Pak J Sci Ind Res 2002 45 (2) 133-139

Spectroscopic Studies of Fluorescent Complexes of Tyrosine-8-Hydroxyquinoline and Tyrosine-8-Hydroxyquinaldine in Aqueous Phase

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(Received 6 December 2000; accepted 12 April 2001)

A new method has been developed by preparing complexes involving condensation of tyrosine with 8-hydroxyquinoline (Oxine) and 8-hydroxyquinaldine (Quinaldine) respectively, producing fluorescent products. The products obtained have been investigated for identification and quantitative estimation using different spectroscopic techniques including fluorescence activity of newly synthesized products. 8-hydroxyquinaldine and 8-hydroxyquinoline (Oxine) condensed with tyrosine separately produced water-soluble fluorescent complexes. The complexes have been investigated for identification and quantitative estimation of amino acids. Identification of amino acids in nano mole or below than nano mole has become possible by present fluorometric activity of these complexes involving different excitation and emission wavelengths. The fluorometric activity of complexes has been observed to be 100 to 1000 times higher than assay method involving ninhydrin and amino acid analyzer.

The method adopted in our laboratory is rapid, versatile with good reproducibility and provides excellent results for adoption by analytical, agricultural and biomedical laboratories to estimate amino acids and metals in composite matrix.

Key words: Tyrosine, Fluorescent complexes, 8-Quinolinol, 8-Quinaldine.

Introduction

In present work new complexes not reported elsewhere have been studied exclusively in our laboratories by condensing tyrosine with oxine and 8-quinaldine separately. A detailed investigation has been carried out using multiple analytical techniques involving fluorometric and infrared spectroscopic studies are reported in this paper. While nuclear magnetic resonance and mass spectroscopic studies to be ivestigated. The use of fluorescence-based analysis is becoming of increasingly popular in many branches of chemical and biological science (Asrar *et al* 1985). It has been established from fluorescence activity of complexes that stoichiometry of complexes follows trend of 1:1, 1:2, 1:3, and so on at various mole ratios of amino acids oxine or quinaldine (Ligand) present in reaction. Derivatization to create fluorescence is widely used technique for the analysis of biological compounds or synthetic chemicals (Jakhrani *et al* 2000).

Detection limits of the fluorescence derivatives are usually of the order of nano to femto moles (Maqsooda *et al* 1990).

The condensation of all amino acids with 8-hydroxyquinaldine or its homologue 8-hydroxquinaldine produces products of definite stoichiometry. Above mentioned complexes have been prepared and extensively studied in our laboratories using spectrofluorometric method for their characteristic excitation and emission wavelengths. The optimisation of experimental conditions provides quantitative and detection limits for amino acids and their complexes at nano mole level (Jat *et al* 1997).

Tyrosine is complexed with 8-hydroxyquinaldine and 8-hydroxyquinoline respectively and stoichiometric investigations have been carried out. Determination of λ_{max} of fluorescent complex tyrosine using thin layer chromatographic scanner and excitation and emission wavelengths by spectrofluorometer were found to be of immense importance for estimation of amino acids down to nano mole or even below above range (Maqsooda *et al* 1993 and 1994).

Tyrosine is strongly resembles phenylalanine but it contains a hydroxyl group on the ring. The bulky ring gives the molecule a hydrophobic nature. Hence the hydroxyl group is polar and will readily interacts with water and the conjugated rings π electrons are also able to interact with other molecules containing π electrons. This residue can be found both at surface and interior of protein molecules (Shaikh *et al* 1997 ; Tasneem *et al* 1980).

Tyrosine complexed with oxine and 8-quinaldine separately shows fluorometric activity at its excitation and emission lines are at λ_{Ex} 350 and λ_{Em} 480 respectively.

