

**A Study of Experimental Data for the Determination of Formation Constant  
Lamber -Beer's Law Method**

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**Abstract**

Literature survey shows that the stability constants of amino acids have been the subject of study by many workers [2] and there is ample scope for the spectrophotometric and kinetic study of amino acids [2-7]. But, no systematic attempt has been made so far on the chemical kinetic and spectrophotometric investigations of amino acids in presence of anions, cations, micelles and catalysts. In view of the importance and growing interest in the field of chelation, kinetics and mechanism of oxidation of amino acids in presence of different oxidants, catalysts and micelles [8-12], it is thought worthwhile to study in detail the title investigation. In the present investigation, kinetic measurements of oxidation of various amino acids in acidic, neutral and alkaline medium were studied. The  $K_{\text{obd}}$  value for the reactions obtained from calculations and from graphical representations was evaluated. The order of reaction was confirmed from the slopes of plots of  $\log K_{\text{obd}}$  Vs  $\log C$ . In all the systems studied, the order was found to be one with respect to each reactant. The rates of reactions were evaluated by using the usual expression. The effect of varying concentration of amino acids, oxidants, Br<sup>-</sup>, Fe<sup>++</sup>, surfactants like T-80 and T-X-100 on rate of reaction has also been studied. In order to study the effect of ionic strength and temperature on  $K_{\text{obd}}$ , the systems were studied at different concentrations of the electrolyte and at elevated temperatures.

**Key Words:** The oxidation of amino acids viz. glycine, DL-alanine, DL-leucine, DL-aspartic acid, DL-glutamic acid, L-lysine and L-arginine by potassium permanganate in sulphuric acid

**Introduction:**

Biologically active compounds like amino acids, proteins and vitamins containing heteroatoms are receiving considerable attention in various fields like nutrition, pharmaceutical, clinical and biochemical research. The amino acids have received considerable attention as possible constituent of proteins. These biologically important amino acids have been used as chelating agents, with certain metal ions at

different experimental conditions [1]. An exhaustive work has been carried out on the thermodynamics and complexometric investigations of these chelating reagents. There has been a great deal of interest in the reactions between the amino acids and metal ions because of their importance in chemistry and biology. Simple and mixed complexes of amino acids with certain metal ions in solution have also been studied by some workers [2]. The binary and ternary chelates of some amino acids

have been studied exhaustively in aqueous and aquo organic media by few workers [2-5] using pH metry, potentiometry and polarography. However no systematic study of the chemical kinetics of oxidation of amino acids in aqueous acidic, neutral, alkaline medium has been done so far in presence of surfactants, cations, anions and at elevated temperatures.

### **Methods:**

Chemical kinetics is gaining importance in pure and applied fields and it leads to find out optimum conditions required to get desired product which is economically viable. In practice the decrease in concentration of a reactant or increase in concentration of a product can be measured with time. Numbers of methods are available for the measurements of kinetic parameters, few of which are summarised as under:

- (i) Periodic or continuous spectral measurement: The reaction mixture under investigation can be subjected for kinetic measurements using spectrophotometric (UV, VIS, IR, NMR, ESR), chromatographic, polarographic methods.
- (ii) Quenching and analysing: Series of reactions can be performed at different experimental conditions either by lowering the temperature or by adding an inhibitor. The reaction mixture can be then subjected for analysis using usual procedures, depending upon the nature of the reactants or product formed.
- (iii) Removal of aliquots at intervals: Each aliquot from the reaction mixture can be subjected for analysis.

- (iv) Measurement of change in total pressure for gas phase reactions [6, 7].
- (v) Spectrophotometric methods: of the methods available for the kinetics of oxidation of amino acids, spectrophotometric method is most suitable since it finds wide applications because of its quick, precise and continuous means of monitoring changes in concentration of the reactants and or products.

### **Experimental Results**

#### **Part - A: Kinetic Measurements of Lamber -Beer's Law Method**

The kinetic measurements of the oxidation of amino acids viz. glycine, DL-alanine, DL-leucine, DL-aspartic acid, DL-glutamic acid, L-lysine and L-arginine by potassium permanganate in sulphuric acid medium keeping excess of amino acids have been studied spectrophotometrically using Shimadzu UV-VIS-160-1A Spectrophotometer in aqueous acidic, neutral and alkaline medium in presence and absence of anions, cations and surfactants. Sulphate ions and bromide ions were used as anions in the form of Na<sub>2</sub>SO<sub>4</sub> and KBr. Fe (III) and copper (II), Ag (I) were studied in presence of few amino acids. Polyoxyethylene sorbitan monooleate, octyl phenoxy polyethoxy ethanol and sodium dodecyl sulphate were used to study the effect of surfactant on the rate of reaction. The dependence of ionic strength, on the rate of reaction has been studied using sodium sulphate, sodium perchlorate and potassium nitrate as electrolytes.

