Comparative Study of Fluorescent Complexes of Amino Acids with 8-Hydroxyquinoline and 8-Hydroxyquinaldine Using Spectrofluorophotometer RF-510 and Thin Layer Chromatographic Scanner CS-910 in Aqueous Phase

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Summary: A comparative study using Spectrofluorophotometeric and Thin layer chromatographic techniques in acidic media has been carried out for fluorescent complexes of amino acids formed with 8-hydroxyquinoline and 8-hydroxyquinaldine at noted excitation and emission wavelengths.

Present study of new fluorescent complexes of amino acid were carried out using SPF on different excitation and emission wavelengths .The λ_{max} of each wavelengths values were confirmed using TLC scanner. The fluorescent complexes of amino acids with 8-hydroxquinoline have higher fluorescent intensity as compared with 8-hydroxyquinaldine.

Method adopted in these laboratories is rapid, versatile and provides encouraging results for adoption by analytical, agricultural and biomedical laboratories.

Introduction

Quinine and its derivatives are analytically and medicinally very important heterocyclic compounds. These have been extensively used as in pharmaceutical and oil industries, in analysis of metal ions, as an antioxidant and catalyst for polymerization [1].

In the present work new complexes not reported elsewhere have been studied exclusively in our laboratories by condensing glycine, alanine, valine, leucine, tyrosine and phenylalanine with oxine and 8-quinaldine separately. The glycine, alanine, leucine and valine are aliphatic amino acids they have no fluorescence activity alone [2-3], but when they complexed with 8-hydroxyquinaldine and 8-hydroxyquinoline separately they show their fluorescence activity on respective excitation and emission wavelengths [4-5].

Phenylalanine possesses a weakly absorbing benzene ring and does not emit fluorescence intensity enough for measurement in trace quantities. Several methods for estimating phenylalanine in biological samples are available [6].

Tyrosine strongly resembles phenylalanine but hydroxyl group on the ring. The hydroxy group is polar and readily interacts with water [7-9].

A detailed investigation using multiple analytical techniques involving fluorometry and thin layer chromatography is reported here.

The condensation of all amino acids with 8-hydroxyquinoline and its homologue 8-hydroxquinaldine produces products of definite stoichiometry. Above mentioned complexes have been prepared and extensively studied using spectrofluorometric method at their characteristic excitation and emission wavelengths [10]. The optimisation of experimental conditions provides quantitative and detection limits for amino acids complexes at nano mole level can be achieved applying this method.

Results and Discussion

The use of 8-quinaldine reagent for complexing with aliphatic and aromatic amino acids and its comparison to 8-hydroxyquinoline amino acids complexes indicate decrease in fluorescence signal of 8-quinaldine-tyrosine, glycine, alanine, valine, leucine and phenylalanine complexes (Table-1). This is in confirmity with theoretical predication that steric effect due to methyl group in 2-position with respect to nitrogen creates partial steric hindrance in complexation. Whereas no steric effect is present when amino acids complexes with oxine.

Table 1: Comparative study of fluorescent complexes of amino acids-8-quinolinol (a) and Amino acids -8-hydroxyquinaldine (b) using spectrofluorophoto meter model RF-510.

Amino acids complexes	Excitation wave-Lengths (nm)	Emission wave-Lengths (nm)	Detection limits (μmole/ml)
Glycine-8-quinolinol	375nm	430nmn	$2x10^{-4}(5.8x10^{-7})$
Glycine-quinaldine	355nm	435nm	
Alanine -8-quinolinol	330nm	420nm	$4x10^{-4}$ $(2x10^{-4})$
Alanine-quinaldine	345nm	430nm	
Valine-8-quinolinol	340nm	420nm	$2x10^{-4}$ $(5.8x10^{-7})$
Valine-quinaldine	365nm	440nm	
Leucine-8 quinolinol	330nm	400nm	$2x10^{-4}(1x10^{-6})$
Leucine-quinaldine	350nm	425nm	
Phenylalanine-8- quinolinol	315nm	400nm	$2x10^{-4}$ $(7x10^{-7})$
Phenylalnine-quinaldine	360nm	440nm	
Tyrosine-8-quinolinol	325nm	425nm	$2x10^{-4}(3.60x10^{-7})$
Tyrosine-quinaldine	350nm	480nm	

Key. Values in parenthesis indicate theoretical values calculated at S / N equal to two.

