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Analytical Investigation of Fluorescent Complexes of Alanine and Phenylalanine with 8-hydroxyquinaldine and 8-hydroxy quinoline in Aqueous Phase

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Abstract: A new method has been developed by preparing complexes involving condensation of amino acids with 8-hydroxyquinaldine using various experimental conditions. The products so obtained are being investigated for identification and quantitative analysis using different spectroscopic techniques including fluorescence activity of newly synthesized products. In present studies the identification of amino acids in nano mole quantities has become possible by fluorometric activity of amino acids-quinaldine complex involving different excitation and emission wavelengths. This fluorometric activity of complexes is 10 to 100 times more sensitive method than assay method involving ninhydrin. 2- methy1-8-hydroxyquinoline (8-hydroxyquinaldine) condensed with Alanine and Phenylalanine produced fluorescent complexes. The complexes separately have been investigated for identification and quantitative estimation of amino acids. The use of 8-hydroxyquinaldine for various purposes and its comparison to 8-hydroxyquinoline for similar purpose indicates decrease in fluorescence signal of 8-hydroxyquinaldine- amino acid complexes.

Key words: Fluorometric activity, Alanine-8-hydroxyquinaldine, Phenylalanine-8-hydroxyquinaldine

Introduction

Alanine occurs in body fluids in considerable amounts and is involved in various metabolic functions, such as together with Glycine it makes a considerable fraction of the amino nitrogen in human plasma. Previously the Alanine is indirectly measured in protein hydrolysate by the use of non specific degradation methods to convert amino acid to acetaldehyde, which in turn estimated quantitatively (Jat *et al.,* 1997). The aromatic amino acid phenylalanine possesses a weakly absorbing benzene ring and does not emit fluorescence intensity enough for measurement in trace quantities. Because of much higher tyrosine and tryptophan fluorescent intensity, the phenylalanine is not visible in tyrosine and tryptophan containing peptides and proteins. Several methods for estimating phenylalanine in biological samples are available (Hans *et al.,* 1981). In present work Alanine and phenylalanine complexed with 8-hydroxyquinaldine its stoichiometric investigations have been carried out involving Spectrofluorometric IR and other techniques. Determination of A_{max} of fluorescent complexes of Alanine and phenylalanine 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.

Materials and Methods

All the chemicals and reagents were used of AR Grade.

1. 0.01 M Acetic acid = 0.8 ml of glacial acetic acid diluted up to 1000 ml with deionized water
2. 0.01 M 8-hydroxyquinaldine = 0.40 g of 8-ydroyquinaldine dissolved in 0.01 M acetic acid made up to 250 ml
3. 0.01 M Alanine =0.089 g of alanine dissolved in 0.01 M acetic acid and volume made up to 100 ml volumetric flask
4. 0.01 M Phenylalanine = 0.165 g of phenylalanine dissolved in 0.01 M acetic acid and volume made up to 100 ml volumetric flask

Preparation of complexes of Alanine and Phenylalanine with 8-hydroxyquinaldine for spectrofluorometric study: Equal volumes of 0.01 M Alanine and phenylalanine solution were pipetted out in several pre labelled conical flasks and known volumes of 8-hydroxyquinaldine solutions were added to these flasks to maintain ratio of Alanine-8-hydroxyquinaldine and Phenylalanine-8-hydroxyquinaldine 1:1, 1:2, 1:3, 1:4 ,1:5 and so on. The flasks were then covered with watch glasses and the solutions in labelled flasks were heated to gental reflux for one hour and then watch were removed and reaction mixture evaporated to semi dried residue which was subjected to recrystallisation to obtain light yellow product. Equal volume of 0.01 M acetic acid was added to dissolve the complex in respective flasks. The resulting transparent and clear solution was examined spectrofluorometrially to determine excitation and emmission wave lengths (Table 1, 2).

