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The Infleunce of the Substituent on the Kinetic of Amino Acids Oxidation by Permanganate Ion

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الخلاصة

تضمن هذا البحث دراسة حركية اكسدة تسع من الاحماض الامينية وهي الكلايسين والالانين والفالين والليوسين والنورليوسين والسيرين والثريونين والاسبارتك واللاسين وذلك باستخدام ايون البرمنكنات كعامل مؤكسد. وقد اظهرت النتائج ان عملية الاكسدة تحدث بطريقتين الاولى غير محفزة وتعتمد سرعتها على تركيز الحامض الاميني والعامل المؤكسد والثانية محفزة ذاتيا بالناتج ثاني اوكسيد المنغنيز (MnO_2) وسرعته تعتمد على تركيز الحامض الاميني والعامل المؤكسد والمحفز الذاتي. ومن اعتماد ثوابت السرع على درجة الحرارة تم حساب الدوال الثرموداينميكية للتفاعل ومناقشتها.

في هذا البحث اعطيت اهمية خاصة لتاثير تركيب المجموعة المرتبطة بالكاربون الكيرالي على حركية وميكانيكية تفاعل الاكسدة. وقد اعطت النتائج الحركية دلائل تؤكد صحة الميكانيكية المقترحة للتفاعل. كما ان دراسة تفاعل الاكسدة في اوساط حامضية مختلفة مكنتنا من حساب ثابت معدل سرعة تفاعل الاكسدة للايون السالب والايون ثنائي الشحنة للحامض الاميني.

ABSTRACT

The kinetics of the oxidation of nine amino acids (glycine, alanine, valine, leucine, norleucine, serine, threonine, spartic and lysine) by permanganate ion were studied in an aqueous medium. The results showed that the oxidation reaction proceeds through two different pathways. The first is noncatalyzed and the second is autocatalyzed by the colloidal inorganic product (MnO_2). The rate of the noncatalyzed reaction is first-order in concentration of both amino acid and oxidizing agent, while the autocatalyzed reaction is first-order in concentration of amino acid, oxidizing and autocatalytic agent. Depending on the relation between the rate constant and temperature the thermodynamic parameters for the two pathways were calculated. A special care has been given to the effect of the structure of the group attached to chiral carbon atom of the amino acids on the rate and mechanism of oxidation reactions. The

kinetic results gave evidences supporting the proposed mechanism for the reaction. On the other hand, by measuring the rate of oxidation at different pH medium, the rate constant of oxidation of ananionic and zwitterionic form are calculated.

INTRODUCTION

The kinetic studies of amino acids oxidation by common oxidants have been given a large concern because of their biological significance[1-4,12]. One of these studies[5] is the oxidation by permanganate ion, which has been shown[4] that these reactions are started by electron transfer from the amino acids to the permanganate ion in the rate-determining step followed by other fast steps leading to the product as shown in scheme (1).

$$RCH(^{+}NH_3)COO^{-} + MnO_4^{-} \xrightarrow[k_1]{\text{slow step}} RCH(^{+}NH_3)COO^{\cdot} + MnO_4^{2-}$$

$$\xrightarrow[\text{fast steps}]{MnO_4^{-}} RCHO + NH_3 + CO_2 + MnO_2 \text{ (colloidal)}$$

Scheme (1)

It has been found that these reactions are first order in the concentration of both amino acid and oxidizing agent as shown by equation (1).

$r = k_1 [AA][MnO_4^{-}]$ 1

Where k_1 is rate constant and [AA] stands for the amino acid concentration. After that the reaction is autocatalyzed by manganese dioxide resulting from the reaction which is present in the solution as colloidal particles absorbs the amino acids on its surface and catalyzed the oxidation reaction by acting as autocatalytic agent. The mechanism of autocatalyzed reaction is similar to the mechanism of noncatalyzed reaction[5], the only difference, that reaction in the autocatalyzed process occurs between adsorbed amino acid and permanganate ion in the solution as summarized in scheme (2).

$$\underset{\text{(colloidal form)}}{(MnO_2)_n} + \text{Amino acids} \longrightarrow \text{Adsorbate}$$

$$\text{Adsorbate} + MnO_4^{-} \xrightarrow[K_2]{\text{slow step}} \text{intermediate} \xrightarrow{\text{fast}} \text{product}$$

Scheme (2)

The rate for the autocatalyzed reaction can be represented by equation 2.

