Quantitave determination of 8-hydroxyquinoline complexes of amino acids by fluorometric method

T. IQBAL, G.H. KAZI[+] AND Z.H. ZAIDI*

National Centre of Excellence in Analytical Chemistry, University of Sind, Jamshoro, Pakistan,
**H.E.J. Postgraduate Institute of Chemistry University of Karachi, Karachi-32, Pakistan.*

(Received 6th July, 1980)

Summary: The use f 8-hydroxyquinoline as fluorometric developer for amino acids has been reported[1] recently. In present studies detailed investigations have been carried out to measure quantitatively the fluorometric signal for sixteen 8-hydroxyquinoline derivatives of amino acids. The intensity of fluorceseence signal at various filter combinations and slit widths has been measured and limits of detection calculated by statistically analysing the experimental data.

The secondary effects encountered in similar fluorometric procedures[2,3] have been eliminated in this procedure.

The present method offers needed sensitivity, accuracy and simplicity, for both screening and confirmatory tests of all amino acids.

Introduction

The use of 8-hydroxyquinoline reagent for fluorometric studies of metals such as magnesium, aluminium and gallium is reported by many workers[4].

In our earlier communication[1] the use of 8-quinolinol in flourometric detection of all amino acids has been reported Subsequently the present work involved detailed quantitative estimation of amino acids by preparing their corresponding 8-quinolinol adducts in aqueous acidic solutions and measuring their flourescence intensity at 405 nm. The results are encouraging and offer a versatile method of quantitative estimation of amino acids with possible application in quantitative determination.

Experimental

10^{-3}M master solutions of 8-hydroxyquinoline and each amino acid were prepared in water acidified with acetic acid. Four test solutions of each amino acid 8-hydroxyquinoline adducts were prepared by pippetting out 5, 10, 15 and 20 ml of amino acids in different flasks and adding similar volumes of 8-hydroxyquinoline. The final volume of each test solution was made 5.0 ml by evaporation. Thus amount of amino acid in flasks Number 1 to 4 corresponded to 5, 10, 15 and 20μ moles/5ml respectively. Solutions were examined for fluorescent signal on Turner model 110 fluorometer, using various filter combinations, slit widths and meter sensitivity to obtain maximum signal .for each amino acid under investigation show in graphs I – IX.

The fluorescence intensity of some amino acids (Phenylalanine and Tryptophan) was very high with various filter combinations and slit opening. Therefore, they were further diluted proportionately so as to investigate the lowest possible concentration at which reasonable signal (S/N ratio= 2) could be obtained with the available instrument.

Results and Discussion

Ninhydrin is a powerful oxidizing agent; it causes oxidative decarboxylation of amino acids, producing carbon dioxide, ammonia and an aldehyde with one carbon atom less than the parent amino acid. The reduced ninhydrin than reacts with the liberated ammonia forming a blue complex, namely Ruhemann's purple having a maxima at 570 nm[5,6].

The intensity of blue colour produced under standard conditions is the basis of an extremely useful quantitative test for α-amino acids. The amines also react with ninhydrin forming blue colour without evolving carbon dioxide and ammonia. Proline and hyrdroxy-

[+]To whom all correspondence may be made.

Table I. Slopes and intercepts of linear graphs of concentration versus fluorescence intensity data of standard solutions of amino acids 8-hydroxyquinoline adducts, using Turner Model 110 fluorometer.

(Concentration range = 5.0 to 20 μ moles/5ml test solution).