For other physicochemical studies Alanine-8-hydroxyquinaldine and Phenylalanine-8-hydroxyquinaldine complexes was prepared by same procedure as above. For structural elucidation of alanine-8-hydroxyquinaldine complex involving SPF, IR and other techniques have been used to established stoichiometry and calculate detection limit of newly prepared complexes (Table 1b, 2b).

Results and Discussion

The newly synthesized complexes of amino acids with 8-hydroxyquinaldine developing new analytical methodology for identification and estimation of amino acid in various matrices. It has been established from fluorescence activity of complexes that stoichiometry of complexes follows a definite trend of 1:1, 1:2, 1:3 at various mole ratio of amino acid with 8-hydroxyquinaldine (Legend) in liquid and solid phase which is understandable behavior of Zwitter ions and Polydentate molecules. Alanine is non fluorescent aliphatic amino acid (Jat *et al*., 1997) aftercomplexation the flourescence intensity starts increasing at these excitation and emission lines and the flourescence intensity of complex appeared at λ_{Ex} = 345 nm and λ_{EM} = 330 nm. Phenylalanine is aromatic amino acid , it have weakly absorbing benzene ring (Hans *et al.,* 1981). Phenylalanine complexed with 8-hydroxyquinaldine, showed fluorescent activity at λ_{Ex} 360 and λ_{Em} 440 nm.

Infrared spectra of Alanine, 8-hydroxyquinaldine and complex: Infrared spectra of Alanine, 8-hydroxyquinaldine and newly

Table 1: Determination of excitation and Emission wavelengths and stoichiometric investigation of alanine-8-hydroxy-quinaldine complex

Mole ratio	Amino acid (ml)	8-hydrox-quinaldine (ml)	% Fluorescence intensity
1:1	0.5	0.5	50
1:2	0.5	1.0	72
1:3	0.5	1.5	92
1:4	0.5	2.0	80
1:5	0.5	2.5	65

Experimental parameters:
B.W λ_{Ex} = 10 nm B.W λ_{Ex}, 10 nm, Gain = 1, Respons = Medium
Excitation wavelength = 345 nm. Emission wavelength = 430 nm
Remarks
1:3 mole ratio confirm soichiometric complex formation at maximum fluorescence intensity
Note: At λ_{Ex} 345 nm and λ_{Ex} 430 nm, the reactants 8-hydroxyquinaldine, Alanine and acetic acid showed no fluorescence activity
Fluorescence intensity determined for working solution prepared from 0.5 ml of 0.01 M amino acid with 8-hydroxyquinaldinediluted up to 25 ml

Table 1 (b): Detection of alanine-8-hydroxyquinaldine complex

Aminoacia Solution Conc: (M)	λ_{Ex},345BW	λ_{Em},430 nm BW	Flu: Intensity	Blank	FSD (x)mV	Chart Div:	Gain
(A)	10	10	92	0	100	92	1
(B)	10	10	470	450	500	94	5
-	-	-	1060	1020	2000	53	10
-	20	40	325	12	500	65	2
-	-	-	920	50	1000	92	5
-	-	-	1380	115	2000	69	10
(C)	20	40	124	16	200	62	2
-	-	-	340	62	500	68	5
-	-	-	680	141	1000	68	10
-	-	-	1320	300	2000	66	20
(D)	20	40	96	16	200	48	2
-	-	-	260	66	500	52	5
-	-	-	540	150	1000	54	10
-	-	-	1120	310	2000	56	20
-	-	-	1380	820	2000	69	50

Detection Limit Experimental = $2\times10^{-4}\mu$, moles/ml = Theoretical = 7×10^{-7} moles/ml
Key:
Dilution steps
O.S = 0.089 g alanine/100 ml 0.01 M = 0.5 ml of O.S is diluted up to 25 ml = (A) 2×10^{-4}M
Stoichiometric composition 1:3 Alanine 8-hydroxyquinaldine
5 ml of A/50 ml, (B) 2×10^{-5} M, 5 ml of B/50 ml = (C) 2×10^{-5} M of = 5 ml C/50 ml = (D) 2×10^{-7} M