$r_n = k_2 [Adsorbate][MnO_4^-]$ ..... (2)

On the other hand relation between the concentration of adsorbate, colloidal MnO_2 and amino acids at equilibrium can be extracted from Freundlich adsorption isotherm as shown in equation (3).

$[Adsorbate]/[MnO_2] = a [AA]^b$ ..... (3)

where a and b are adsorption constants.

So that the rate of autocatalyzed reaction can be represented by equation (4).

$r_a = ak_2[MnO_4^-][MnO_2][AA]^b$ ..... (4)

According to the above assumption the total rate for the oxidation reaction by permanganate ion can be represented by equation (5).

$r = k_1[MnO_4^-][AA] + ak_2[MnO_4^-][MnO_2][AA]^b$ ..... (5)

Since the reaction is started by the electron transfer from the amino acids to the oxidizing agent. One can anticipate that the electron density at the chiral carbon atom of the amino acids plays an important role on the rate of oxidation. Owing to the fact that the electron density at the chiral carbon atom is highly affected by the nature of alkyl group attached to it. The present work is concerned with the oxidation of amino acids having different structure by permanganate ion, in order to asses the effect of R-group attached to chiral carbon atom on the rate and mechanism of the oxidation reaction. On the other hand Arrhenius equation is employed to calculate the thermodynamic parameters which are utilized in the discussion of the reaction mechanism.

EXPERIMENTAL

Materials: All amino acids, $KMnO_4$, KH_2PO_4, K_2HPO_4 and all other chemicals used in this work were purchased from Fluka, G.A. and B.D.H. Chemical Ltd.

Measurement of permanganate ion concentration:

The inorganic product (MnO_2) resulting from the reaction absorbs light at λ_{max} of the permanganate ion (526 nm). So that it is necessary to correct the absorbance of permanganate ion at (526 nm) by measuring the absorbance of the mixture at 418 nm (where only the product absorbs light). After that the concentration of permanganate ion was calculated using equation (6).

$$C = \frac{A^{526} - A^{418}.E_p^{526}/E_p^{418}}{E_R^{526}} \quad \ldots\ldots (6)$$

Where the subscripts R and P stand for the reactant and product respectively. The extinction coefficient of permanganate ion E_p at 526 is

equal to 2.4×10^3 $mol^{-1}.dm^3.cm^{-1}$, and the extinction coefficient for the product at 418 and 512 nm (E_p^{418} and E_p^{512}) can be measured from the final absorbance of the reaction mixture at these two wave lengths.

The kinetic measurements: A buffer solution of K_2HPO_4 and KH_2PO_4 was used to keep the pH of the medium constant and preventing manganese dioxide from precipitation during the reaction course. The permanganate ion concentration was chosen as a rate monitoring species, which has been followed spectrophotometrically using Shimadzu UV-210 double beam spectrophotometer equipped with a digital printer DP 802. The temperature of the cell was controlled by circulating water in its outer jacket from the a thermostat of type Julaboo Pattern PT 40 PS. The pH of the solution was measured using pH-meter Orion type. A linear regression method was employed to calculate the rate constants and the activation energies for the reaction. Correlation coefficient not less than 0.96 is accepted.

RESULTS AND DISCUSSION

The kinetic treatments: The kinetic measurements were carried out under pseudo first-order condition, where the amino acids concentration is always ten times greater than the concentration of permanganate ion. The reactions were followed by monitoring the permanganate ion concentration spectrophotometrically. The total rates (r) for the reaction at different times were calculated using equation (7) which provides a simple and accurate method[7] for this purpose.

$$\left.\begin{aligned} r_2 &= C_3 - C_1 / t_3 - t_1 \\ r_3 &= C_4 - C_2 / t_4 - t_2 \end{aligned}\right\} \ldots\ldots\ldots (7)$$

where C stands for the concentration of permanganate ion. The calculated rate values for oxidation of thereonine, as representative results, are tabulated in Table (1).

