

Kinetics and Mechanism of Oxidation of Alanine and Phenylalanine by Fenton's Reagent

Mohamed Nuri Rahuma¹ and Iftikhar Ahmad²

¹Chemistry Department, University of Benghazi, Benghazi, Libya.

²AGOCO Oil Company, Benghazi, Libya.

ABSTRACT

The combination of hydrogen peroxide and ferrous salt is frequently referred to as Fenton's reagent. The comparative kinetics of the oxidation of alanine and phenylalanine by hydrogen peroxide in the presence of ferrous sulphate have been studied. The reaction is first order with respect to ferrous sulphate and amino acids (alanine and phenylalanine), and zero order with respect to hydrogen peroxide. The reaction rate is higher in case of phenylalanine than that of alanine. The reaction mechanism for both the amino acids have been proposed and rate laws derived.

INTRODUCTION

The combination of hydrogen peroxide and ferrous salt is frequently referred to as Fenton's reagent. It has been well established (Walling 1975) that the action of ferrous sulphate on hydrogen peroxide in acidic aqueous solution brings about the formation of hydroxyl radicals. The Fenton's reagent has been used in the initiation of vinyl polymerisation, in the oxidation of alcohols (Merz and Water 1949, Walling and El-Taliawi 1973). A survey of the literature indicates that few reports (Merz and Waters, 1949; Kalyankar, 1955) described the oxidation of some amino acids by the Fenton's reagent. These studies refer mainly to their oxidation products, but only few reports (Ahmad, et. al/1983; 1980) appears to have been done on the kinetics of deamination of amino acids by this reagent. Recently we have studied the kinetics of oxidation of glycine (Rahuma et.al 2006). Mohamed Rahuma <moh_n_r@yahoo.co.uk>

EXPERIMENTAL

All the chemicals used were of Analar (B.D.H.) grade. The freshly prepared ferrous sulphate solution was used to avoid the termination of free radicals with oxygen. The concentration of hydrogen peroxide in kinetic runs was measured colorimetrically (Reichert, et. al, 1939).

After the reactants had reached thermostat temperature, the reaction was started by addition of the hydrogen peroxide. The kinetics were followed by examining 5 mL aliquots of the reaction mixture for hydrogen peroxide content. The aliquots were added to 5 mL of titanium sulphate solution to stop the reaction. Optical density was measured at 420 nm, and the concentration of hydrogen peroxide was read from the calibration graph. Every run was followed until the reaction was at least 80% complete. The same reaction flask was used throughout our investigations. In each set the initial volume of the reaction mixture was constant. All the reactions were studied in an open atmosphere. However, the dependence of the reaction on atmosphere was checked by studying two identical reaction mixtures, one under nitrogen and the other without nitrogen. The rate constants in both the cases were almost equal.

Identification of Products

The reaction mixture was heated on a water bath at about 60 °C and gaseous products formed were passed through freshly prepared lime water solution which turned milky and thus confirmed the presence of carbon dioxide. The formation of formaldehyde in gaseous products was tested with chromotropic acid which gave violet-pink colour. The reaction mixture was made alkaline with sodium hydroxide and heated on a water bath. The characteristic smell of ammonia was noted in the issuing gases. The ammonia evolved was also confirmed by reaction with Nessler's reagent. The reaction mixture was treated with acidified 2,4-dinitrophenylhydrazine solution, which yielded a hydrazone. The IR spectrum of this

hydrazone superimposed on the spectrum of a corresponding hydrazone of a standard sample of benzaldehyde. Moreover, the mixture melting point of the hydrazone did not show any depression.

RESULTS AND DISCUSSION

The progress of the reaction was observed by measuring the disappearance of hydrogen peroxide concentration in the presence of high concentration of amino acid (alanine or phenylalanine). Straight line plots for hydrogen peroxide concentrations against time indicates zero order dependence of rate on hydrogen peroxide concentration. The pseudo-zero-order rate constants (k_{obsd}) were calculated from the slope of these lines and reported in Table- 1.