Filters	Slit Pass	Wave length nm.	Statistical Analysis.	Phe.	Alo.	Glu.	Try.	Pro.	Thr	Cys	His.	Tyr.	Leu.	Gly.	Met	Lys.	Arg.	Asp.	Val
Primary 405 Secondary 3	1 X	405	Slope "m"	13.94	7.02	7.08	4.02	4.86	4.19	2.98	2.29	2.43	3.33	6.13	3.08	1.87	0.95	1.95	3.10
			Intercept "c"	-0.80	-.0.22	-0.73	0.20	-0.39	-0.29	-0.29	-0.40	0.20	-.0.20	0.80	-0.80	0.00	0.00	-0.20	-0.39
			Corr. Coeff. "r"	0.999	0.997	0.996	1.12	0.997	0.995	1.16	0.986	0.996	0.996	1.06	0.980	0.995	0.82	0.991	0.994
			"o x"	1.17	0.65	0.99	1.63	0.81	0.84	1.18	1.47	1.28	0.93	0.53	1.05	1.28	1.48	0.94	0.82
			"o y"	16.28	4.43	7.08	5.82	3.96	3.55	3.03	3.44	3.12	3.12	3.30	3.31	2.42	1.76	1.85	2.56
	3 X	405	Slpe "m"	39.51	21.28	22.91	11.86	14.41	12.93	9.63	7.27	7.95	9.71	14.23	7.51	5.49	3.61	5.26	7.06
			Intercept "c"	-2.90	-0.85	0.19	0.24	-0.80	0.99	-1.2	-1.78	-1.80	-0.51	-1.20	-0.80	0.00	0.00	0.20	-0.19
			Corr. Coef. "r"	1.26	0.980	1.00	1.12	0.997	0.994	0.989	0.989	0.973	0.987	1.09	0.997	0.999	0.997	0.998	0.994
			"o x"	1.17	0.65	0.99	1.63	0.81	0.84	1.18	1.47	1.28	0.93	0.53	1.05	1.28	1.48	0.94	0.82
			"o y"	36.54	13.52	22.93	17.16	11.77	10.97	11.51	10.87	10.46	9.22	6.78	7.93	7.07	5.39	4.95	5.83
	10 X	405	Slope "m"	–	51.68	55.10	–	36.32	39.13	23.43	21.72	25.30	33.94	35.20	22.82	14.39	11.14	16.24	25.38
			Intercept "c"	–	1.50	-0.17	–	-0.42	-3.18	0.00	-3.40	-3.00	-4.00	-1.00	-1.80	1.00	-0.80	1.20	-1.84
			Corr. Coef. "r"	–	0.996	0.999	–	0.995	0.995	0.995	0.989	0.985	0.989	0.999	0.999	0.998	0.995	0.997	0.996
			"o x"	–	0.49	0.60	–	0.65	0.84	0.98	1.47	1.28	0.93	0.53	1.05	1.28	1.48	0.94	0.82
			"o y"	–	25.81	33.70	–	23.59	33.13	22.93	32.43	32.98	32.01	18.74	24.07	18.56	16.63	15.31	20.90

Table II. Slopes and intercepts of linear graphs of concentration versus fluoroscence intensity data of standard solutions of amino acids 8-hydroxyquinoline adducts, using Turner Model 110 fluorometer.