Table (1): The kinetic data for oxidation of threonine by $KMnO_4$ at pH = 7.43 and 30 °C

Step No.	t/min	A (418 nm)	A (526 nm)	$C \times 10^{-5}$ mol/dm^3	$r \times 10^6$ mol.dm^{-3}.min^{-1}	$r/c \times 10^2$ min^{-1}
1	0	0.084	0.545	21.67	-	-
2	1.5	0.104	0.529	20.76	7.9	3.81
3	3	0.117	0.498	19.30	8.6	4.46
4	4.5	0.154	0.482	18.18	10.4	5.74
5	6	0.175	0.440	16.17	11.7	7.26
6	7.5	0.240	0.432	14.66	11.00	7.51
7	9	0.280	0.392	12.87	10.84	8.44

At high concentration of amino acids the rate equation (5) is reduced to equation (8).

$r = k_nC + k_AC(C_o - C)$ ..... (8)

where k_n and k_A stand for the rate constants for non and autocatalyzed reactions respectively.

(where $k_n = k_1[AA]$ and $k_A = ak_2[AA]^b$) and C stands for the concentration permanganate ion.

By rearranging of equation (8), one can get equation (9).

$r/C = k_n + k_AC_o - k_AC$ ..... (9)

A plot of r/C against C gave a linear relationship up to 75% of the reaction as shown in Figure (1), which confirms the validity of equation (9). From the slope and the intercept the values of k_n and k_A were evaluated and summarized in Table (2).

Table (2): The calculated rate constants for oxidation reaction by permanganate ion at temperature 303 °K and pH = 7.43

Amino acids	Structure	K_n $min^{-1} \times 10^4$	K_A $M^{-1}.min^{-1}$
Glycine	H-CH(NH_2)COOH	4.97	75.81
Alanine	CH_3-CH(NH_2)COOH	40.44	43.78
Valine	$(CH_3)_2$CH-CH(NH_2)COOH	11.43	22.40
Leucine	$(CH_3)_2$CH-CH_2- CH(NH_2)COOH	38.41	30.47
Norleucine	$CH_3CH_2CH(CH_3)$-CH(NH_2)COOH	35.20	51.45
Phenyl alanine	Ph-CH_2-CH(NH_2)COOH	16.03	176.22
Serine	HOCH_2-CH(NH_2)COOH	78.03	231.93
Threonine	CH_3CH(OH)-CH(NH_2)COOH	414.00	605.00
Spartic	HOOC-CH_2-CH(NH_2)COOH	292.40	347.20
Lysine	$NH_2(CH_2)_4$-CH(NH_2)COOH	403.00	601.20

Effect of amino acids structure: Referring to Table (2), the values of the rate constants for the amino acids oxidation in the non-catalyzed reaction are increased in the following order.

Glycine < valine < norleucine < leucine < alanine

A careful look at the structure of the above amino acids reveals that they are different in the structure of R-group attached to the chiral carbon atom where R equal to H, CH_3-$CH(CH_3)$-, CH_3CH_2-$CH(CH_3)$-, CH_3-$CH(CH_3)$-CH_2 and CH_3 respectively. It is clear that in all cases the replacement of the hydrogen atom (for glycine) by alkyl group facilities the oxidation rate of the reaction. This result is attributed, in general, to the fact that the presence of alkyl group increases the electron density at reaction centre and enhances the electron transfer from amino acid to oxidizing agent i.e. facilitates the oxidation rate. From the order of the rate constant one can observe that the values of the rate constant for both valine and norleucine are smaller than the values of alanine. These decreases may be resulting from the bulk of the isopropyl and isobutyl

group which decreases the plannarty of the molecule and decreases the stability of the resulting radical leading to a decrease in the oxidation rate. On the other hand, the electron densities at the chiral and carbonyl carbon atoms are theoretically calculated using the theoretical programme "Chem Office, 2002" of Cambridge Univ. The result shows that the electron density at the chiral carbon atom increases approximately in the same order as the reaction rate as shown in Table (3).