Experimental

Preparation of solutions. 0.01 M tyrosine, 0.01M oxine and 8-quinaldine were prepared separately in 0.01M acetic acid solution in 250ml volumetric flask and stored as stock

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Table 1
Determination of excitation and emission wavelengths & stoichiometric investigation of Tyrosine-8-hydroxy-quinaldine complex

Sr. No.	Amino acid (ml)	8-hydroxy-quinaldine (ml)	Mole ratio	Fluorescence intensity
1	0.5	0.5	1:1	140
2	0.5	1.0	1:2	280
3	0.5	1.5	1:3	400
4	0.5	2.0	1:4	370
5	0.5	2.5	1:5	315

Experimental parameters response= Medium, Aver,Time = Isec, Scan Speed = 100nm/min, B. Wλ_{EX}=10nm B.W λ_{EM}=10nm B.W λ_{EM} =10nm, Gain =01, Excitation wavelength=350 nm, Emission wavelength=480 nm

Note: At the λ_{EX} 325nm and λ_{Em} 480, the reactants 8-hydroxyquinaldine, Tyrosine and Acetic acid showed no fluorescence activity. Fluorescence intensity determined for working solution prepared from 0.5ml of 0.01M amino acid with 8-hydroxyquinaldine diluted up to 25 ml.

solutions. All the chemicals and reagents used were of analytical grade.

Measurement of spectra. Fluorescence spectra were recorded using Shimadzu Model RF-510 Spectrofluorophotometer equipped with Xenon discharge lamp pulsed at line frequency (50Hz). All experiments were conducted using 1x1 cm rectangular quartz cell. Excitation and emission slit widths were fixed at 10nm for all experiments and varied for detection limits measurement only.

Preparation of Tyrosine-oxine and Tyrosine-8-quinaldine complexes. Equal volumes of 0.01M Tyrosine were pipetted out separately in labelled conical flasks and known volumes of 0.01M oxine and 8-quinaldine solutions were added separately to these flasks to maintain ratio of tyrosine to 8-hydroxyquinaldine or tyrosine to 8-hydroxyquinoline as 1:1, 1:2, 1:3, 1:4, and so on.

The flasks were covered with watch glasses and the solutions in flasks were heated to gentle reflux for one hr and then the watch glasses were removed and reaction mixture evaporated to semi dried residue which was dissolved in acetic acid subjected to recrystallisation and to obtain light yellow products.

Table 2
Detection limit of Tyrosine-8-hydroxyquinaldine complex

Sr. No	Amino acid solution Conc: (M)	λ 350 nm B.W	λ 480 nm B.W	Flu: Intensity	Blank	FSD (x) mV	Chart Div:	Gain
1	(A)	10	10	400	0	500	80	1
2	(B)	10	10	40	0	50	80	1
	-	-	-	110	25	200	55	2
	-	-	-	275	104	500	55	5
	-	-	-	570	230	1000	57	10
	-	20	40	860	0	1000	86	1
	(C)	10	10	325	50	500	65	10
	-	20	40	100	0	100	100	1
	-	-	-	250	32	500	50	2
	-	-	-	560	128	1000	56	5
3	(D)	20	40	120	0	200	60	1
	-	-	-	280	34	500	56	2
	-	-	-	630	137	1000	63	5
	-	-	-	1400	290	2000	70	10
		Detection Limit:	Experimental	= $2x10^{-4}$ μ moles/ml				
			Theoretical	= $3.60x10^{-7}$ μ moles/ml				

Experimental parameters response = Medium, Scan speed = 100 nm/min, Ave: Time = Isec

Key: Dilution steps. O.S =0.181 g Tyrosine/100 ml = 0.01 M, 0.5 ml of O.S is diluted up to 25 ml = (A) 2 x 10^{-4} M

Stoichiometric composition 1:3 Tyrosine 8-hydroxyquinaldine

I. 5ml of A/50ml = (B) $2x10^{-5}$ M

II. 5ml of B/50ml = (C) $2x10^{-6}$ M

III. 5ml of C/50ml = (D) $2x10^{-7}$ M

Table 3

Determination of excitation and emission wavelengths and stoichiometric investigation of tyrosine-8-hydroxy-quinoline complex