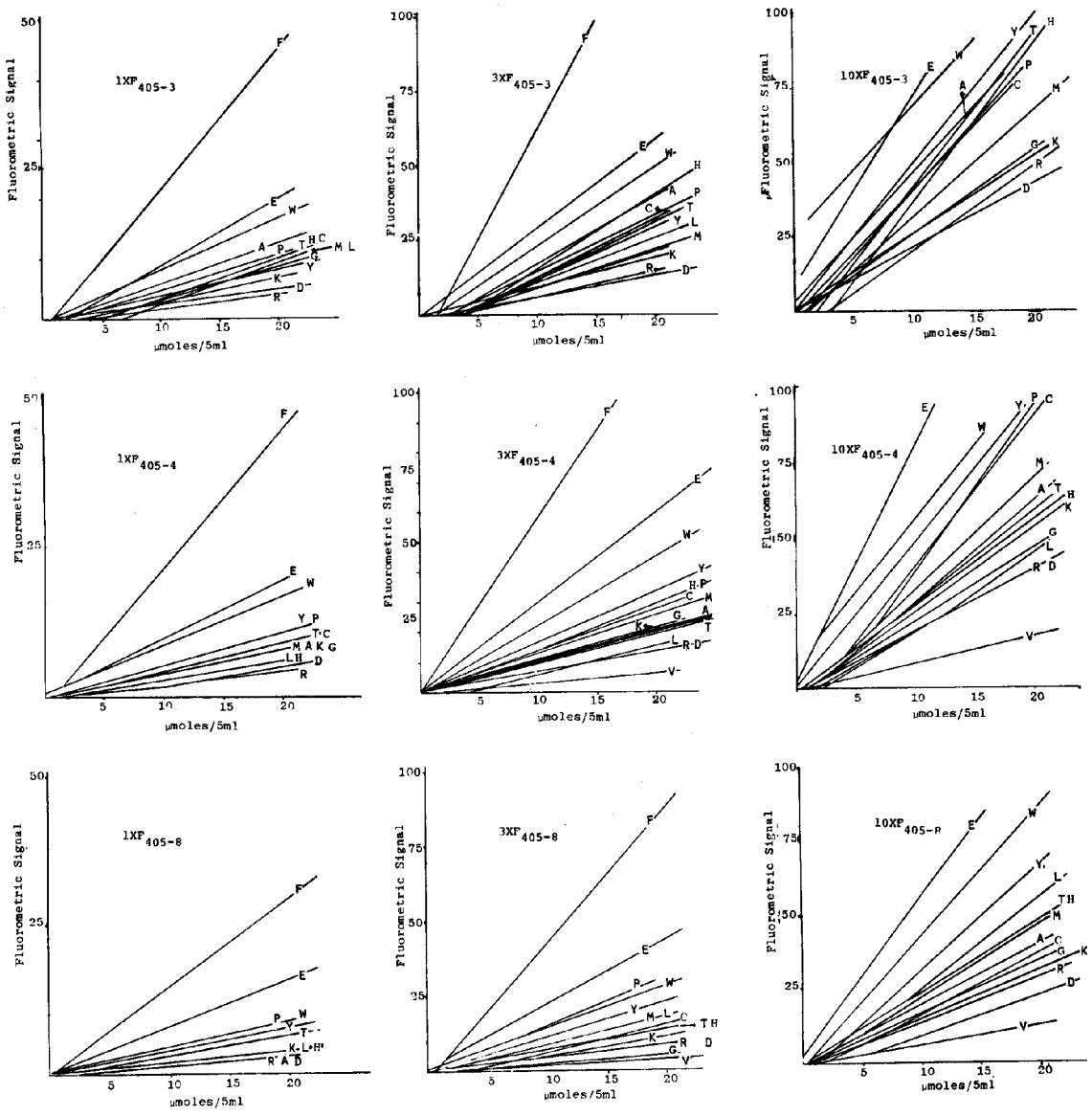
(Concentration range 5.0 to 20 μ moles/5ml test solution)

Filters	Slit Pass	Wave Length nm.	Statistical Analysis.	Phe.	Ala.	Glu.	Try.	Pro.	Thr.	Cys.	His.	Tyr	Leu.	Gly.	Met.	Lys.	Arg.	Asp.	Val.
Primary 405 Secondary 4	1 X	405	Slope "m"	14.42	4.30	6.24	3.52	4.86	3.02	2.75	1.54	2.98	2.57	5.33	2.82	2.19	0.95	1.95	–
			Intercept "c"	-0.40	-0.02	0.48	0.53	-0.39	-0.39	-0.19	-0.180	0.20	-0.60	0.00	-0.06	0.00	0.00	-0.2	–
			Corr. Coef "r"	0.998	1.03	0.990	1.09	0.997	0.994	0.998	0.999	0.998	0.968	0.999	0.987	0.999	0.999	0.991	–
			"o x"	1.17	0.65	0.99	1.63	0.81	0.84	1.18	1.47	1.28	0.93	0.53	1.05	1.28	1.48	0.94	–
			"o y"	16.86	2.71	6.28	5.23	3.96	2.56	3.26	2.28	3.82	2.48	2.83	3.01	2.83	1.41	1.85	–
	3 X	405	Slope "m"	9.21	11.78	19.43	10.68	13.19	8.56	8.94	4.68	9.50	6.06	12.27	8.86	5.46	3.21	4.96	2.42
			Intrecept "c"	-1.0	0.36	1.00	1.40	-1.39	-0.59	-1.08	0.20	-0.60	-1.0	-.06	-1.2	0.85	0.40	0.20	0.19
			Corr. Coef "r"	0.997	0.992	0.995	1.12	0.995	0.998	0.994	0.995	0.997	0.982	0.988	0.992	1.03	0.998	0.991	0.959
			"o x"	1.17	0.65	0.99	1.63	0.81	0.84	1.18	1.47	1.28	0.93	0.53	1.05	1.28	1.48	0.94	0.82
			"o y"	10.78	7.41	19.97	15.51	10.79	7.22	10.63	6.95	12.19	5.76	6.56	9.42	6.79	4.69	4.75	2.13
	10 X	405	Slope "m"	–	32.78	55.78	–	40.08	25.69	28.02	13.59	28.84	16.66	30.93	25.36	11.21	9.43	13.98	7.24
			Intercept "c"	–	1.29	0.33	–	-4.58	-1.59	-1.39	0.00	-0.90	-0.8	-1.2	-3.0	0.00	0.20	2.40	0.40
			Corr. Coef. "r"	–	0.989	0.996	–	0.992	0.998	1.07	0.999	1.61	0.989	1.02	0.994	0.995	0.999	0.986	0.998
			"o x"	–	0.65	0.60	–	0.81	0.84	1.18	1.47	1.01	0.93	0.53	1.05	1.28	1.48	0.94	0.82
			"o y"	–	20.71	33.48	–	32.93	21.71	30.85	22.09	18.09	15.73	16.45	26.87	14.49	14.01	13.33	5.95