Table (3): The theoretical calculated charge densities at chiral carbon and carbonyl atom of some amino acids

Amino acid	Charge density at chiral carbon atom	Charge density at C=O atom
Alanine	4.061	6.602
Leucine	4.049	6.601
Valine	4.048	6.597
Isoleucine	4.043	6.604
Glycine	3.986	6.602

On the same bases a comparison between the rate constans for non-catalyzed oxidation reaction for alanine, serine, threonine, spartic and lysine [where R group are CH_3, CH_2OH, CH_3CHOH, $HOOCCH_2$ and $(CH_2)_4$-NH_2 respectively] reveals that the presence of these groups activate the oxidation rate by increasing the electron density at the reaction centre. Conversely the replacement of CH_3 group in alanine by Ph-CH_2 (phenyl alanine) is accomplished by a pronounce decrease in the oxidation rate (Table 2) revealing that the phenyl group deactivates the oxidation by decreasing the electron density at reaction centre. These results validate the proposed mechanism for the oxidation reaction that the main factor affecting the rate is the availability of electrons at the reaction centre.

For autocatalyzed oxidation reaction the constant (k_A) for glycine, alanine, valine, leucine and norleucine, showed no significant relationship between them. These abnormal results may be due to the fact that the value of (k_A) is highly affected by the value of Freundlish constant as shown in equation (3). So that the effect of structure may be compensated by the effect of adsorption constants.

On the other hand, the oxidation rate constant for alanine, serine, threonine, spartic and lysine reflects the fact that presence of OH, COOH or NH_2 activates reaction in the same way as seen in the noncatalyzed reactions. This result can be explained by the fact that the values of Freundlich constant are almost having similar effects in all cases and do not tolerate the effects resulted from the difference in the structure of the amino acid. This result supports the idea that the two pathways may be proceed through the same mechanism as proposed in literature[5].

Effect of temperature: At temperatures ranging between (298-318 °K), where the rate of decomposing of the oxidizing agent in negligible. The temperature dependences of the rate constants is investigated. Application of Arrhenius equation and a plot of lnk against 1/T gave a straight line (as shown in Figures 2 and 3). This result allowed us to calculate the activation parameters for the non-and autocatalyzed reaction which are gathered in Tables (3) and (4). The activation energies for the oxidation in all cases showed a decrease in their values as the rate constants increased which means that the reaction is mainly controlled by the energy barrier of the rate determining step. The negative values for the entropy of activation ΔS^* reflect that, the transition state is more arranged than the reactant. This result is consistent with the proposed mechanism in which the electron transfer from the amino acid to the oxidizing agent producing highly charged intermediate, i.e. the transition state is more arranged the reactant.

Table (4): The thermodynamic parameters of activations for non-catalyzed oxidation reaction at pH = 7.43

Amino acids	Ea KJ/mol	ΔH^* KJ/mol	ΔS^* KJ/mol	ΔG^* KJ/mol
Glycine	86.960	81.839	- 9.90	84.890
Alanine	67.530	62.165	- 78.90	87.890
Valine	75.673	72.987	- 107.60	107.742
Leucine	71.000	68.314	- 45.22	82.920
Norleucine	74.597	71.912	- 52.27	88.795
Phenyl alanine	78.475	73.437	- 29.474	82.367
Alanine	67.530	62.165	- 78.9	87.890
Serine	66.386	63.908	- 53.396	79.820
Threonine	36.510	34.074	- 137.28	84.832
Spartic	38.243	35.765	- 117.56	73.276
Lysine	34.310	31.916	- 135.32	73.282

Table (5): The thermodynamic parameters of activations for aut-catalyzed oxidation reaction at pH = 7.43

Amino acids	Ea KJ/mol	ΔH* KJ/mol	ΔS* KJ/mol	ΔG* KJ/mol
Glycine	73.700	68.550	- 26.47	76.570
Alanine	54.856	49.486	- 40.198	62.469
Valine	58.875	56.190	- 33.861	69.812
Leucine	64.610	61.916	- 27.49	73.489
Norleucine	63.953	61.351	- 25.00	69.176
Phenyl alanine	82.886	77.347	- 63.44	102.108
Alanine	54.856	49.486	- 40.19	62.469
Serine	56.00	50.440	- 25.06	58.224
Threonine	27.297	24.86	- 109.93	59.510
Spartic	36.082	33.604	- 85.81	61.655
Lysine	24.262	21.868	- 118.78	58.471