Table 2: Determination of excitation and emission wavelengths and stoichiometric investigation of phenylalanine-8-hydroxyquinaldine complex

Amino acid (ml)	8-hydroxy-quinaldine (ml)	Mole ratio	%Fluorescence intensit
0.5	0.5	1:1	60
0.5	1.0	1:2	110
0.5	1.5	1:3	170
0.5	2.0	1:4	160
0.5	2.5	1:5	150

Experimental parameters
B.W λ_{Ex} = 10 nm, B.Wλ_{Ex} = 10 nm, Gain = 01
Respons = Medium, Excitation wavelength, 360 nm Emission wavelength = 440 nm
Remarks: 1:3 mole ratio confirm soichiometric complex formation at maximum fluorescence intensity
Note: At λ_{Ex} 360 nm and λ_{Ex} 440 nm, the reactants 8-hydroxyquinalcline, Phenylalanine and Acetic acid showed no fluorescence activity Fluorescence intensity determined for working solution prepared from 0.5 ml of 0.01 M amino acid with 8-hydroxyquinaldine diluted up to 25 ml

Table 2 (b): Detection limit of phenylalanine-8-hydroxyquinaldine complex

Amino acid (M) solution Conc:	λEx 360 B.W	λEm 440 nm B.W	Flu:Intensity	Blank	FSD(x)mV	Chart Dlv:	Gain
(A)	10	10	74	0	100	74	1
(B)	10	10	98	70	200	49	5
-	-	-	200	158	500	40	10
-	-	-	425	325	500	85	20
-	20	40	430	18	500	86	2
-	-	-	1100	70	2000	55	5
-	-	-	1380	160	2000	69	20
(C1	20	40	148	18	200	74	2
-	-	-	420	70	500	84	5
-	-	-	890	160	1000	89	10
-	-	-	1360	320	2000	68	20
(D)	20	40	184	70	200	92	5
-	-	-	520	160	1000	52	10
-	-	-	700	325	1000	70	20
-	-	-	1360	840	2000	68	50

Detection Limit Experimental = $2\times10\times^{-4}$ μ, moles/ml
Theoretical = 7.7×10^{-7} μ moles/ml Key, Dilution steps = O.S = 0.165 g Phenylalanine/100 ml 0.01 M = 0.5 ml of O.S is diluted up to 25 ml = (A)2×10^{-4}M
Stoichometric composition 1:3 Phenylalanine-8-hydroxyquinaldine
5 ml of A/50 ml =(B2$\times10^{-6}$ M = 5 ml of B/50 ml = (C) 2×10^{-5}M 5 ml of C/50 ml = (D) 2×10^{-7} M. Fluorescence intensity determined for working solution prepared from 0.2 ml of 0.01 M Alanine with 8-hydroxyquinaldine and 8-quinolinoi diluted up to 25 ml

prepared complex were recorded by Hitachi model 260-50 in KW, over the range of 4000 cm^{-1} to 250 cm^{-1}. Alanine shows bands at 1560 (anti symmetric stretching of COO^{+}) and 1460 cm^{-1} (symmetric stretching of COO^{+}). The finger print comparison of three spectra viz: of the reactants and product showed that frequency bands of COON and >NH groups in Alanine at 1575 and 1510 cm^{-1} of and frequency band 8-hydroxyquinaldine at 1575 cm^{-1} are affected due to complexation. Also comparison of the IR spectra of complexes with free 8-hydroxyquinaldine indicated that -OH frequency in quinaldine molecule in the region of 1360 cm^{-1} affected due to complexation. In complex frequency bands at 920, 870, 730, 420 cm^{-1} are due to quinaldine -CH rocking and were not present in Alanine. The spectral evidences show that the complex is present in its definite structure (Table 3).