The effect of [H<sup>+</sup>] ion concentration has also been studied for all the systems selected in the present investigation.

The kinetic runs were carried out at 0.1 M Na<sub>2</sub>SO<sub>4</sub> ionic strength and at 298°K in a thermostatic serological water bath (± 0.1 °C). Some of the systems, which were investigated earlier, were reinvestigated here to get the kinetic data under identical experimental conditions maintained for present kinetic runs. The reaction was initiated by adding to an equilibrated mixture of respective

amino acid, sodium sulphate, sulphuric acid requisite quantity of preequilibrated solution of potassium permanganate. The experimental details of first order rate constants with respect to amino acids and potassium permanganate are given here in a tabular form (Table 3.1a- 3.1.f-40) for some representative systems. However, since the experimental conditions were the same for the remaining systems they are not described to avoid duplication.

**Table 3.1.G-4. Effect of varying concentration of T-80 on the first order rate constant of glycine at 298°K.**

[Gly] = 0.2M

Medium = Acidic

[Oxi] = 0.8x10<sup>-4</sup> M

[H<sub>2</sub>SO<sub>4</sub>] = 2M

Time (in min)	O.D.			
	[T-80] %			
	4 x 10 <sup>-3</sup>	8x10 <sup>-3</sup>	1.2x10 <sup>-2</sup>	106x10 <sup>-2</sup>
0.0	0.135	0.135	0.135	0.135
2.0	0.125	0.118	0.110	0.100
4.0	0.114	0.107	0.100	0.090
6.0	0.107	0.100	0.092	0.081
8.0	0.101	0.095	0.086	0.071
10.0	0.097	0.091	0.080	0.065
12.0	0.093	0.085	0.075	0.060
14.0	0.089	0.082	0.070	0.052
16.0	0.085	0.077	0.064	0.046
18.0	0.081	0.072	0.057	0.040
20.0	0.078	0.069	0.051	0.034
22.0	0.074	0.064	0.047	0.028
24.0	0.071	0.060	0.041	0.024
26.0	0.068	0.057	0.035	

28.0	0.064	0.054	0.030
30.0	0.060	0.052	
	$K_{\text{mean}} =$	$K_{\text{mean}} =$	$K_{\text{mean}} =$
	$3.876 \times 10^{-2} \text{ Sec}^{-1}$	$5.004 \times 10^{-2} \text{ Sec}^{-1}$	$6.393 \times 10^{-2} \text{ Sec}^{-1}$
	$K_{\text{graph}} =$	$K_{\text{graph}} =$	$K_{\text{graph}} =$
	$3.811 \times 10^{-2} \text{ Sec}^{-1}$	$4.499 \times 10^{-2} \text{ Sec}^{-1}$	$8.502 \times 10^{-2} \text{ Sec}^{-1}$