The order of reaction with respect to catalyst (ferrous sulphate) was determined by varying its concentration and keeping other variables constant. The plot of k_{obsd} against the concentration of ferrous sulphate was a straight line (Fig. 1) which passes through the origin. This shows first order dependence of rate on catalyst concentration and it also confirms that there is no reaction in the absence of ferrous sulphate. Similarly the order of reaction with respect to substrate (alanine or phenylalanine) was determined by varying its concentration and keeping all other variables constant. The observed pseudo-zero order rate constants (k_{obsd}) when divided by substrate concentration, gave constant values (Table 2), indicating that the order of reaction with respect to substrate (alanine or phenylalanine) is unity.

Table 1: Pseudo-zero-order dependence of rate for the oxidation of alanine and phenylalanine by hydrogen peroxide in presence of Fe(II) ions

[Alanine or Phenylalanine] = 0.01 M ; [FeSO ₄] = 5.00 x 10 ⁻⁴ M [H ⁺] = 0.20 M ; Temp., 45 °C		
[H ₂ O ₂] x 10 ³ M	$k_{\text{obsd}} \times 10^2$ (mol dm ⁻³ min ⁻¹)	
	Alanine	Phenylalanine
1.25	1.35	2.01
2.50	1.37	1.95
3.25	1.34	1.87
5.00	1.35	1.98
6.25	1.36	1.92

Table 2: First order dependence on the Fe(II) ion catalysed oxidation rate of alanine and phenylalanine on their concentrations

[Substrate] x 10 ²	Alanine	Phenylalanine
	$k_{\text{obsd}} \times 10^2$ (moles dm ⁻³ min ⁻¹)	$k_{\text{obsd}} \times 10^2$ (moles dm ⁻³ min ⁻¹)
5.00	0.67	0.99
7.50	1.01	1.49
10.00	1.35	1.98
12.50	1.69	2.47
15.00	2.03	2.97
[H ₂ O ₂] = 2.50 x 10 ⁻³ M ; [FeSO ₄] = 5.00 x 10 ⁻⁴ M ; [H ⁺] = 0.20 M ; Temp., 45 °C		

All the experiments have been carried out in the presence of aqueous sulphuric acid. The concentration of this acid used in all experiments was ≥ 0.2 M which was sufficient to prevent the hydrolysis of Fe(II) ions and the self decomposition of hydrogen peroxide as well. Preliminary studies have shown that under our experimental conditions, hydrogen peroxide does not bring about oxidation of phenylalanine without ferrous sulphate. Also ferrous sulphate alone without hydrogen peroxide did not oxidise phenylalanine. It is fairly well established (Bakore and Dwivedi, 1968) that hydrogen peroxide in the presence of Fe(II) ions produces hydroxyl free radical (.OH) which is very likely to attack phenylalanine. Two probable mechanisms of oxidation of phenylalanine involving this free radical are represented in scheme I and scheme II.

The hydroxyl free radicals generated in step 1 can attack on α - or β - carbon to the carboxylic group of phenylalanine giving respective free radicals as proposed in scheme I and scheme II. The benzylic free radical, Ph - $\dot{\text{C}}\text{H}$ - CH(NH₃⁺) - COOH, depicted in scheme I is resonance

stabilised. Therefore, it is likely to be more stable than the free radical, Ph – CH₂ - .C (NH₃⁺) - COOH suggested in scheme II. These free radicals indicate the formation of α – ketoacid in scheme II and β-ketoacid in scheme I as intermediate products. Unfortunately none of these could be detected under our experimental conditions. But β-ketoacids are generally less stable than α – ketoacids. Nevertheless, both the intermediates, although vary in stability, may give the same products through different routes as detailed in schemes I and II.

The rate of reaction in both the schemes (I and II) is

$$-\frac{d[\text{Phenylalanine}]}{dt} = k_2 [\text{Phenylalanine}] [\text{OH}\cdot] \quad \dots\dots\dots (1)$$

Applying the steady state approximation for the concentrations of all intermediate free radicals in scheme I, we obtain

$$[\text{OH}\cdot] = \frac{k_1 [\text{Fe}^{2+}]}{k_{10}} \quad \dots\dots\dots (2)$$

