Sindh Univ. Res. Jour. (Sci.Sr) Vol: 29 (2): 95-104 (1997)

ANALYTICAL IVESTIGATIONS OF NEWLY PREPARED COMPLEX OF ALANINE AND 8-HYDROXYQUINOLINE USING MULTIPLE SPECTROSCOPIC METHODS

M.M. Jat*, S.W. Shah*, T.G. Kazi* and G.H. Kazi**

National Centre of Excellence in Analytical Chemistry, Jamshoro-76080, Sindh Pakistan

Abstract

Alinine occurs in body fluids in considerable amounts and is involved in various metablolic functions, such as together with Glycine it makes a considerable fraction of the amino nitrogen in human plasma. The use of 8-hydroxyquinoline (Oxine) for identification and analysis of amino acids on TLC plates and flurometerically in nano mole quantity has already been reported by new methods accomplished in our laboratories (1-3).

In present work Alanine-Oxine complex has been repared and its stoichiometric investigations has been carried out involving Spectrofluorometric, IR, NMR, TGA-DTA, Mass spectrometeric techniques.

The results revealed that alanine was stoichiometrically bound with 8-hydroxquinoline molecules in 1:2 ratio for amino acid to reagent.

Determination of λ_{max} of flourescent complex of alanine-oxine in UV region using TLC scanner and excitation and emission wavelengths by spectrofluorometer were found to be of immense importance for estimation of amiro acids down to nano mole range.

Introduction

Alanine always occur in body fluids in essential amounts. In diabetic mellitus and other diseases, increased level of alanine was accomponied with increased amount of pyruvate. No adequate specific colorimetric or titrimetric procedure for the direct estimation of alanine has yet been devised. Indeed, the determination of alanine in a mixture of amino acids, e.g., protein hydrolysate is based almost exclusively upon the use of non specific degradation methods to convert amino acid to acetaldehyde which, in turn may be estimated quantitatively and hence provide an indirect measure of the amount of alanine intially present. Alanine can be quantitated by an enzymatic microfluorometric method.[1]

** Institute of Chemistry, University of Sindh, Jamshoro-78060, Sindh, Pakistan

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Experiment

Preparation of solutions

- 0.01 M Acetic acid = 4.5 ml of glacial acetic acid diluted upto 1000 ml with deionized water.
- 0.01 M 8-hydroxyquinoline = 1.45 g of 8-hydroxyquinoline dissolved in 0.01 M acetic acid made upto liter.
- 0.01 M Methionine = 0.089 g alanine dissolved in 100ml volumetric flask in 0.01 M acetic acid.

Preparation of Alanine and Oxine complex complex for spectrofluorometric study:

Equal volumes of 0.01 M Alanine soluion i.e. 0.2ml of 0.01 M alanine were pipetted out ion several labelled concial flasks and known volumes of oxine solutions were added to these flasks to maintain ratio of alanine: oxine :: 1:1, 1:2, 1:3, 1:4 and so on.

The flasks were covered with watch glasses and the solutions in labelled flasks were heated to reflux for one hour, removed the watch glassess and evaporated to semi dried residue which was subject to recrystallistaion to obtain light yellow product. Equal volume of 0.01 M acetic was added to dissolve the complex in respective flasks. The resultig transparent and clear solution was examined spectroflourometrially to determine excitation and emission wave lengths (λ Ex. And λ Em) of alanine - oxine complex and detection linit of newly prepared complex.
(table 1)

For other analytical investigation alanine-oxine complex was prepared on same procedure as above. For structural elucidation of alanine-oxine complex by physico-chemical methods involving SPF, TGA, IR, UV-VIS, CH N, NMR, and mass spectrometry have been used to established stoichiometry.

Results and discussion

Infrared spectra of Alanine, Oxine and complex.

Infrared spectra of alanine, 8-hydroxyquinoline (Oxine) and newly prepared complex were recorded by Hitachi model 260-59 in KBr, over the range of 4000 to 250 cm^{-1}. Alanine show bands at 1580 (antisymmetric stretching of COO - and 14400 cm^{-1} (symmetric stretching of COO)

The finger print comparison fo three spectra viz: of the reactants and product showed that frequency bands of COOH and >NH groups in alanine at 1580 and 1500 cm^{-1} of and frequency band 8-hydroxyquinoline at 1585 cm^{-1} are affected due to complexation. Also comparisio of the IR spectra of comples with free 8-hydroxyquinolinol indicated that - OH frequency in oxine molecule in the

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region of 1385 cm^{-1} was affected due to complexation in complex freqeuency bands at 920, 870, 700, 450 cm-1 are due to quinolinol - CH rocking and were no present in alanine.