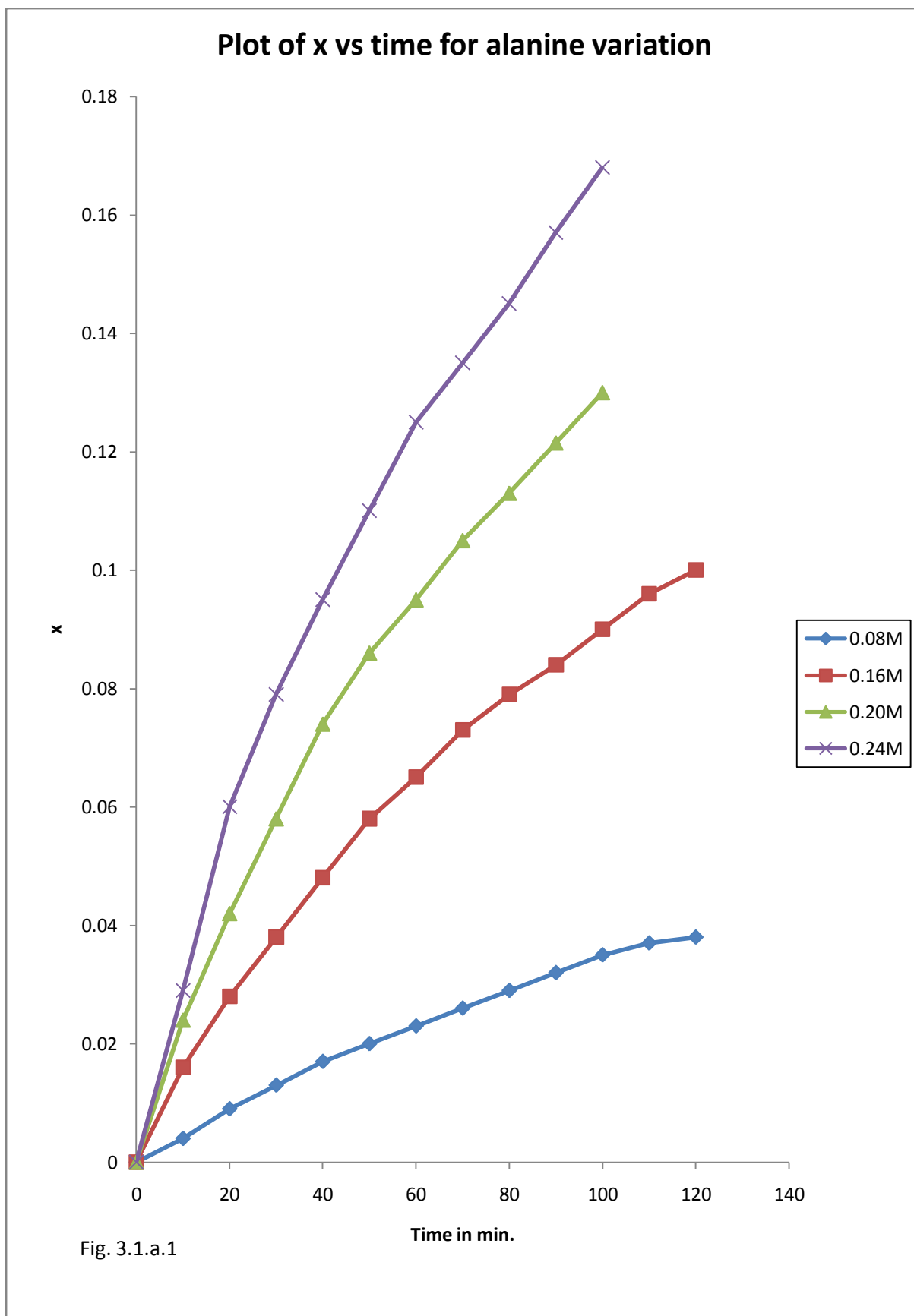
**Table 79.1.G-5. Effect of varying concentration of T-X-100 on the first order rate constant of glycine at 298°K.**

[Gly] = 0.2M  
 [Oxi] =  $0.8 \times 10^{-4}$  M  
 Medium = Acidic  
 [H<sub>2</sub>SO<sub>4</sub>] = 2M

Time (in min)	O.D.			
	[T-X-100] %			
	$4 \times 10^{-5}$	$8 \times 10^{-5}$	$1.2 \times 10^{-4}$	$1.6 \times 10^{-4}$
0.0	0.135	0.135	0.135	0.135
2.0	0.124	0.117	0.109	0.099
4.0	0.113	0.106	0.099	0.089
6.0	0.108	0.101	0.091	0.079
8.0	0.102	0.094	0.084	0.070
10.0	0.098	0.090	0.078	0.064
12.0	0.094	0.084	0.073	0.058
14.0	0.090	0.081	0.068	0.050
16.0	0.086	0.076	0.062	0.044
18.0	0.082	0.071	0.055	0.038
20.0	0.077	0.068	0.049	0.032
22.0	0.073	0.063	0.049	0.026
24.0	0.070	0.059	0.039	0.022
26.0	0.069	0.056	0.033	
28.0	0.063	0.053	0.028	

30.0	0.059	0.51		
	$K_{\text{mean}} =$	$K_{\text{mean}} =$	$K_{\text{mean}} =$	$K_{\text{mean}} =$
	$3.719 \times 10^{-2} \text{ Sec}^{-1}$	$4.834 \times 10^{-2} \text{ Sec}^{-1}$	$6.576 \times 10^{-2} \text{ Sec}^{-1}$	$8.931 \times 10^{-2} \text{ Sec}^{-1}$
	$K_{\text{graph}} =$	$K_{\text{graph}} =$	$K_{\text{graph}} =$	$K_{\text{graph}} =$
	$3.712 \times 10^{-2} \text{ Sec}^{-1}$	$4.814 \times 10^{-2} \text{ Sec}^{-1}$	$6.612 \times 10^{-2} \text{ Sec}^{-1}$	$8.899 \times 10^{-2} \text{ Sec}^{-1}$

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## Discussion of the Results

### Part - A: Kinetics of oxidation of amino acids in aqueous acidic, neutral and alkaline medium.

The methods and experimental results related with the kinetics of oxidation of amino acids and the stability constants of L-lysine and L-arginine at various experimental conditions are given in Chapters I and II.