The effect of medium pH: At the pH range covered by the experimental ranging between (5.0-8.0) most of the amino acids present as zwitterionic form[9] $RCH(^{+}NH_3)COO^{-}$, while the concentration of the cationic form $RCH(^{+}NH_3)COOH$ and anionic form $RCH(NH_2)COO^{-}$ are too low to be oxidized. The concentration of anionic form increases with the pH raising and it's oxidation rate becomes as an important factor in the total rate of oxidation. According to the above assumption the total rate of non-catalyzed oxidation reaction results from the sum of two rates. The first one is the rate of oxidation of zwitterionic form and the second is the rate of oxidation of anaionic form. As shown in equation (9).

$$r_n = k_{HA}[MnO_4^-][HA] + k_A^-[MnO_4^-][A^-] \quad \ldots\ldots (9)$$

by comparison one can find that:

$$k_n = k_{HA}[HA] + k_A^-[A^-] \quad \ldots\ldots (10)$$

The concentrations of these two forms were calculated by supposing that the total concentration of the amino acid [AA] is always equal to the sum of all species present in the solution. The concentration of the zwitter ion [HA] and the anion $[A^-]$ are calculated using the equations (11) and (12).

$$[HA] = [AA][H^+]/(K_2 + [H^+] + \frac{[H^+]}{K_1}) \quad \ldots\ldots (11)$$

$$[A^-] = [AA]/(1 + \frac{[H^+]}{K_2} + \frac{[H^+]}{K_1K_2}) \quad \ldots\ldots (12)$$

where K_1 and K_2 are the dissociation constants for the cationic and zwitterionic forms respectively. The results of these calculations for glycine are summarized in Table (6). These results showed that at high concentration of the amino acids the value [HA] becomes practically constant and the relation between k_n and $[A^-]$ becomes linear as shown clearly from equation (10). The validity of this assumption is confirmed by plotting k_n against $[A^-]$ at different pH which gives a straight line as shown in Figure (4). From the slope and intercept the values of k_{HA} and k_{A^-} were calculated and found equal to (0.046) $M^{-1}.min^{-1}$ and (50.067) $M^{-1}.min^{-1}$ respectively. It is clear from Table (6) that the calculated values of the term $(k_{HA}[HA]+k_{A^-}[A^-])$ are similar to the experimental values of k_n which again prove the validity of the above assumption. Also the contribution percentage of the term $k_{A^-}[A^-]$ shows an increase in its value as the pH of reaction medium increase. On the other hand the value k_{A^-} is larger than the value of k_{HA} which reveals that the anionic form oxidized more faster than the zwitter ionic form. This result gave additional evidence supporting the proposed mechanism because the availability of the electron at the reaction centre in the anionic form is higher than zwitter ionic form. The same result has been noticed when the amino acids are oxidized by peroxomonosulphate[9-11].

Also at high pH there is a deviation from linearity this may be due to the increase in the reactivity of oxidizing agent as the pH of the medium increase.

Table (6): The rate constant of the non-catalyzed reaction at different pH values and respective contribution of each amino acid forms of glycine at total concentration equal to (0.05) M

pH	5.0	6.0	7.0	8.0
$10^2 \times K_n$	2.2	2.89	6.30	19.05
$10^6 [A^-]$	0.80	8.0	79.87	787.4
$10^2 \times [HA]$	4.988	4.998	4.991	4.921
$10^3 (K_{HA}[HA]+K_{A^-}[A^-])$	2.35	2.72	6.31	41.73