Substituting the concentration of OH· from eqn. (2) into eqn. (1), we get,

$$-\frac{d[\text{Phenylalanine}]}{dt} = \frac{k_1 k_2 [\text{Phenylalanine}] [\text{Fe}^{2+}]}{k_{10}} \quad \dots\dots (3)$$

or

$$-\frac{d[\text{Phenylalanine}]}{dt} = k' [\text{Phenylalanine}] [\text{Fe}^{2+}] \quad \dots\dots (4)$$

where,

$$k' = k_1 k_2 / k_{10}$$

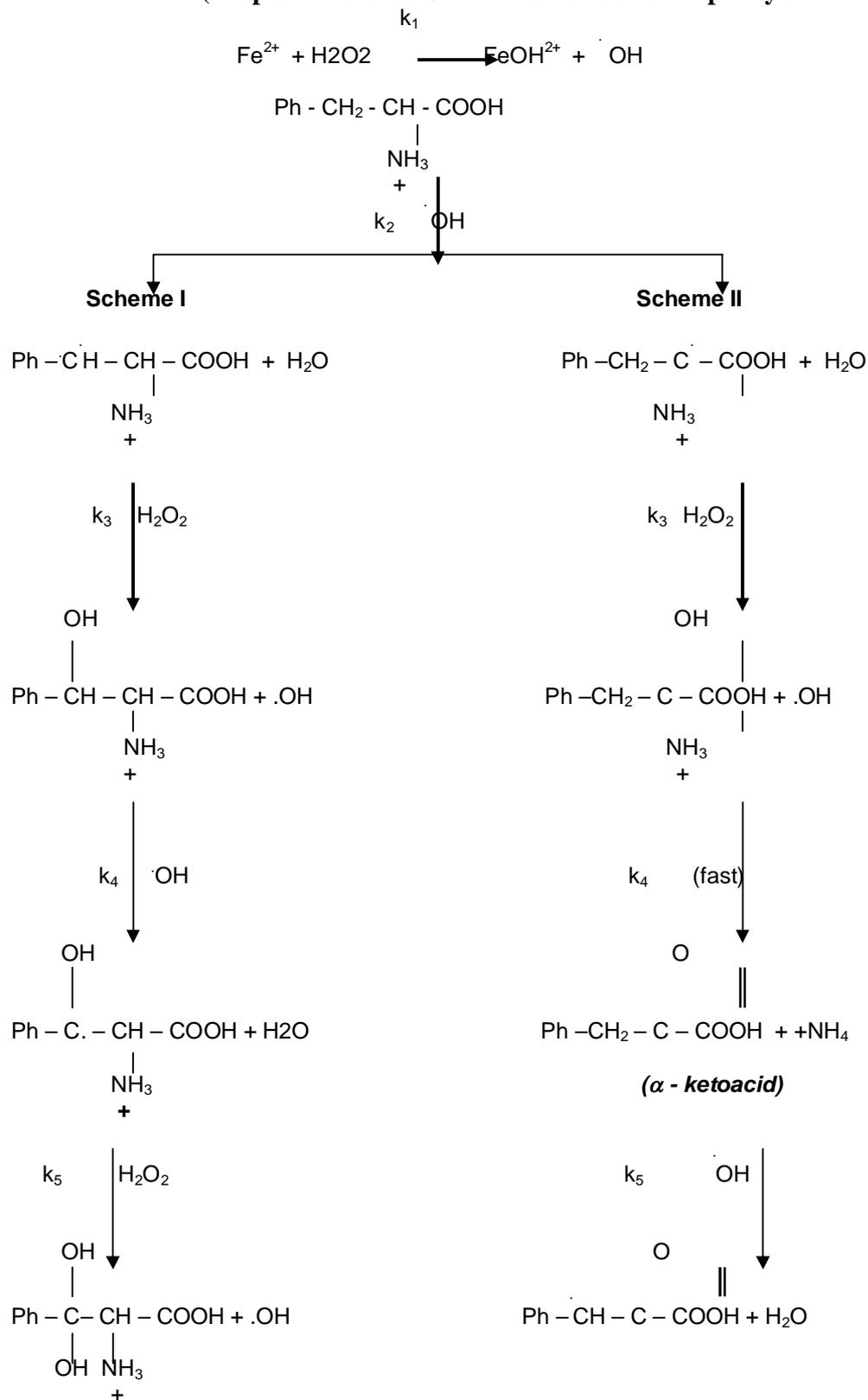
The eqn. (4) can also be written as:

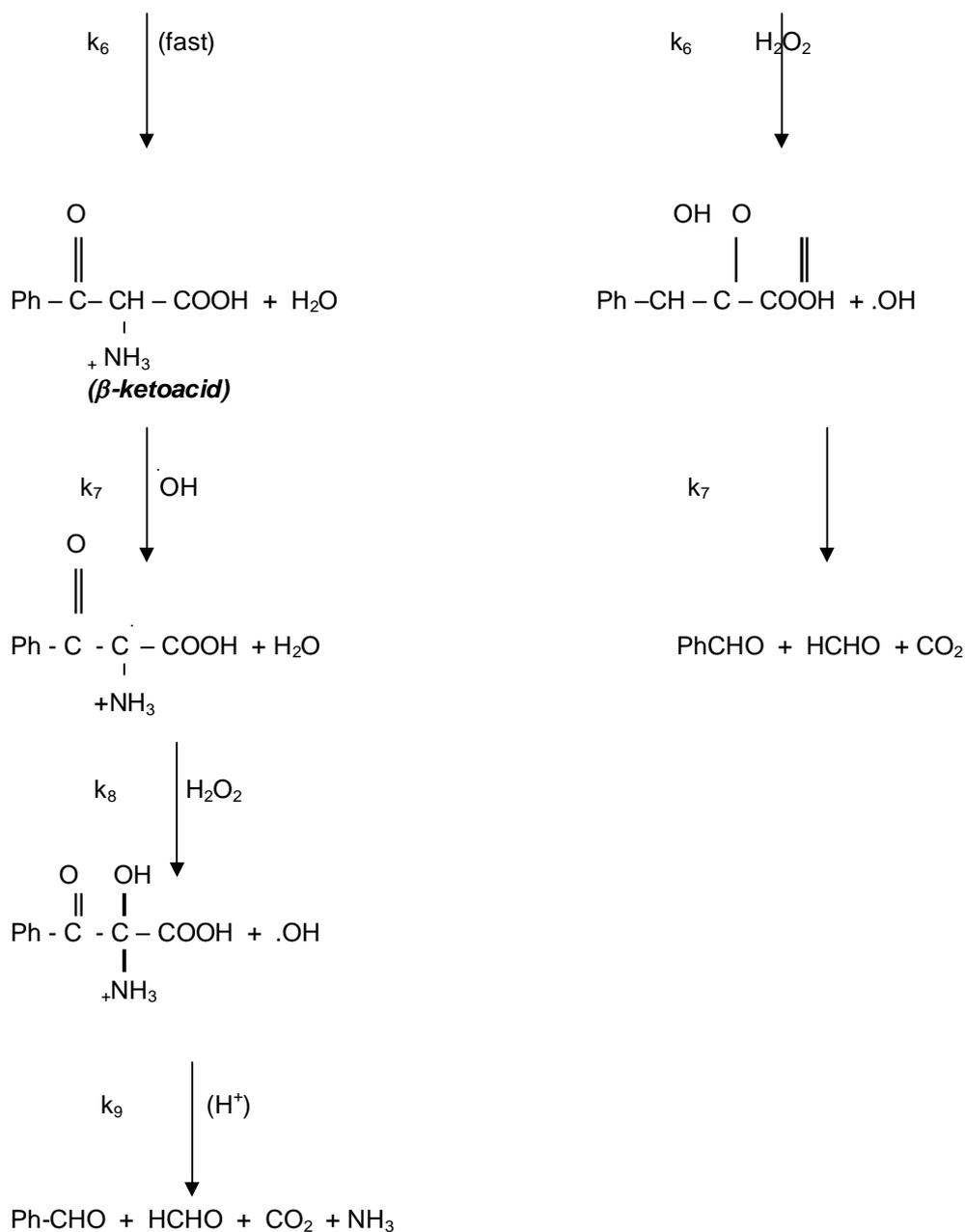
$$\text{Rate of reaction} = k_{\text{obsd}} [\text{H}_2\text{O}_2]^0 \quad \dots\dots\dots (5)$$

$$\text{Where, } k_{\text{obsd}} = k' [\text{Phenylalanine}] [\text{Fe}^{2+}] \quad \dots\dots\dots (6)$$

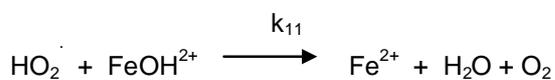
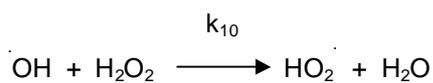
Equation (5) clearly explains the observed order of reaction with respect to phenylalanine, Fe(II) ions and hydrogen peroxide concentrations.