Chemical kinetics is receiving considerable attention in recent years due to the role of anions, cations, micells and different catalysts in biological systems. It is also a known fact that the role of metal ions in biological systems is gaining significant attention since last few decades.

Keeping in mind the importance of amino acids, it was decided to undertake systematic study of oxidation of amino acids using some oxidants in aqueous acidic, neutral and alkaline medium.

The literature survey reveals that there is an ample scope for the title investigation. Thus in view of the role of metal ions and importance of amino acids in biological and medicinal research, the spectrophotometric and kinetic study of glycine, DL-alanine, DL-leucine, DL-aspartic acid, DL-glutamic acid, L-lysine and L-arginine in aqueous acidic, alkaline and neutral as well as in aquo-organic media has been undertaken.

Literature survey also reveals that the amino acids are playing a key role as Table 4.1.a-l.

complexing reactants and are useful in number of biological systems<sup>2</sup>. It was observed from the literature survey that the kinetics of oxidation of some amino acids has been studied in aqueous acidic medium using some anions and cations<sup>3-6</sup>. The order and rate of reaction has been a subject of study by many workers<sup>7-40</sup>.

However very little is known about the spectrophotometric and kinetics of oxidation of amino acids in presence of cation, anions, micelles and other catalysts.

### Kinetic measurements of oxidation of glycine by $\text{MnO}_4^-$ in aqueous acidic medium with varying concentration of oxidant.

The kinetics of oxidation of glycine by  $\text{MnO}_4^-$  has been reinvestigated by carrying out the kinetic runs in aqueous  $\text{H}_2\text{SO}_4$  medium at 298 K in order to get the data under identical experimental conditions. The measurements of absorbance with time are given in Table 3.1.G-1 a10ng with the first order rate constants. The results are in agreement with the literature values. However, slight differences are observed which may be accounted towards changes in experimental conditions. The kinetic measurements were carried out at different concentrations of glycine ranging from 0.08 M to 0.24 M, keeping constant concentration of  $\text{MnO}_4^-$  ( $8 \times 10^{-5}$  M) and also at different concentrations of oxidant with fixed concentration of substrate. The observed first order rate constants are given in table 4.1.a-l.

[Gly]	$K_{\text{obsd}} \times 10^{-2} \text{ min}^{-1}$	$[\text{MnO}_4^-]$	$K \times 10^{-3} \text{ min}^{-1}$
0.08 M	1.796	$4 \times 10^{-5}$ M	2.111
0.16 M	3.727	$8 \times 10^{-5}$ M	3.127



0.20 M	4.605	$12 \times 10^{-5}$ M	3.912
0.24 M	5.343	$16 \times 10^{-5}$ M	4.951

The system has also been studied in presence of  $\text{Br}^-$  ion. The bromide ion catalysed rate constants are given in table 4.1.a-2.

**Table 4.1.a-2.** Effect of varying bromide ion concentration on the rate of oxidation.

[Gly] = 0.2 M,	$[\text{MnO}_4] = 8 \times 10^{-5}$ M,	T = 298 K <sup>o</sup>
[Br <sup>-</sup> ]	$K_{\text{obsd.}} \times 10^{-2} \text{ Sec}^{-1}$	
	0.7587	
$4 \times 10^{-5}$	1.279	
$8 \times 10^{-5}$	1.833	
$12 \times 10^{-5}$	2.530	
$16 \times 10^{-5}$		

The effect of surfactants on rate of reaction has also been studied. The results are summarised and are presented in table 4.1.a-3.

#### **Kinetics of oxidation of DL-alanine in aqueous acidic and alkaline medium:**

The kinetics of oxidation of DL-alanine system was studied in aqueous acidic and

alkaline medium. The kinetic measurements made are given in chapter III. The observed data for first order rate constant at different experimental conditions are depicted in tables 4.1.a-4 and 4.1.a-5.

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