Table III. Slopes and intercepts of linear graphs of concentration versus fluorescence intensity data of standard solutions of amino acids 8-hydroxyquinoline adducts using Turner Model 110 fluorometer.

(Concentration range 5.0 to 20 μ moles/5ml test solution).

Filters	Slit Width	Wave Length nm.	Statistical Analysis	Phe.	Ala.	Glu.	Try.	Pro.	Thr.	Cys	His.	Tyr.	Leu.	Gly.	Met.	Lys.	Arg.	Asp.	Val.
Primary 405 Secondary 8	1 X	405	Slope "m"	9.21	2.13	5.93	2.16	3.99	2.85	1.71	0.985	2.09	2.25	–	2.15	0.87	0.71	1.11	--
			Intercept "c"	-1.0	-0.37	-0.28	0.40	0.20	-0.59	-0.11	0.00	0.00	-0.34	–	-0.80	-0.4	-0.17	-0.17	–
			Corr. Coef. "r"	0.997	0.939	0.990	1.12	0.997	0.970	0.978	0.999	0.990	0.982	–	1.07	0.97	0.982	0.982	–
			"o x"	1.17	0.65	0.99	1.63	0.81	0.84	1.18	1.47	1.28	0.93	–	1.05	1.28	1.48	0.94	–
			"o y"	10.78	1.48	5.67	3.12	3.26	2.48	2.05	1.41	2.71	2.24	–	2.24	1.17	1.11	1.12	–
	3 X	405	Slope "m"	26.79	4.99	15.16	6.67	11.28	6.38	4.74	3.27	6.07	7.08	4.0	6.30	3.18	2.19	2.71	2.23
			Intercept "c"	-2.0	0.37	-0.88	0.80	-0.39	-0.39	-1.50	-0.02	0.40	-1.6	-0.4	-1.2	-0.2	-0.20	0.40	-0.48
			Corr. Foef. "r"	0.989	0.996	0.995	1.11	0.999	0.996	0.969	0.997	0.995	0.956	0.984	0.986	0.993	0.997	0.975	0.989
			"o x"	1.17	0.65	0.99	1.63	0.81	0.84	1.18	1.47	1.28	0.93	0.53	1.05	1.28	1.48	0.94	0.82
			"o y"	31.29	3.12	15.19	19.73	9.20	5.38	5.78	5.23	7.11	6.74	2.15	6.73	4.19	3.26	2.61	1.85
	10 X	405	Slope "m"	–	16.34	35.52	20.17	35.54	19.99	11.04	11.37	17.12	20.15	22.40	17.85	7.91	6.67	7.22	5.52
			Intercept "c"	–	1.72	1.73	1.52	-4.80	-1.59	-2.88	-0.32	1.00	-3.60	-0.2	-0.20	1.0	-2.0	2.20	0.02
			Corr. Coef. "r"	–	0.981	0.998	1.10	0.990	0.998	0.976	0.998	0.985	0.967	0.987	0.987	0.995	0.999	0.955	0.999
			"o x"	–	0.65	0.78	1.63	0.81	0.84	1.18	1.47	1.28	0.93	0.53	1.05	1.28	1.48	0.94	0.82
			"o y"	–	10.38	28.06	29.73	29.15	16.87	13.37	16.56	22.24	19.45	2.15	19.07	10.93	9.90	7.11	1.85



proline produce a yellow colour rather than Ruhemann's purple colour and has a maxima at 440 nm.