Table 3: Infrared spectral band assignments for 8-hydroxyquinaldine, alanine alanine 8-hydroxyquinaldine complex

Bands cm^{-1}	Aanine cm^{-1}	8-hydroxy-quinaldine cm^{-1}	Complex cm^{-1}	Remarks
3740	+	-	+	
3200	-	+	-	
3080	-	-	+	due to complexation
2980	+ (B)	-	-	
2720	+ (B)	-	-	
2600	+	-	+	
2320	+ (S)	+ (S)	+ (S)	
2120	+ (S)	-	+	
1720	-	-	+	new band
1700	+	-	+	
1650	-	-	+	new band
1620	-	+	+ (S)	
1600	-	+	-	
1575	+	+	+	
1560	+ (W)	-	+	
1540	+	-	+	
1510	+	+	+ (S)	
1460	+ (S)	-	+ (S)	
1415	+ (S)	-	+ (S)	
1360	+ (S)	+	+ (S)	
1310	+ (S)	+ (S)	+ (S)	
1240	+	+	+ (S)	
1190	-	+	-	
1155	+	-	+	
1115	+ (S)	-	+ (S)	
1020	+ (S)	-	+ (S)	
920	+	-	+ (S)	
900	-	+ (W)	-	
870	-	+	-	
850	+ (S)	+ (S)	+ (S)	
800	-	+	-	
770	+ (S)	+	+	
730	-	+	-	
650	+	-	+	
550	+ (S)	+	+ (S)	
420	+ (S)	+	-	
400	+ (S)	-	+ (S)	

Table 4: Infrared spectral band assignments for 8-hydroxyquinaldine, alanine alanine 8-hydroxyquinaldine complex

Bands cm^{-1}	Phenylalanine cm^{-1}	8-hydroxy-quinaldine cm^{-1}	Complex cm^{-1}	Remarks
3745	+ (W)	-	+	
3200	-	+ (B)	+	
3000	+ (B)	-	+	
2960	-	-	+	new band
2340	+ (S)	+	+ (S)	
2120	+	-	+	
1695	+ (W)	-	+ (W)	
1645	+ (W)	-	+	
1630	-	+	+	
1600	-	+ (S)	-	
1565	-	+	+ (B)	broad due to complexation
1540	+ (B)	-	+	
1520	+	-	+	
1510	+	+	+	
1490	+	-	+	
1460	+	+	+	
1410	+ (S)	-	+ (S)	
1360	-	+ (W)	-	
1340	+	-	+	
1315	+ (S)	+ (S)	+ (S)	
1250	-	+	-	
1230	+ (W)	-	+	
1190	-	+	-	
1165	+	-	+ (S)	
1130	-	-	+	
1080	+ (S)	-	+	
1035	+ (W)	-	+	
1005	+ (W)	-	+	
960	+ (W)	-	+ (W)	
920	+	-	+	
900	-	+ (W)	-	
870	-	+	-	
850	+ (S)	+	+ (S)	
800	-	+	-	
780	+	-	+	
750	+ (S)	+	+ (S)	
730	-	+	-	
700	+ (S)	+	+ (S)	
685	+ (S)	-	+	
610	+	-	+ (W)	
545	+ (S)	+	-	
530	-	-	+ (S)	
475	+	-	+	

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

Table 5: Comparative study of fluorecent complexes of phenylalanine-8-quinolinol (a) and phenylalanine-8-hydroxyquinaldine (b) by spectrofluorophotometer model RF-510

Mole ratio	Amino acid	Reagent (ml)	%Fluorescence intensity	
			Phenylalanine-8Auinolinol	Phenylalanine-8-hydroxyquinaldine
1:1	0.2	0.2	66	24
1:2	0.2	0.4	136	44
1:3	0.2	0.6	199	68
1:4	0.2	0.8	195	64
1:5	0.2	1.0	180	60