(Comparison of three spectra seen in table 3). The spectral evidence supports presence of complex of definite composition.

Proton magnetic resonance spectrum of Alanine-Oxine complex.

PMR of alanine - oxine complex was run on high resolution 300 MHZ NMR Spectrometer and following major chemical shift were noted.

At 1.25 ppm shift for >NH protons of amino acid were noted. For CH_2 of alanine shift appear at 2.2 ppm. At 4.2 ppm, shift for methylene group attached to -COOH and also due to aromatic ring attached to CH_2-COOH group. From 0.7 to 8.0 ppm due to resonance of phenolic proton. Between 8.0-93 ppm, proton resonance is obtained due to heterocyclic ring oxine molecule.

Mass spectrometry fo Alanine - Oxine complex

Mass spectrum of alanine-oxine was obtained at low resolution on mass spectrometer. The zwitter ionic character of amino acids lead to very low vapour pressure and hampered the study of free amino acids into simple derivatives, which would exhibit a sufficient vapour pressure to be analyzed by conventional techniques.

Taking into consideration 1:2 composition of oxline and alanine and p = 344 fragmentation pattern was noted. (see in table)

Thermogravimetric and Differential Thermal Analysis for Cleavage and Stoichiometric Analysis.

The thermogravimetric analysis and differential thermal studies of 8-hydroxyquinolinol, alanine, and the stoichiometrically prepared comples were accomplished using shimadzu system DT 30-B. The sample weight in the range of 5-20 mg and temperature interval of 10°-500°C under nitrogen stream were examined.

1. **Thermogram of Alanine**: Alanine was a volatile compound, TG curve showed 100% loss in one step.

1. Alanine, between 190° - 260° C 100% loss of sample weights was observed.

2. **Thermogram of Alanine - Oxine complex.**

On the basis of 1:2 stoichiometry the TG / DTA interpreted as follow.

1. Between 100 - 170° C loss was 60% of sample weight (corresponding to 207 out of 344 of molecular weight of complex), probably one molecule of oxine with alanine.

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2. In second stage between 170 - 260°C a loss of 38% of sample weight (corresponding to 131 out of 344 molecular weight) probably second molecule of oxine.

DTA curve of alanine at 200° - 280° C exothermic peak showed melting followed decomposition. DTA of complex showed three exothermic peaks at 120° to 280° showed change in crystal lattice melting, decomposition and volatility of complex.

Elemental Analysis for Percentage of Hydrogen, Carbon and Nitrogen

Percentage of C, H and N in alanine, 8-hydroxyquinoline and complex were determined by Yanaco, MT-3 high speed analyzer. Experimental results are in agreement with the theoretical results and conform that the stoichiometric product is comprised of one molecule of alanine and two molecules of 8-quinolinol reagent.

Referecnes

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TABLE 1

DETERMINATION OF EXCITATION AND EMISSION LINES, AND DETECTION LIMIT OF ALANINE - 8 - QUINOLINOL BY SPECTROFLUOROMETER SHIMADZU - RF 510

Experimental parameter :-

Excitation wavelength = 330 nm.

Emission wavelength = 420 nm.

S.No	Amino acid (ml)	Oxine (ml)	Mole ratio	Fluorescence Intensity *	Remarks
1.	0 . 2	0 . 2	1 : 1	56	1:3 mole ratio is confirm stoichiometric complex formation in solution form have maximum fluorescence intensity.
2.	0 . 2	0 . 4	1 : 2	116	
3.	0 . 2	0 . 6	1 : 3	141	
4.	0 . 2	0 . 8	1 : 4	134	
5.	0 . 2	1 . 0	1 : 5	127	

Note :- At Ex. 330 and Em. 420 nm the-reactants, Oxine , Alanine and Acetic acid showed no fluorescence activity.

* Fluorescence intensity determined for working solution prepared from 0.2 ml of 0.01M amino acid with different ratio of oxine diluted upto 25 ml.