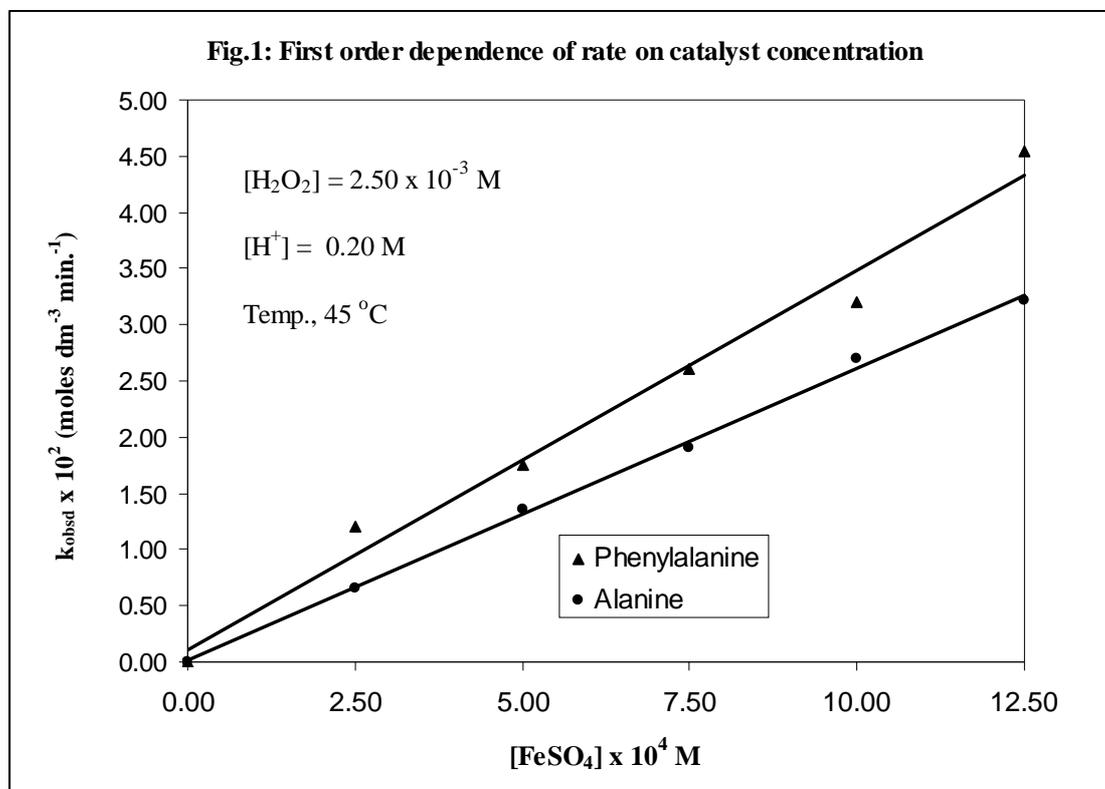
(Proposed reaction Scheme for oxidation of phenylalanine)



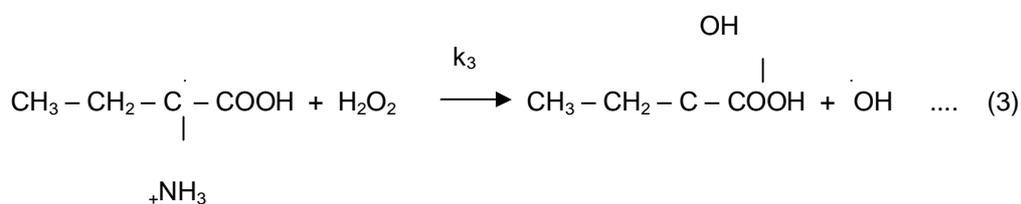
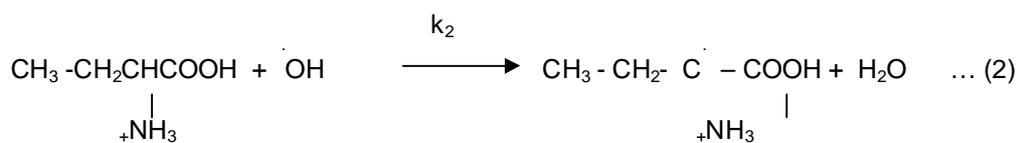