Experimental parameters: Gain = 01,Response = Medium, B. W λ_{Ex} =10nm, B. W λ_{EM}=10nm, Gain= 1

Note: At above mentioned λ_{Ex} and λ_{Em} reactants showed no fluorescence activity before complexation.

The fluorescence studies in solution indicated 1:3 stoichiometry as shown by maximum intensity in all complexes of tyrosine, glycine, alanine, valine, leucine and phenylalanine-oxine as well as with 8-quinaldine complexes.

The graphical representation shows the comparison of amino acids complexes with both reagents. It has been noted that starting from 1:1 mole ratio the fluorescence intensity goes to increase till it reaches to 1:3 mole ratio then it is decreased slowly. Such activity of complexes is observed in most of the amino acid complexes with both reagents. This probability due to forming ion pair, vanderwalls forces and hydrogen bonding plays an important role in association of extra molecule of free reagent with complexes. In most cases this is experimentally for maximum fluorescence intensity. However at 1:4 and 1:5 ratio of amino acid to reagent fluorescence intensity of complexes persists or decreases gradually probably due to quenching effect.

The method due to its versatility and rapidity can also be useful for biomedical and other laboratories as it has been found to be superior in comparison to existing analytical methods presently in use. 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 8-hydroxyquinoline in direct as well as indirect estimation procedures.

In the light of results as shown in tables (2-4 and 6-8) it has become apparent the fluorescence properties of alanine, glycine, leucine, valine, phenylalanine, tyrosine-8-quinaldine and alanine, glycine, leucine, valine, phenylalanine, tyrosine–8-hydroxyquinoline derivatives of amino acids would be of great interest for analysts. These reagents form highly fluorescent derivatives with above amino acids and it is a breakthrough 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.

Table 2

Mole ratio	% Fluorescence intensity		% Fluorescence intensity	
	Glycine-8-quinolinol	Glycine -8-quinaldine	Alanine-8-quinolinol	Alanine-8-quinaldine
1:1	48	24	55	20
1:2	72	34	116	29
1:3	96	40	141	37
1:4	90	36	134	32
1:5	87	30	127	26

Table 3

Mole ratio	% Fluorescence intensity		% Fluorescence intensity	
	Phenylalanine -8-quinolinol	Phenylalanine -8-quinaldine	Tyrosine-8-quinolinol	Tyrosine-8- quinaldine
1:1	66	24	100	56
1:2	136	44	210	112
1:3	199	68	450	160
1:4	195	64	429	148
1:5	180	60	408	126

Table 4

Mole ratio	% Fluorescence intensity		% Fluorescence intensity	
	Leucine-8-quinolinol	Leucine-8-quinaldine	Valine-8-quinolinol	Valine-8-quinaldine
1:1	54	32	40	36
1:2	111	42	91	48
1:3	200	58	185	72
1:4	190	46	180	80
1:5	180	40	175	50

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, biological and physiological laboratories for direct identification, separation and estimation of amino acids.

Experimental

Preparation of Solutions

0.01M solution of each amino acid and 0.01M reagents were prepared separately in 0.01M acetic acid solution in 250ml volumetric flask and stored as stock solutions. All the chemicals and reagents used were of analytical reagent grade.

Measurement of Spectra

Fluorescence spectra were recorded using Shimadzu Model RF-510 Spectrofluorophotometer equipped with Xenon discharge lamp. All experiments were conducted using 1cm^3 quartz cell. Excitation and emission band widths were fixed at 10 nm for all experiments.

TLC Chromatograms were recorded using Shimadzu Model CS-910 thin layer chromatographic scanner equipped with Xenon discharge lamp. All experiments were conducted using 20x20 cm standard glass pre-coated TLC plates supplied by M/S Merck, of Germany. A 0.25mm thick layer of silica gel 60 was spread on TLC plates, which were spotted with 2 to 8 µl solution. All the experiments were carried out using fluorescence mode.

Preparation of Tyrosine, Valine, Leucine, Glycine, Alanine and Phenylalanine-oxine and Tyrosine. Valine, Leucine, Glycine, Alanine and Phenylalanine -8- Quinaldine Complexes.