DETECTION LIMIT

Sr No	Sample solution conc.(M)	Ex. 330 nm. B.W	Em. 410 nm B.W	% fluore-scence	Blank	FSD (x) mv	chart div.	gain	Detection limit	
									µ mol/ml	ppm
1.	(B)	10	10	70	1	100	70	1		
2.	(C)	20	40	82	40	100	82	2	1.0×10^{-5}	0.0089
	---	-	-	260	120	500	55	5		
3.	(D)	-	-	132	120	200	66	5	Theoretical detection limit	
	---	-	-	267	235	500	54	10	4.1×10^{-7}	
	---	-	-	548	500	1000	55	20		

Key :- Dilution steps :

Stock solution = 0.089 g. Alanine / 100 ml = 0.01 M. (A)

1. 0.2 ml of A / 50 ml = 4.0×10^{-5} M (B).
2. 1.0 ml of B /100 ml = 4.0×10^{-7} M (C).
3. 5.0 ml of C / 20 ml = 1.0×10^{-7} M (D).

TABLE 2

INFRARED SPECTRAL BAND ASSIGNMENTS OF 8-QUINOLINOL , ALANINE AND ALANINE -8-QUINOLINOL COMPLEX.

S. No.	Bands cm-1	Alanine cm-1	8-Quinolinol cm-1	Complex cm-1	Remarks on complex
1.	3150	+	–	+ (b)	
2.	3050	–	+	+ (b)	Broad band due to -CH
3.	2800	+	–	+	-CH Stretching and
4.	2100	+	–	+	-NH band
5.	1640	–	–	+ (sh)	
6.	1600	+	+	+ (s)	
7.	1585	–	+	–	
8.	1490	–	+ (w)	+ (b)	New broad band due to
9.	1410	+	+	+	complex.
10.	1385	–	+	–	
11.	1360	+	–	+ (s)	The complexation
12.	1300	+	–	+ (s)	involve - OH
13.	1270	–	+	–	
14.	1220	+	+	+	
15.	1160	–	+	+	
16.	1120	+	–	+	
17.	1020	+	+ (w)	+ (s)	strong band.
18.	970	–	+	+ (w)	
19.	920	+	–	+ (s)	Bands between 930 and
20.	870	+	+	+	870 due to aromatic
21.	820	–	+	–	resonance of complex
22.	780	+	+	+	
23.	760	+ (w)	+	+ (s)	
24.	730	–	+	–	
25.	700	–	+	+ (s)	
26.	620	–	+	+	
37.	540	+	+	+	
28.	500	+ (w)	–	+ (s)	

Key:-

w= (weak) , s (strong) , and (b)(braoad).