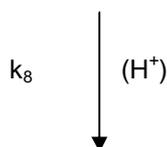
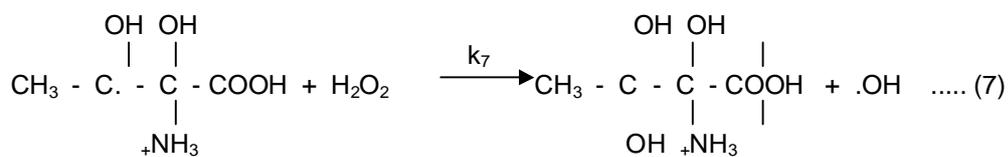
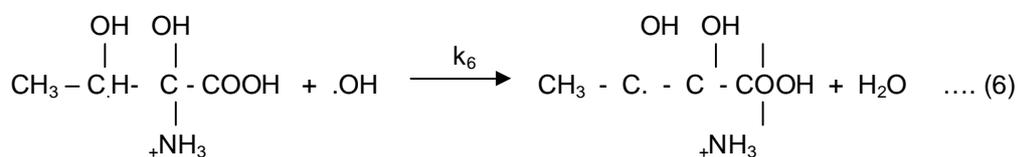
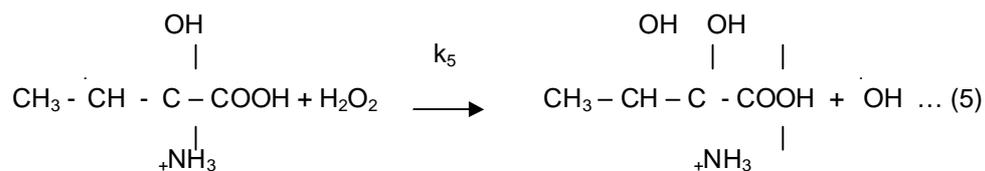
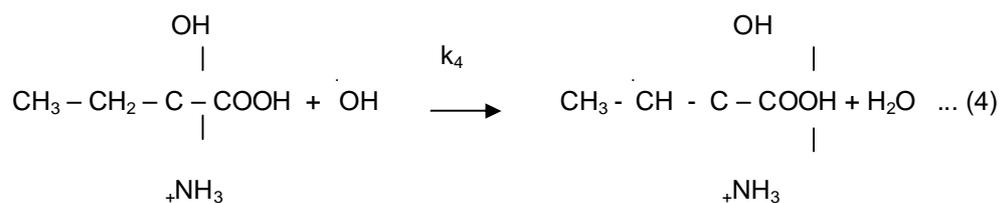
Chain termination steps common in both schemes



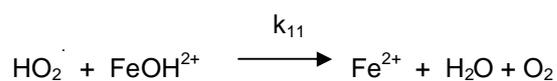
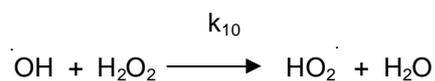


Proposed Reaction Scheme of Oxidation of Alanine





Chain termination steps common in both schemes



REFERENCES

1. Bakore GV. and Dwivedi VJ. Indian J Chem. 1968;6:651.
2. Kalyankar GD, Vaidyanathan CS and Giri KV. Experientia. 1955; 11:348.
3. Merz JH and Waters WA. J Chem Soc. 1949;2427-2440.
4. Merz JH and Waters WA. J Chem Soc. 1949;(Supplement)15- 25.
5. Reichert JS, Mc Neight SA and Rudel HW. Ind Eng Chem Anal Ed. 1939;11:194.
6. Walling C. Acc Chem Res. 1975; 8:125-130.
7. Walling C and Taliawi CM. J Am Chem Soc. 95:844 – 847.