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Analytical Investigation of Fluorescent Complexes of Valine-8-hydroxyquinoline and Valine-8-hydroxyquinaldine in Aqueous Phase Using Spectroscopic Techniques

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Abstract: A new method has been developed by preparing complexes involving condensation of amino acids with 8-hydroxyquinoline and 8-hydroxyquinaldine. The products so obtained are being investigated for identification and quantitative estimation of amino acids using different spectroscopic techniques including fluorescence activity of newly synthesized products. The method adopted in our laboratories is rapid, versatile, reproducible and provides excellent results for adoption by analytical, agricultural and biomedical laboratories to estimate amino acids and metals in composite matrix at economical costs. 8-hydroxyquinoline (Oxine) and 2-methyl-8-hydroxyquinoline (8-hydroxyquinaldine) condensed with valine produced fluorescent complexes. The complexes have been investigated for identification and quantitative estimation of amino acids. By this method identification of amino acids in nano mole quantities has become possible by fluorescence activity of valine-8-hydroxyquinoline and valine-8-hydroxyquinaldine complexes involving different excitation and emission wave lengths. This fluorescence activity of complexes is 100 to 1000 times higher than assay method involving ninhydrin and amino acid analyzer.

Key words: Valine, fluorometric activity, 8-hydroxyquinaldine, 8-hydroxyquinoline , complexes

Introduction

The use of fluorescence-based analysis is becoming increasingly popular in many branches of the chemical and biological sciences. The principal advantages of this technique, which encourage its use, are its high sensitivity which allows the measurement of low analyte concentrations, its selectivity which is, in part, due to the two characteristic wavelengths (excitation and emission) of each fluorescent species and the verity of sampling methods available (Momin and Narayanaswamy, 1992).

The condensation of amino acids with 8-hydroxyquinoline or its 2-alkyl homologue viz., 8-hydroxquinaldine produces products of definite stoichiometry, Such complexes have been extensively studied in our laboratories by using spectrofluorometric method for their characteristic excitation and emission wavelength (Kazi *et al*., 1997). The optimisation of experimental conditions provides quantitation and detection limits for amino acids and their complexes down to nanomol range (Asrar *et al*., 1985). Valine is complexed with 8-hydroxyquinoline and 8-hydroxyquinaldine, its stoichiometric investigations have been carried out involving Spectrofluorometric activity and Infrared spectral band asssignment. Determination of X_{max} of fluorescent complexes of valine in UV region using TLC scanner and excitation and emission wavelengths by spectrofluorometer were found to be of immense importance for estimation of amino acids down to nano mole range.

Valine is an essential amino acid. An amino acid is defined by amino group (NH_3) and an acid group (COOH). The amino acids than have an R group attached which in valine case is an aliphatic side chain. The essential part means that the human body cannot synthesis valine itself (Bremer *et al*., 1981). There are nine essential amino acids in human body. If valine is not ingested by outside means the body will break down proteins in the body in order to get the necessary valine. Valine can be synthesised in plants and bacteria from pyrvate (Berkow, 1992). A limiting amino acid is an essential amino acid supplied in less than the amount needed to provide support for protein synthesis. The following are the mg/kg of body weight of valine that is needed to be consumed. Infant, child 10-12 years, adult male and adult female 95, 33, 14, and 11 mg/kg respectively. A complete protein is one that supplies all the essential amino acids in the necessary amounts for human survival (Whitney and Rolfes, 1993).

Materials and Methods

All chemicals and reagents used were of Analytical reagent Grade:

1. 0.01 M Acetic acid = 0.6 ml of glacial acetic acid diluted up to 1000 ml with deionized water
2. 0.01 M 2-methylquinaldine = 0.40 g of 8-hydroxyquinaldine dissolved in 0.01 M acetic acid and volume made upto 250 ml
3. 0.01 M 8-hydroyqunoline = 0.36 g of 8-hydroxyquinoline dissolved in 0.01M acetic acid and volume made up to 250 ml
4. 0.01 M Valine = 0.117 g of Valine dissolved in 100 ml volumetric flask in 0.01 M acetic acid

Preparation of valine-8-hydroxyquinaldine and valine 8- hydroxyquinoline complexes for spectrofluorometric study: 0.01 M Valine were pipetted out separately in several pre labelled conical flasks and known volumes of 0.01 M 8-hydroxyquinaldinel 8-hydroxyquinoline solutions were added to these flasks to maintain ratio of valine to 8-hydroxyquinaldine and valine to 8-hydroxyquinoline as 1:1, 1:2, 1:3, 1:4 and so on.