Sr.No.	Amino acid (ml)	8-hydroxyquinoline (ml)	Mole ratio	Fluorescence intensity
1	0.2	0.2	1:1	100
2	0.2	0.4	1:2	210
3	0.2	0.6	1:3	450
4	0.2	0.8	1:4	429
5	0.2	1.0	1:5	408

Experimental parameters response = medium, aver, time = Isec, scan speed = 100nm/min, B.W λ_{EX} =10nm, B.W λ_{EM} =10nm, Gain =01, Excitation wavelength = 325 nm, Emission wavelength =425 nm

Note At The λ_{EX} 325 and λ_{Em} 425 nm, the reactants, Oxine, Tyrosine and acetic acid showed no fluorescence activity.

Fluorescence intensity determined for working solution prepared from 0.2 ml of 0.01 M amino acid with Oxine and diluted to 25. 0 ml

Known volume of 0.01M acetic acid was added to each product to dissolve the complexes in respective flasks. The resulting transparent and clear solutions were examined for fluorescence intensity.

The excitation and fluorescence spectra were obtained at room temperature using Spectrofluorophotometer RF-510. For identification and quantitative studies, the excitation wavelengths were 350 and 325nm; emission wavelengths were 480 and 425 nm for respective complexes. Results are shown in Table 1 for Tyrosine-8-quinaldine complex and in Table 3 for Tyrosine-oxine complex.

Detection limits of newly prepared complexes were also determined using same method but with variable experimental parameter for maximum detection. Results are shown in Table 2 for tyrosine-8-quinaldine complex while that of tyrosineoxine is listed in Table 4.

For other analytical investigations such as Infrared spectroscopic study of the tyrosine-8-quinaldine and tyrosine-oxine complexes were prepared under same procedure as above and stoichiometry were established using above methodology. For IR study, samples were dried and measurement made on Hitachi 260-50 infrared spectrophotometer. The results are summarised in Table 5 and 6. Comparative studies of amino acid cmplexes with reagents oxine and 8-quinaldine indicated decrease in the fluorescence intensity of complex 8-quinaldine, as shown in Table. 7

Results and Discussion

Fluorometric study. The fluorescence studies in solution indicated 1:3 stoichiometry as shown by maximum intensity in most of the tyrosine complexes. In solution form ion pair, vanderwall forces and hydrogen bonding play an important role in association of extra molecule of free reagent with complexes and in most cases this is experimentally for maximum fluorescence intensity. However at 1:4 and 1:5 ratio of aminoacid to reagent fluorescence intensity of complexes persists or decreases gradually due to quenching effect.

Table 4

Detection limit of tyrosine-8-hydroxyquinaldine complex

Sr. No	Amino acid solution Conc: (M)	λ_{EX} 325 B.W	λ_{Em} 425 nm B.W	Flu: Intensity	Blank	FSD (x) mV	Chart Div:	Gain
1	(B)	10	10	450	1	500	42	1
2	(C)	-	-	166	16	200	83	2
3	(D)	20	40	124	16	200	62	2
	-	-	-	250	16	500	50	5
4	(E)	20	40	44	84	50	88	5
	-	-	-	120	84	500	56	10
	-	-	-	235	161	500	47	20
		Detection Limit:	Experimental	$= 8x10^{-6}$ μ moles/ml				
		Theoretical		$= 2.1x10^{-8}$ μ moles/ml				

Key:- Dilution steps: O.S. = 0. 181.0g. Tyrosine/100 ML = 0.01 M (A) Stoichiometric composition 1:3:Tyrosine:quinolinol

i. 0.2 ml of A / 25ml $= 8.0X\ 10^{-5}$ M (B)
ii. 1.0 ml of B / 100 ml $= 8.0\ X\ 10^{-7}$ M (C)
iii 1.0 ml of C / 10 ml $= 8.0\ X\ 10^{-8}$ M
iv. 1.0 ml of D / 10ml $= 8.0\ X\ 10^{-9}$ M (E)

