was observed to affect by the concentration of oxidant, reductant, alkali and ionic strength. Variation of temperature and solvent polarity also affects the rate of chemical reaction. The important thermodynamic paramers can be calculated. The reaction was initially preceded by forming the complex between RP and permanganate. This complex was also reacted to alkali and proceed to the formation of manganate, tri ketone form of ninhydrin and imine. The results are given in scheme7.

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