The flasks were then covered with watch glasses and the solutions were heated to gentle reflux for one hour and then the watch glasses were removed and reaction mixture evaporated to semidried residue which was subsequently subjected to recrystallisation to obtain light yellow product. Known volume of 0.01 M acetic acid was added to each product to dissolve the complex in respective flasks. The resulting transparent and clear solutions were examined spectrofluorometrically and excitation and emission wave

Table 1: Determination of excitation and emission wavelengths and stoichiometric investigation of valine-8- hydroxyquinaldine complex

Amino acid (ml)	8-hydroxyquinaldine	Mole ratio	Fluorescence intensity
0.5	0.5	1:1	90
0.5	1.0	1:2	120
0.5	1,5	1:3	180
0 5	2.0	1:4	150
0.5	2.5	1:5	125

Experimental parameters: Excitation wave length = λ 365 nm, Emission wave length = λ 440 nm, Response = medium, Gain-1, B.Wλ_{Ex}-10 , 8.Wλ_{Em}-10

Note: At λ_{Ex} 365 nm and λE_{Em} 440 nm, the reactants 8-hydroxouinaldine, Valine and acetic acid showed no fluorescence activity. Fluorescence intensity was determined for working solution prepared from 0.5 ml of 0.01 M Valine with 8-hydroxyquinaldine and diluted up to 25 ml

lengths were found as mentioned in (Table 1) for valine-8-hydroxyquinaldine complex and in (Table3) for valine-8-hydroxyquinolinecomplex.

Detection limits of newly prepared complexes were also recorded as mentioned in Table 2 for valine-8-hydroxyquinaldine complex and in Table 4 for valine-8- hydroxyquinoline complex.

Comparison studies of amino acid complexes with reagent 8-hydroxyquinoline and its homologue are given in (Table 7). For other analytical investigations such as Infrared spectroscopic study valine-8-hydroxyquinaldine and valine-8-hydroxyquinoline complex were prepared under same procedure as mentioned above and stoichiometry was established. Results are in (Table 5) for valine-8-hydroxyquinaldine complex and in (Table 6) for valine-8-hydroxyquinoline complex.

Results and Discussion

Fluorescence derivatization is a widely used technique for the analysis of biological compounds or synthetic chemicals. Detection limits of the fluorescence derivatives are usually on the order of femto moles, therefore, a sensitative determination should be possible (Hamase *et al*., 2000).

The reaction of amino groups with DNS-Cl (1-dimethylaminonaphthalene-5-sulfonyl chloride) was used for model of fluorescence derivatization, and molecular selective masking was performed during the reaction (Hamase *et al.,* 2000).

Fluorescence properties of O-phthaladehyde (OPT)

Table 2: Detection bruit of valino-8-hydroxyquina/dine complex

Amino acid solution conc. (M)	λ_{Ex} 365 B.W	λ_{Em} 440 nm B.W	Flu: Intensity	Blank	FSD (x) mV	Vhart Div:	Gain
(A)	10	10	180	0	200	90	1
(B)	-	-	24	0	50	48	1
-	-	-	165	114	200	83	2
-	-	-	560	440	1000	56	5
-	-	-	1200	980	2000	60	10
-	20	40	1100	0	2000	55	1
-	20	40	140	0	200	70	1
-	-	-	360	113	500	72	2
-	-	-	1100	420	2000	55	5
-	-	-	1260	980	2000	63	10
(D)	20	40	40	0	100	40	1
-	-	-	196	113	200	96	2
-	-	-	620	430	1000	62	5
-	-	-	1260	980	2000	63	20

Detection Limit: Experimental = 2×10^{-4} μ moles/ml Theoretical = 2.2×10^{-6} moles/ml

Key: Dilution steps. O.S = 0.117 g Valine/100 ml = 0.01 M

Stoichlometric composition 1:3 Valine-8-hydroxyquinaldine

0.5 ml of O.S is diluted up to 25 ml = (A) 2×10^{-4} M 5 ml of A/50 ml = (B) 2×10^{-5} M 5 ml of B/50 ml = (C) 2×10^{-6} 5 ml of C/50 ml = (D) 2×10^{-7} M

Table 3: Determination of excitation and emission wavelengths and stoichiometric investigation of valine-8-quinolinol complex

Amino acid (ml)	8-quinolmol (ml)	Mole ratio	Fluorescence intensity
0.2	0.6	1:1	40
0.2	0.4	1:2	91
0.2	0.6	1:3	185
0.4	0.8	1:4	180
0.2	1.0	1:5	175

Experimental parameters: Excitation wave length = λ340 nm, Emission wave length = λ 420 nm, Response = medium, Gain = 1, B.Wλ_{Ex}-10, B.Wλ_{Em} = 10 =