Table 5

Infrared spectral band assignments for 8-hydroxquinoline, tyrosine and tyrosine-8-hydroxyquinoline complex

S.No.	Bands cm^{-1}	Tyrosine cm^{-1}	8-hydroxyquinoline cm^{-1}	Complex cm^{-1}	Remarks
1	3400	+	-	+(B)	Broad band due to -CH and -NH stretching
2	3200	-	+	-	
3	3050	+	+	+(B)	
4	2060	+	-	+	
5	1760	-	+	+	
6	1620	+(S)	-	+	
7	1585	-	-	+	New broad band -OH of oxine involve
8	1520	+	-	+	
9	1500	-	+	+(W)	
10	1480	-	+	+	
11	1460	+	+(W)	+	
12	1440	+(W)	-	-	
13	1420	+	+	-	
14	1400	-	+	-	
15	1385	-	+	+	
16	1360	+(S)	-	+	
17	1320	+(S)	-	+	
18	1280	-	+	+(B)	
19	1260	+	-	+	
20	1200	+	+	-	
21	1180	+	-	-	
22	1160	+	+(W)	-	
23	1140	-	-	-	
24	1090	+	+(W)	+(S)	The complexation involve -OH and -NH groups
25	1060	+(S)	-	+	
26	1040	+(S)	-	-	
27	1000	+(W)	-	-	
28	970	+	+(W)	-	
29	940	+(W)	-	+(W)	
30	890	+(S)	-	-	
31	870	+(S)	+	+(B)	
32	840	+(S)	+	+(W)	
33	820	+(W)	+(W)	+(S)	
34	800	+	+	-	
35	780	-	+	+	
36	760	-	+	+(S)	
37	740	+	+	-	
38	700	+	-	+(W)	
39	680	+	-	-	
40	650	+	+	-	
41	620	-	-	+(W)	
42	560	+(S)	-	+(W)	
43	480	+(S)	+(W)	+(W)	

Key. W= weak, S =strong and B= broad

The use of 8-quinaldine reagent for complexing and its comparison to oxine indicates decrease in fluorescence signal of 8-quinaldine-tyrosine complex. This is in confirmity with theoretical predication that steric effect due to methyl group in 2-position with respect to nitrogen creates steric hindrance in complexation. Whereas no steric effect is present when tyrosine complexed with oxine.

Table 6

Infrared spectral band assignments for 8-hydroxquinoline, tyrosine and tyrosine-8-hydroxyquinoline complex

S.No.	Bands cm^{-1}	Tyrosine cm^{-1}	8-hydroxyquinoline cm^{-1}	Complex cm^{-1}	Remarks
1	3500	-	-	+(W)	
2	3400	-	-	+(S)	New band due to
3	3250	-	+	-	complexation
4	2950	+(B)	-	+(S)	
5	2500	+	+	+(B)	Broad band due to
6	2400	-	+	+	complexation
7	2100	+	-	+(W)	
8	1580	+(S)	+(W)	+(S)	
9	1560	-	-	-	
10	1500	-	-	+	New band
11	1480	+	+	+	
12	1450	-	-	+	
13	1430	-	+(W)	-	
14	1400	+	+(W)	+	Due to complexation
15	1380	-	+	+(S)	
16	1320	+	+	+	
17	1300	+	-	-	
18	1280	+	-	-	
19	1240	+(W)	+(S)	+(S)	
20	1180	+(W)	+(S)	+(W)	
21	1120	+(S)	+(S)	+(W)	
22	1050	+	+	+	
23	1000	+	+	+	New band
24	980	+(W)	-	+	
25	940	+(W)	-	+(S)	
26	900	+	+	+	
27	840	+	+	+	
28	760	+(S)	+(S)	+(W)	
29	720	-	+	-	
30	660	+	-	+	
31	520	+	-	+	

Key: W= weak, S=strong and B=Broad

Tyrosine is aromatic amino acid and exhibits no fluorescence in free form. However, when it is complexed with 8-quinaldine or oxine it shows fluorescence activity at λ_{Ex} 350nm λ_{Em} 480nm for 8-quinaldine and at λ_{Ex} 325nm λ_{Em} 425nm for oxine. Results are shown in Table 1 and 2. After complexation of tyrosine with above two reagents it gave strong fluorescent products due to its' stable complexation and as such detection of tyrosine becomes possible by this method.