The blue colour of complex is unstable, however, when sprayed with cupric nitrate, cadmium acetate or strontium solution[7,8,9], the complex obtained is stable. Copper ninhydrin complex dissociates reversibly between pH 7 and 9, but above pH 9 the decomposition of complex occurs and reaction becomes irreversible. It can be used for quantitative analysis of amino acid at nanomole range detection. Recently flourescamine reagent has also been employed and a fluorometric spectrophotometer is used as a detector for quantitative determination in amino acid analysers. [10]

Considering the limitations and disadvantages of existing colour developing reagents of ninhydrin for amino acids we have carried out investigations on the use of 8-hydroxyquinoline in forming adducts or complexes which produce fluorescence and the products are stable in pH range 4 to 9 and can be measured in aqueous or non-aqueous media.

The mechanism of formation of the fluorescent amino acid 8-hydroxyquinoline species is not yet clear. The products are possibly some type of adducts or weak complexes with carboxylic and amino groups of amino acids involved with quinoline ring nitrogen and hydroxyl group. More investigation are needed for establishing exact stoichiometric composition.

The fluorescence intensity of various amino acid 8-hydroxyquinoline species has successfully been measured with Turner Model 110 fluorometer using different combinations of primary and secondary filters and available slit pass. The meter reading equal to two was taken as legitimate for calculating the minimum concentration of amino acids by appropriate dilution of the stock solutions. The instrumental noise level was negligi-

and the matrix interferences were automatically levelled off by using blanks prepared by mixing all the ingredients under identical conditions except acid under investigation. The reading for water was also recorded to check blank flourescence.

The enhancement of fluorometric signal is observed when oxine and amino acid solutions are heated and evaporated to semi-solid residue and redissolved in water to make appropriate volume.

The quantitative determination of amino acids by fluorometric method has been carried out by using a simple and easily available reagent (8-hydroxyquinoline). It offers simplicity and cuts down long procedure involved in existing methods.

The fluorometer turner Model 110 has been used, in these experiments. A more sensitive instrument may provide better limits of detection possibly in picomole range. In present work the limit of detection achieved ranges between 10 and 100 nanomoles/liter. Further experimental work is required to identify and quantitatively estimate various amino acids present in unknown mixtures by investigating known mixtures.

References

1. T. Iqbal, G. H. Kazi and Z. H. Zaidi, *J. Chem. Soc. Pak.* **2**, 47 (1980).
2. M.W. McCaman and E. Kobins, *J. Lab. Clin. Med.* **59**, 885 (1962).
3. T.P. Waales and S. Udenfriend, *J. Lab. Clin. Med.* **50**, 733, (1957).
4. D. Schachter, *J. Lab. Clin. Med.* **54**, 763 (1959).
5. R. Consden, A. H. Gordon and A.J.P. Martin, *Biochem. J.* **41**, 590 (1947) Ibid **38**, 224 (1944).
6. Tsukamoto T., and T. Komori, *Chem. Pharm. Bull* (Tokyo) **8**, 913 (1960).
7. E. Kawerau and Th. Wieland, *Nature* **168**, 77 (1951).
8. Moor, S. and Stein, W.H, *Ann. N. Y. Acad. Sci* **49**, 265 (1948).
9. A. Abbasi, A. Rashida and Z.H. Zaidi, *J. Biochem. Biophys. Method.* **3**, 311 (1980).
10. Weigle, M. De Bernardo S.L., Tengi, J.P; Leimgruber W., *J. Amer. Chem. Soc.* **94 (16)**, 5927 (1972).