Note: At λ_{Ex} 340 nm and λ_{Em}, 420 nm, the reactants 8-hydroxquinoline, Valine and acetic acid showed no fluorescence activity, Fluorescence intensity was determined for working solution prepared from 0.2 ml of 0.01 M Valine with 8-quinolinol and diluted up to 25 ml

Table 4: Detection limit of valine-8-quinolinol complex

Amino acid solution conc. (M)	λ_{Ex} 365 B.W	λ_{Em} 440nm B.W	Flu: Intensity	Blank	FSD (x) mV	Vhart Div:	Gain
(B)	10	10	90	1	200	45	1
(C)	1	10	46	1	50	92	1
-	20	40	96	28	100	96	1
-	-	-	196	55	200	98	2
(D)	20	40	60	55	100	60	2
-	-	-	142	120	200	71	5
-	-	-	285	250	500	57	10
-	-	-	570	500	1000	57	20

Detection Limit: Experimental = 8.0×10^{-5} μmoles/ml Theoretical = 2.2×10^{-6} moles/ml

Key: Dilution steps. -0.5 = 0.117 g Valine/100 ml =0.01 M = Stoichlometric composition 1:3 Valine = quinalinol 1, 0.2 ml of A/50 ml (B) 4.0×10^{-5} M 2. 1.0 ml of 18/100 ml = 4.0×10^{-7} M 3. 2.0 ml of C/10 ml = 8.0×10^{-8} M

Table 5: Infrared spectral band assignments for 8-hydroxyquinaldine, valine and valme-8-hydroxyquinaldine complex

Bands (cm^{-1})	Valine (cm^{-1})	8-hydroxy-quinatdine (cm^{-1})	Complex (cm^{-1})	Remarks
3400	-	-	-	new band
3250	-	+	-	
2950	+	-	+	
2600	+	-	+	
2100	+	-	+	
1600	-	+	+	
1580	+(s)	+	+(b)	due to complexation
1500	$+(_w)$	+ (w)	+(s)	
1420	+	+	-	
1380	+	+(w)	+	
1340	+(sh)	+(w)	+	
1330	+	+	+	
1270	+	-	+	
1260	-	+(s)	-	
1180	+	+	+	
1140	+(s)	+(s)	+	
1060	+	-	+	
1040	+	-	+	
940	+	-	+	
820	+	+(s)	+	
180	+	+(w)	+	
740	+(w)	+(s)	+(w)	
720	+(s)	+(s)	+(s)	
540	+(s)	+(w)	+(s)	

Key: (w) = weak, (s) = strong and (B) = broad

Table 6: Infrared spectral band assignments for 8-quinolinol, Valine and Valine 8-quinolinol complex

Bands (cm^{-1})	Valine (cm^{-1})	8-quinolinol (cm^{-1})	Complex (cm^{-1})	Remarks on complex
3050	+	+	+(B)	Broad band due to CH and NH group
2600	+	-	-	
2070	+	-	+	
1650	+	+	+(b)	
1585	+	+	+(b)	OH of Oxine involve
1500	+	+	-	
1460	+	+(w)	-	
1420	-	-	+(b)	New broad band due to complex
1400	-	-	+	
1385	-	+	-	
1340	+	-	+	
1280	-	+	-	
1260	+	+	+	
1220		+	-	
1180	+	-	-	
1160	+(w)	+(w)	+	The coplexation involve
1120	+(w)	+(w)	+	
1090	-	+(w)	+	
1020	+	-	+	
970	-	+(w)	-	
930	+(w)	-	+(s)	Strong bands between 930 and 870 due to aromatic ring
890	+	+	+(s)	
780	+	+	-	
760	+	-	+	
700	+	-	+	
680	+	-	-	
650	+(s)	+	+(w)	
620	-	-	+	
460	-	+ (w)	+	

Key: W = (weak), S = (strong) , B = (broad)

derivatives of several iodoamino acids have been studied and compared with those of glycine and tyrosine. Incorporation of successive iodine atoms in tyrosine and theronine structures produces increasing quenching of the fluorescence of OPT derivatives (Miller and Thakrar, 1981). Derivatizing reagent, dansylaminomethylmalic acid (DAM) was synthesized and utilized for the reversible fluorescence labling of amino groups (Sakata *et al*., 1999).

Valine is aliphatic amino acid, as it has no fluorescence activity in free state (Shaikh, 1993; Shaikh *et al*., 1989). When it complexesd with 8-hydroxyquinoline and 8-hydroxyquinaldine the complex· gives fluorescence intensity at excitation and emission lines mentioned in Table 1 and 3.