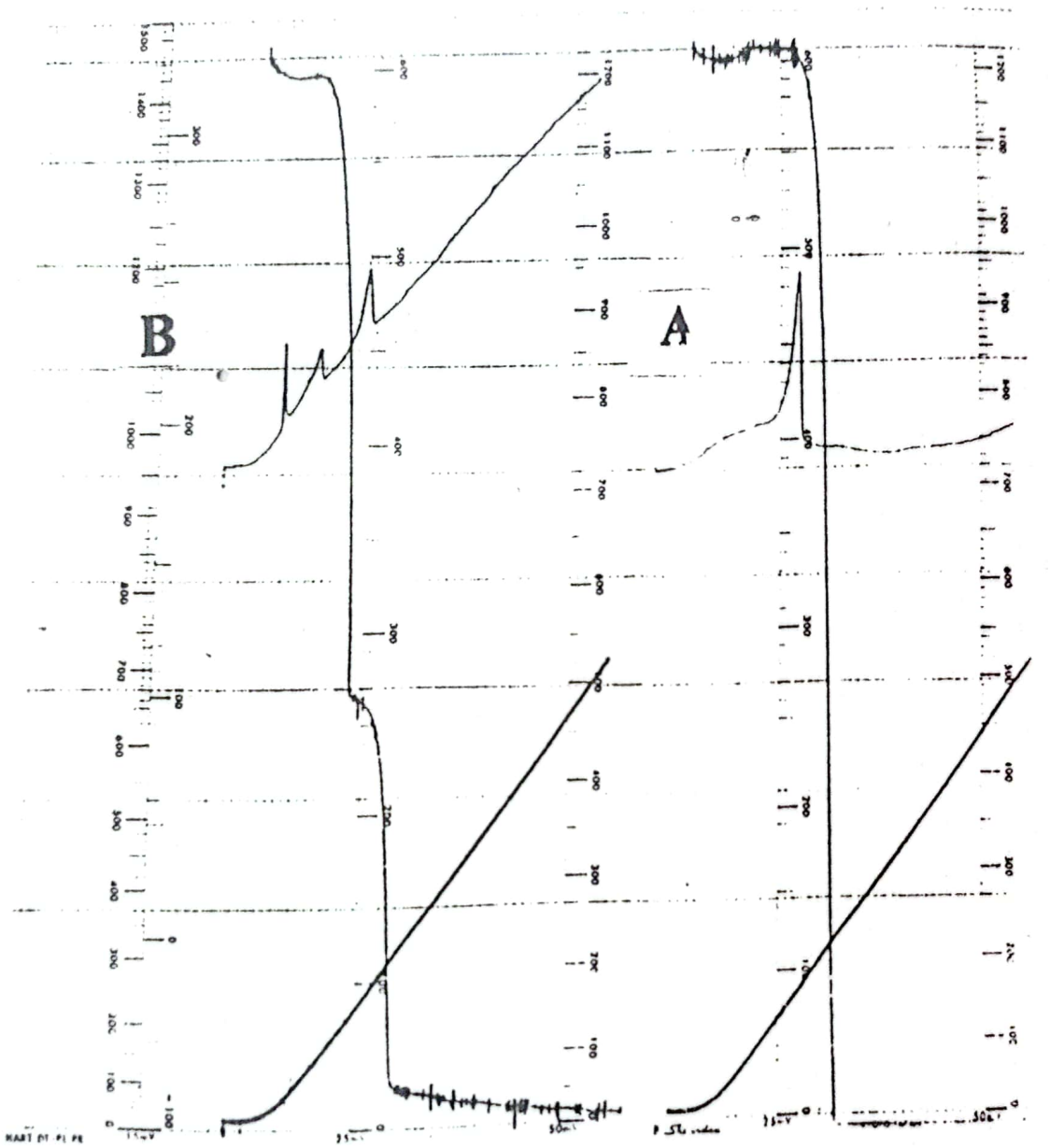
TABLE .3

ELEMENTAL ANALYSIS OF ALANINE , 8-QUINOLINOL, ALANINE - 8 - QINOLINOL COMPLEX BY ELEMENTAL ANALYZER YANACO, MT - 3

S.NO.	Samples	Weight µg	Theoretically calculated %	Experimentally found %	Mean
1.	Oxine	2345	H = 4.8222 C = 74.4006 N = 9.6445	H = 4.7598 C = 75.009 N = 9.6985	H = 4.7788 C = 74.9820 N = 9.6917
		2498		H = 4.7980 C = 74.9550 N = 9.6850	
2.	Alanine	2126	H = 8.9880 C = 40.4490 N = 15.7300	H = 9.1200 C = 39.7850 N = 14.9100	H = 9.1355 C = 40.1170 N = 15.2220
		2445		H = 9.1510 C = 40.1320 N = 15.5340	
3.	Alanine Oxine Complex	2465	H = 5.8000 C = 73.2500 N = 12.2000	H = 5.9080 C = 73.2364 N = 13.0560	H = 5.4940 C = 73.6214 N = 13.0300
		2543		H = 5.0850 C = 74.0064 N = 13.0040	