Equal volumes of 0.01M tyrosine, valine, leucine, glycine, alanine and phenylalnine were pipetted out separately in several pre-labelled conical flasks and known volumes of 0.01M oxine and 8-quinaldine solutions were added separately to these flasks to maintain ratio of above mentioned complexes 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 hour and then the watch glasses were removed and reaction mixture evaporated to semidried residue which was dissolved in acetic acid subjected to recrystallisation and to obtain light yellow products. 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. For identification and quantitative studies, the excitation wavelengths were fixed on specific lines and emission wavelengths also were fixed for respective complexes are given in table 1.

Original solutions of respective amino acids complexes was used for comparative study the results are summarised separately for all amino acids in table 2-4. It is noted that, amino acid complexes i,e tyrosine, valine, leucine, glycine, alanine and

Table 5;Determination of excitation and emission also fluorescence signal of amino acids-8-hydroxyquinaldine complexes using TLC-scanner Shimadzu CS-910.

Amino acids complexes	Excitation Wave-lengths (nm)	Emission Wave-lengths (nm)	Detection Limits (µ mole/ml)
Glycine-8-hydroxyquinaldine	340	450	2x10^{-2} (22x10^{-4})
Alanine-8-hydroxyquinaldine	350	450	8x10^{-2} (24x10^{-4})
Valine-8-hydroxyquinaldine	310	450	8x10^{-2} (17x10^{-4})
Leucine-8-hydroxyquinaldine	330	450	8x10^{-2} (22x10^{-4})
Phenylalanine-8-hydroxyquinaldine	325	450	8x10^{-2} (16x10^{-4})
Tyrosine-8-hydroxyquinaldine	320	450	8x10^{-2} (22x10^{-4})

Key. Values in parenthesis indicate theoretical values calculated at S/N equal to two.

S/N = Signal to the noise ratio

Experimental parameters: *Mode* = *Fluorescence,Response* = *Medium*, Scan = linear,

Scan speed = 20mm/min,Linearizer = (CH1)

Note: At these λ_{Ex} and λ_{Em} no any fluorescence activity has been recorded before complexation of amino acids with above both reagents

Phenylalanine with reagent oxine and 8-quinaldine indicate decrease in the fluorescence intensity of complexes tyrosine, valine, leucine, glycine, alanine and phenylalanine-8-quinaldine

For other analytical investigation such as thin layer chromatographic study 2 to 8μl of 2x10^{-4} M solution of respective complexes were spotted separately on the TLC plate with the help of micro syringe and the plate was run on TLC scanner using fluorescence mode. Detection limits of amino acids complexes has been achieved in nano gram level are given in table 5-8.

Table 6:Alanine-8-hydroxyquinaldine Phenyalanine-8-hydroxyquinaldine

Volume (μl)	Integral intensity	Integral intensity	FSD (mV)
02	06	28	20
04	14	50	20
08	30	100	20

Detection Limits

Alanine-8-hydroxyquinaldine Phenyalanine-8-hydroxyquinaldine

Volume (μl)	Integral intensity	FSD (mV)	Integral intensity	FSD (mV)
04	14	20	30	50
04	30	10	56	20
04	65	05	100	10

Table 7. Glycine-8-hydroxyquinaldin Valine-8-hydroxyquinaldine

Volume (μl)	Integral intensity	FSD (mV)	Integral intensity	FSD (mV)
02	14	10	8	20
04	24	10	17	20
08	37	10	35	20

Detection Limits

Glycine-8-hydroxyquinaldine Valine-8-hydroxyquinaldine

Volume (μl)	Integral intensity	FSD (mV)	Volume (μl)	Integral intensity	FSD (mV)
08	05	100	04	05	100
08	09	50	04	10	50
08	17	20	04	24	20
08	36	10	04	44	10
08	73	05	04	90	05

Table 8: Leucine-8-hydroxyquinaldine Tyrosine-8-hydroxyquinaldine

Volume (μl)	Integral intensity	FSD (mV)	Integral intensity	FSD (mV)
2	16	10	18	20
4	33	10	37	20
8	62	10	75	20

Detection Limits

Leucine-8-hydroxyquinaldine Tyrosine-8-hydroxyquinaldine

Volume (μl)	Integral intensity	FSD (mV)	Integral intensity
04	04	100	09
04	08	50	17
04	17	20	35
04	33	10	72

Conclusions

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.

The fluorescent complexes of amino acids with 8-hydroxyquinaldine are found stable and can be stored for at lest a year with their fluorescence intensity remaining unchanged.

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-droxyquinaldine as compared to 8-hydroxyquinoline, which is due to steric hindrance of α-methyl group on 8-quinaldine.

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