Infrared spectra of Valine, 8-hydroxyquinoline, 8-hydroxyquinaldine and newly prepared complexes were recorded in KB powder using Hitachi 250-60 Infrared spectrophotometer. Valine showed bands at 1585 cm^{-1} for anti symmetric stretching COO^{-}, 1500 cm for NH deformation and 1400 cm^{-1} for symmetric stretching of COO. Also the finger print comparison of three spectra viz. of the reactants and products showed that COOH > -NH groups at 1585 cm^{-1} and 1500 cm^{-1} respectively were the site of reaction with 8-hydroxyquinoline.Also comparison of IR spectra of complex with free 8hydroxyquinoline indicated that -OH frequency in 8-hydroxyquinoline in the region of 1650 cm^{-1} was affected

Table 7: Comparative study of fluorescent complexes of valine-8-euinolinol (a) and valine-8-hydroxyquinaldine (b) by spectrofluorophotometer model RF- 510

Mole ratio	Amino acid (ml)	Reagents (ml)	Fluorescence intensity	
			Valine 8-quinolinol	Valine-8-hydroxyquinaldine
1:1	0.2	0.2	40	36
1:2	0.2	0.4	91	48
1:3	0.2	0.6	185	72
1:4	0.2	0.8	180	60
1:5	0.2	1.0	175	50

Experimental parameters: Response = Medium, Scan speed = 100 nm/min, λve: Time = 1 sec, B.W λ_{Ex} = 10 nm, B.W λ_{Ex} =10 nm, B.W λ_{Em} = 10 nm, Gain = 01, Excitation wavelengths of (a) and (b) complexes = λ340 and 365 nm respectively. Emission wavelengths of (a) and (b) complexes = λ420 and 440 nm respectively.

Note: At λ_{Ex} 340, 365 nm and λ_{Em} 420, 440 nm the reactants 8-quinalinol, 8-hydroxyquinaldine, Valine and acetic acid showed no fluorescence activity.

Fluorescence intensity determined for working solution prepared from 0.2 ml of 0.01 M Valine with 8-quinolinol/8-hydroxyquinaldine diluted up to 25 ml.

due to complexation of Valine with 8-quinolinol.In complex the peaks at 930 cm^{-1}, 890 cm^{-1}, 760 cm^{-1}, 620 cm^{-1} were due to 8-hydroxyquinoline -CH rocking and were not present in Valine comparison of three spectra is shown in (Table 6). The spectral evidence supported presence of complex formation of definite composition.

Also, the finger print comparison of three spectra viz.; of the reactants and products showed that COOH, >NH groups at 1580 cm^{-1} and 1500 cm^{-1} respectively were the site of reaction with 2-methylquinaldine.Also comparison of IR spectra of complex with free 2-methylquinaldine indicated that -OH frequency in 2-methylquFnaldine in the region of 1600 cm^{-1} was affected due to complexation of Valine with 8-hydroxyqyinaldineln complex the peaks at 940 cm^{-1}, 820 cm^{-1}, 740 cm^{-1}, 540 cm^{-1} were due to 8hydroxyquinaldine -CH rocking and were not present in Valine comparison of three spectra is shown in Table 5. The spectral evidence supported presence of complex formation of definite composition.

In present work well defined stoichiometric and fluorescent complexes of amino acids valine with 8-hydroxyquinaldine 8-hydroxyquinoline have been reported.

The fluorescence activity of these complexes at various excitation and emission lines, has been fully exploited and improved detection limits have been achieved for amino acid by SPF compared to previous methods using UV or Amino acid analyzer. These techniques incorporate within them numerous experimental variables such as scale expansion, signal refinement and back ground off 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 in totally absent.

The amino acid complexes showed decrease in fluorescence intensity with 8-hydroxyquinaldine as compared to 8hydroxyquinoline. This is in confirrnity with theoretical prediction that steric effect due to methyl group in 2-position w.r. to OH group creates partial hindrance in complexation.

The fluorescent complexes of amino acid with 8hydroxyquinaldine are stable and can be stored for a year under nitrogen with fluorescence intensity remaining unchanged.

Thus the above experimental findings have paved the way to exploit the method simultaneously for single step direct or indirect estimation of amino acids or 8-hydroxyquinaldine reactant as well as down to nano mole or Pico mole level. 1:3 mole ratio confirms stoichiometric complex formation at maximum fluorescence intensity in solution. The simple, economical and unequivocal preparation procedure of these complexes has also been accomplished successfully, which has provided way to introduction of rapid and new analytical methodology for use in analytical and pathological laboratories for direct identification, separation and estimation of amino acids.

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