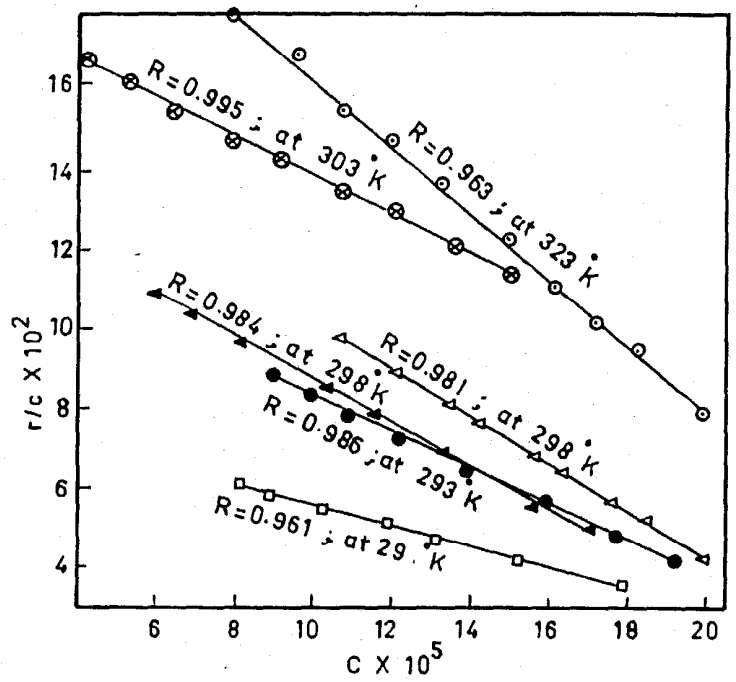

Figure (1): A plot of r/c versus c for oxidation of different amino acid by permanganate ion at pH = 7.43, ⊗ alanine; ⊙ valine; ⊡ spartic; △ threonine; ▲ **lysine; phenyl alanine**

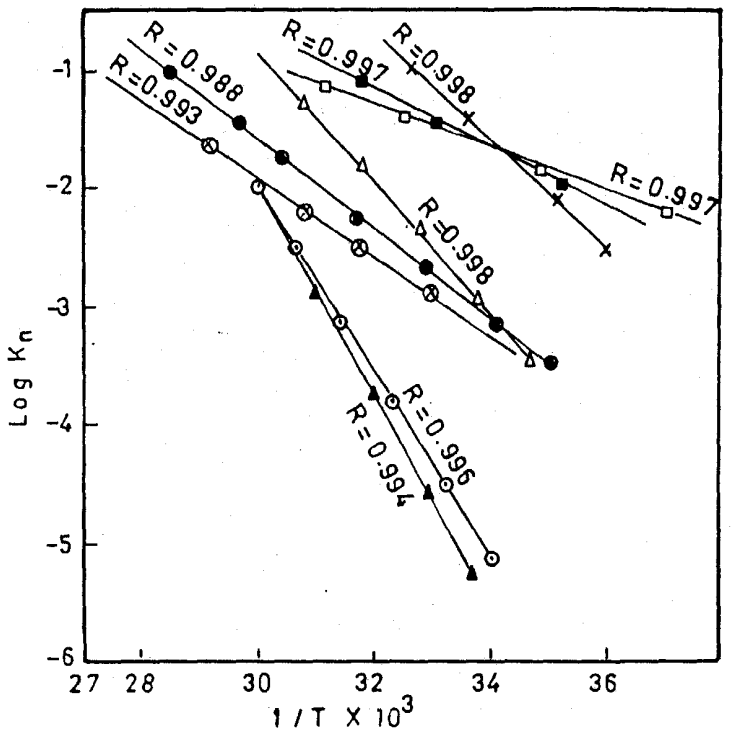

Figure (2): Arrhenius plots for noncatalyzed oxidation reaction; ⊡ **threonine;** □ **spartic;** ⊗ **valine;** • **alanine;** ○ **serine;** △ **glycine;** ▲ **phenyl alanine;** × **lysine**