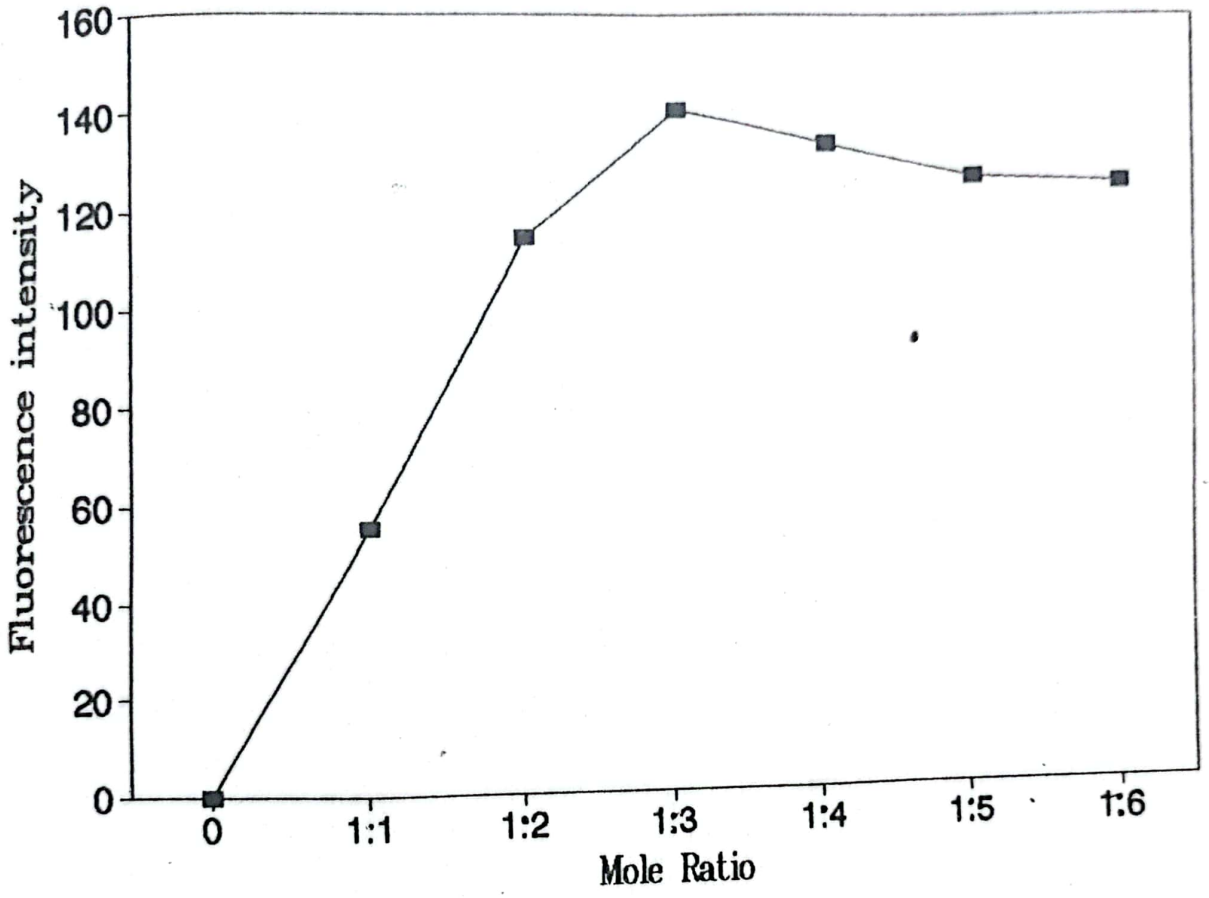
Alanine - Oxine complex confirm 1:2 stoichiometric composition

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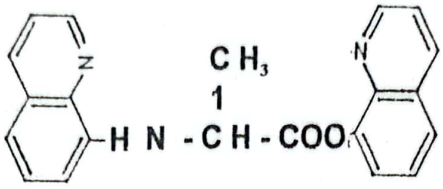
TG -DTA Thermograms
(A) Alanine
(B) Alanine - Oxine complex

PLOT OF FLUORESCENCE VS AMOUNT OF OXINE ADDED TO THE SOLUTION OF ALANINE



MASS SPECTROMETRY OF ALANINE - OXINE COMPLEX

ALANINE : Oxine :: 1: 2 ratio (P = 343)



S.No.	Ion Peak	% Intensity	(P - x)	Loss in Weigh	Remarks
1.	279	5	(343 - 279)	64	
2.	255	5	(343 - 255)	88	Bonds between alanine and oxine break
3.	231	5	(343 - 231)	112	
4.	197	5	(343 - 197)	146	Loss due to quinoline ring
5.	145	100	(343 - 145)	198	Peak at 197 m/e presents relates loss of quinoline ring from main skeleton
6.	117	72	(343 - 117)	226	Fragment of Oxine.
7.	89	34	(343 - 89)	254	Probably molecule of Alanine.
8.	74	35	(343 - 74)	269	Fragment of Alanine NH_2- CH - COOH or stable ring of benzene.
9.	57	26	(343 - 57)	286	Fragment of alanine.

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