The method due to its versatility and rapidity may also be useful for biomedical and other laboratories as it has been found to be superior in comparison to existing analytical methods presently used. The detection limits down to nanomole or even below has been achieved by this method for the estimation of amino acids and complementary molecules of quinaldine and oxine direct as well as indirect estimation procedures.

In the light of above-mentioned results, it has become apparent that, fluorescence properties of tyrosine-8-quinaldine and tyrosine-oxine derivatives of amino acids would be of great interest for analysts. These reagents form highly fluorescent derivatives with tyrosine and it is a break through in the field of amino acids analysis to estimate amino acids qualitatively as well as quantitatively at nano mole level by single step rapid method. The simple, economical and unequivocal preparation methodology of these complexes has provided way to introduction of rapid and new analytical technique for use in analytical and biological/physiological laboratories for direct identification, separation and estimation of amino acids.

Table 7

Comparative study of fluorescent complexes of tyrosine-8-quinolinol (a) and tyrosine-8-hydroxyquinaldine (b) by spectrofluorophotometer model RF-510

Sr. No.	Mole ratio	Amino acid (ml)	Reagents (ml)	Fluorescence intensity	
				Tyrosine -8-quinolinol	Tyrosine -8-hydroxy-quinaldine
1	1:1	0.2	0.2	100	56
2	1:2	0.2	0.4	210	112
3	1:3	0.2	0.6	450	160
4	1:4	0.2	0.8	429	148
5	1:5	0.2	1.0	408	126

Experimental parameters, Response =Medium, Scan Speed =100nm/min, Ave: Time = 1 sec,
B.W λ_{EX}=10nm, B.W λ_{Em} =10nm, Gain =01
Excitation wavelengths of (a) and (b) complexes = 325 and 350 nm respectively.
Emission wavelengths of (a) and (b) complexes = 425 and 480nm respectively.
Note: At λ_{EX} 325,380nm and λ_{Em} 425, 480nm the reactants 8-quinalinol, 8-hydroxyquinaldine, Tyrosine and acetic acid showed no fluorescence activity.
Fluorescence intenstity was determined for working solution prepared from 0.2ml of 0.01M Tyrosine with 8-quinolinol/8-hydroxyquinaldine diluted up to 25 ml.

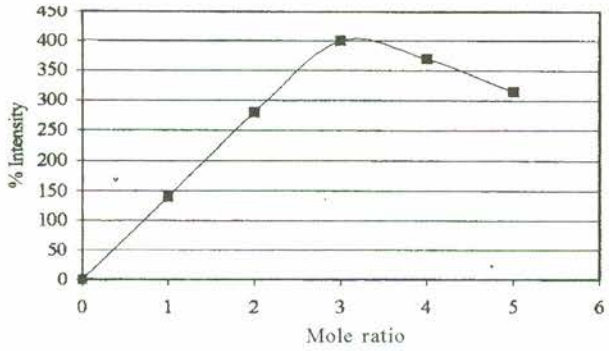

Fig 1. Mole ratio versus fluorescence intensity of tyrosine 8-hydroxyquinaldine complex.