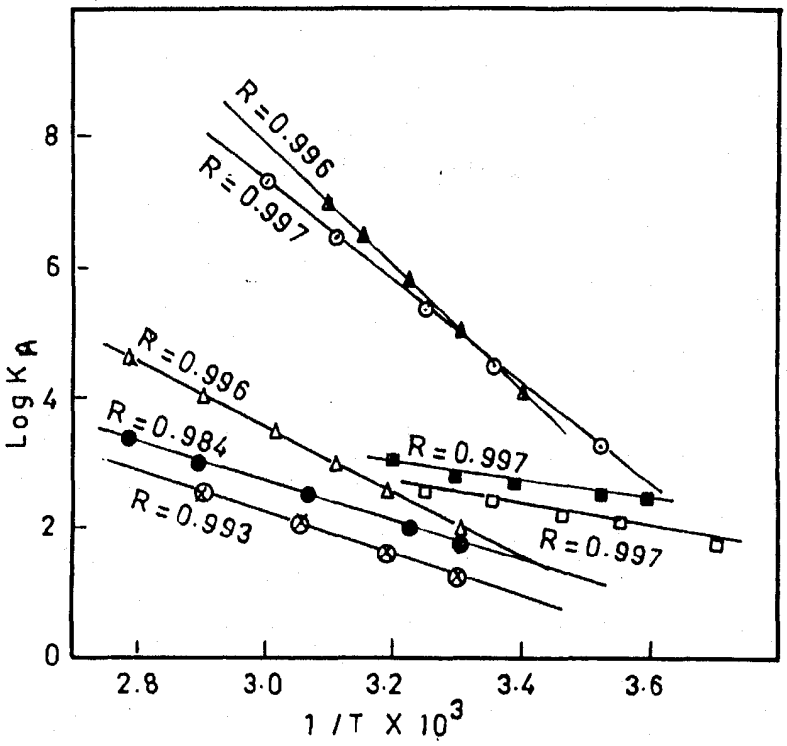

Figure (3): Arrhenius plots for autocatalyzed oxidation reaction; ⊡ threonine; □ spartic; ⊗ valine; • alanine; ○ serine; △ glycine; ▲ phenyl alanine

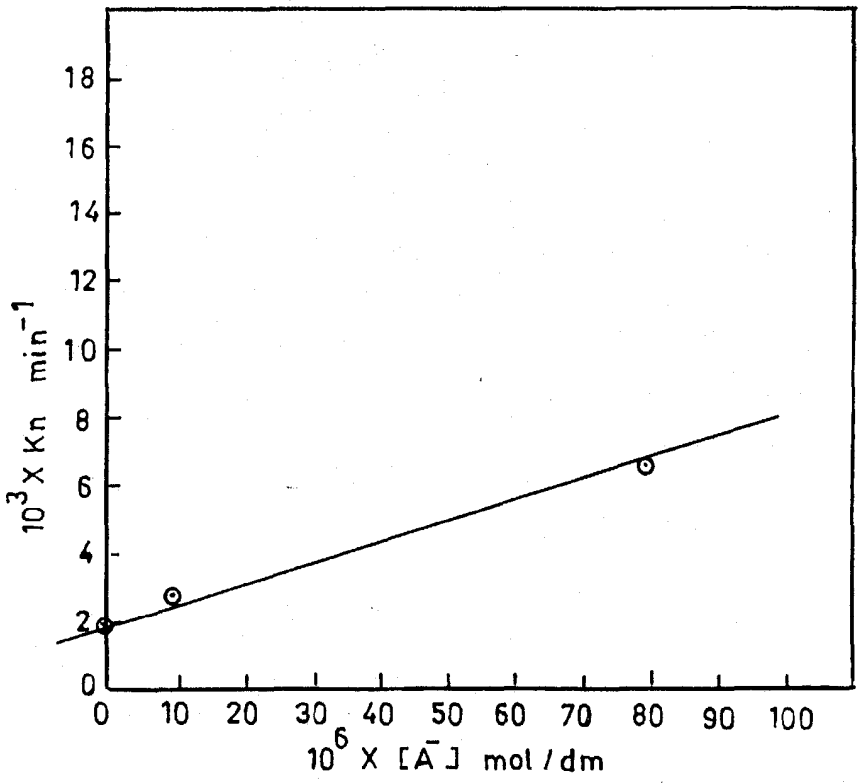

Figure (4): A plot of k_n values versus the anionic form concentration of glycine

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