Experimental parameters. = B.W λ_{Ex}, =10 nm = B.W λ_{Em} = 10 nm = Gain =1 = Respons = medium
Excitation wavelength of (a) and (b) complexes = 315 and 360 nm respectively.
Emission wavelength of (a) and (b) complexes = 400 and 440 nm respectively.
Remarks; The complex of amino acid with reagent 8-quinolinol showed high fluorescence intensity than 8-hydroxyquinaldine. More over the 1:3 mole ratio confirm stoichiometric complex formation at maximum fluorescence intensity in both complexes.
Note: At λ_{Ex} 315, 360 nm and λ_{EM} 400, 440 nm the reactants 8-quinolino1,8-hydroxyquinaldine. Phenylalanine and acetic acid showed no fluorescence activity. Fluorescence intensity determined for working solution prepared from 0.2 ml of 0.01 M Phenylalanine with 8-hydroxyquinaldine and 8- quinolinol diluted up to 25 ml

Table 6: Comparative study of fluorescent complexes of alanine-8-quinolinol (a) and alanine-8-hydroxyquinaldine (b) by spectrofluorophotometer model RF-510

Mole ratio	Amino acid (ml)	Reagent (ml)	% Fluorescence intensity	
			Alanine-8-quinolinol	Alanine-8-hydroxyquinaldine
1:1	0.2	0.2	55	20
1:2	0.2	0.4	116	29
1:3	0.2	0.6	141	37
1:4	0.2	0.8	134	32
1:5	0.2	1.0	127	26

Experimental parameters; B.W λ_{Em}, = 10 nm, B.W λ_{Em} 10 nm, Gain =,1 Respons = medium
Excitation wavelength of(a) and (b) complexes =330 and 345 nm respectively. Emission wavelength of (a) and (b) complexes = 420 and 430 nm respectively.
Remarks: The complex of amino acid with 8-quinolinol showed high fluorescence intensity than 8-hydroxyquinaldine, More over the 1:3 mole ratio confirm stoichiometric complex formation at maximum fluorescence intensity in both complexes.
Note: At λ_{Ex} 330, 345 nm and λ_{EM}, 420, 430 nm the reactants 8-quinolinol. 8-hydroxyquinaldine, Alanine and acetic acid showed no fluorescence activity.

Infrared spectra of Phenylalanine, 8-hydroxyquinaldine and complex: Infrared spectra of Phenylalanine, 8hydroxyquinaldine and newly prepared complex were recorded by Hitachi model 260-50 in KBr, over the range of 4000 cm^{-1} to 250 cm^{-1} .Phenylalanine show bands at 1565 (anti symmetric stretching of $C00^{+}$) and 1460 cm^{-1} (symmetric stretching of COO^{+}). The finger print comparison of three spectra viz: of the reactants and product showed that frequency bands of COOH and >NH groups in Phenylalanine at 1565 and 1510 cm^{-1} of and frequency band 8-hydroxyquinaldine at 1565 cm^{-1} are affected due to complexation. Also comparison of the IR spectra of complexes with free 8-hydroxy quinaldine indicated that -OH frequency in quinaldine molecule in the region of 1360 cm^{-1} was affected due to complexation in complex frequency bands at 920, 870, 730, 425 cm^{-1} are due to quinaldine CH rocking and were not present in Phenylalanine. The spectral evidences show that the complex is present in its definite structure (Table 4-6).

Conclusion

In present work well defined stoichiometric and fluorescent complexes of amino acids Alanine and Phenylalanine with 8-hydroxyquinaldine have been prepared. The fluorescence activity of these complexes at various excitation and emission lines, has been fully exploited.

Improved detection limits have been achieved for amino acid by SPF and TLC-scanner. These techniques incorporate within them numerous experimental variables such as scale expansion, signal refinement and back ground off set system. Thus detection limit 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-8-hydroxyquinaldine complexes showed decrease in fluorescence intensity as compared with Amino acids-8-hydroxyquinoline complexes. The fluorescent complexes of amino acid with 8-hydroxyquinaldine are stable and can be stored for a year with their 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 final products with down to nano mole or pica mole level. The simple, economical and unequivocal preparation procedure of these complexes has been accomplished successfully, which has provide way to introduction of rapid and new analytical methodology for use in analytical laboratories for direct identification, separation and estimation of amino acids.

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