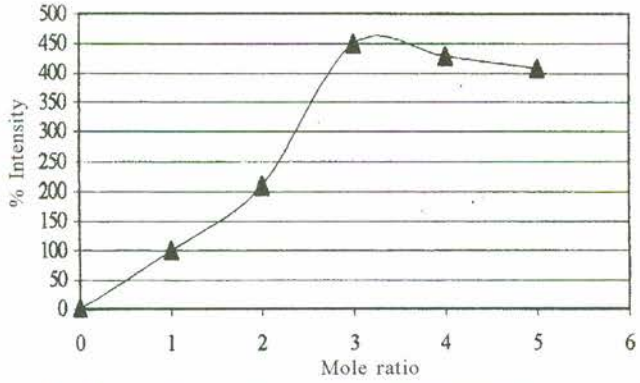

Fig 2. Mole ratio versus fluorescence intensity of tyrosine -8-hydroxyquinoline complex

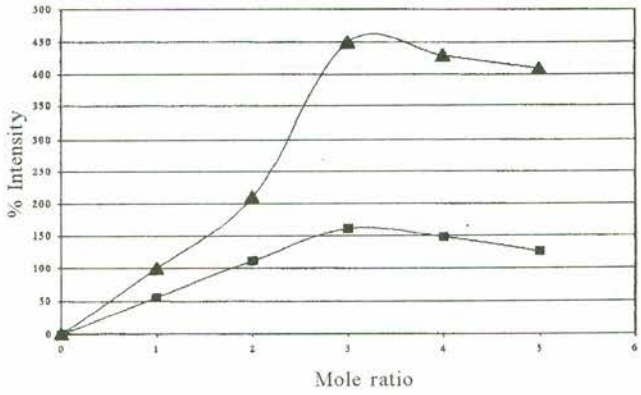

Fig 3. Shows the comparative study of fluorescent complexes of tyrosine-8-hydroxyquinoline complex with tyrosine-8-hydroxyquinaldine complex graph plotted mole ratio versus fluorescence intensity.

Infrared spectral band assignments of tyrosine, quinaldine, oxine and complexes. Infrared spectra of tyrosine, 8-hydroxyquinaldine, 8-hydroxyquinoline and both newly prepared complexes were recorded in KBr powder using Hitachi model 260-50 using range of 4000 to 250 cm^{-1}. Spectral peaks for various functional groups are noted viz., CH stretching 3400 cm^{-1} and 1500 cm^{-1} for -OH 1560 cm^{-1} for asymmetric stretching of COO^- and 1400, 1430, 1450 cm^{-1} for symmetric stretching of COO^-. The finger print comparison of three IR spectra viz: of the reactants and the product showed that frequency bands of -COOH and >-NH groups of tyrosine at 1580 to 1480 cm^{-1} and frequency of 2-methylquinaldine at 1560 cm^{-1} are affected due to complexation. In tyrosine 2-methylquinaldine complex peaks at 1580 to 1400 cm^{-1} were affected at 1120 cm^{-1} change was observed in spectrum of complex. Also comparison of the IR spectra of complex with free 8-hydroxyquinaldine indicated that-OH frequency in 8-hydroxyquinaldine molecule in the region of 1400 cm^{-1} was affected due to complexation. Comparison of three spectra is shown in Table 6. The spectral evidence supports presence of complex of definite composition.

Conclusion

In present paper, well defined stoichiometric complexes having fluorescent properties have been reported. The fluorescence activity of these complexes at various excitation and emission lines has been fully exploited.

For analytical methodology and improved detection limits for amino acid have been reported. This technique incorporates within itself numerous experimental variables such as scale expansion, signal refinement and back ground of set system.

Thus detection limits are enhanced 100 to 1000 fold. The out come of this research is of great importance as fluorescence activity of the complexes lies in regions where interference in signal out put due to reactants, medium and phase were totally absent. The fluorescent complexes of amino acids with 8-hydroxyquinaldine are found stable and may be stored for at lest a year with their fluorescence intensity remaining unchanged. Thus the above experimental findings have paved way to exploit the method simultaneously for single step direct or indirect estimation of amino acids or 8-hydroxyquinaldine reactant as well as final products with down to nano mole or further down level analysis. 1:3 mole ratio for complexes confirms stoichiometric complex formation at maximum fluorescence intensity in solution form. The amino acid complexes showed decrease in fluorescence intensity with 8-hydroxyquinaldine as compared to 8-hydroxyquinoline, which is due to steric hindrance of α-methyl group on 8-quinaldine.

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