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SPECTROPHOTOMETRIC METHOD OF DETERMINATION OF NATURAL INDOLES BY COLOURED COMPLEXES

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ABSTRACT

A new spectrophotometric method has been developed for quantitative determination of naturally occurring indoles via electron donor-acceptor complexes with chloranil. The method is simple, precise and convenient for routine laboratory work. The analysis of each indole can be carried out within half an hour.

Introduction

The indole nucleus have been known to act as a good electron donor in charge transfer complexes (Millie, et al, 1968; Manzar, 1982). In the literature a considerable amount of data involving charge transfer properties of indoles is available, as various biologically important compounds posses the indole ring system (Hutzinger, et al. 1971 Cilento, 1961). The charge transfer phenomena have also been used in quantitiative determination of indoles by spectrophotometric method using various strong acceptors (Manzar, 1981, 1982).

In this article some naturally occurring indoles, which are biologically and physiologically important have been used for quantitative determination by spectrophotometric method, Chloranil is employed as a new complexing agent for their quantitative determination. The mentioned natural indoles have until now not been spectrophotometrically determined via coloured complexes with chloranil.

Results and Discussion

A new spectrophotometric method was developed for the quantitative determination of naturally occuring indoles with choranil. These natural indoles are structurally similar compound in indoles with the same substitution pattern (e.g. 3-indole-CH_2). The colours obtained from natural indoles with chloranil are shown (Table 1, 2). The colour showed little change and were stable for hours in a laboratory atmosphere.

Structurally similar natural indoles gave similar colour with the complexing reagent. The colour form with substituted indoles shows a clear and consistant correlation with the electron-donor acceptor properties of the substitutent (Hutzinger, 1969). The in. crease in complex stability with increasing electron-donor properties of the indole is reflected in these colour changes (Berg and Lam, 1964). The mechanism of colour formation varies with different types of compounds, the colour formed with indoles is unoubtedly due to complex formation (Szent-Gyorgvi and Isenberg, 1960). Result shows that the present

Table 1. Colour complex of Natural Indoles with Chloranil

Indole	Colour	Appearance of colour (min.)	Stability of colour (min.)
Gramine	Violet	At *on*ce	30
Melatonin	Purple-Violet	At once	70
Serotonin	Grayish-Violet	At once	120
Tryptophan	Violet	At once	1S0
Tryptamine	Brown	At once	130

method is suitable for all those substituted natural indoles which form stable coloured complexes with a good accepted like cliloranil. The molar solution of each natural indoles and the reagent were mixed. The immediate appearance of colour is a characteristic feature of charge transfer complexes as shown (Table 1, 2). Results show that the stability of coloured complexes are not less than 30 minutes which are good enough for analytical purposes.

Absorption spectra of these natural indoles were taken in the region of 450-600 tan. In case of serotonin tryptophan and tryptoamine the maximum absorption appeared sharply in the region of 470-500 nm. The intense absorption of the visible and UV region of the spectrum is infact the most prominent feature of 1:1 molar ratio complexes (Muhiken. 1952). Complex composition of natural indoles with chloranil was determined by isomolecular series method and was found in the ratio 1:1. The ratio 1:1 is generally suitable for quantitative analysis. This ratio for the first time allowed a complete quantitation of studied natural indoles with chloranil in the form of charge transfer complexes. Serotonin is a very good donor than tryptophan and tryptamine (Kerremen, et at. 1969). Thus it has a good action with the reagent and forms more stable complexes. Serotonin was also taken to shown 1:1 ratio. Lambert-Beer's law was applied and the concentration of each indole complex solution was calculated under the following wave

Table 2. Conditions for the formation of coloured complexes of Natural Indoles with chloranil

Indole	Wt. of the substance 10 mg	Concentration of the substance 10^{-4} M/1
Gramine	10.0	1.15
Melatonin	25.0	2,16
Serotonin	20.0	2.27
Tryptophan	25.0	2,45
Tryptamine	125	1,56

Table 3. Quantitative determination of natural indoles with chloranil.

Natural Indole	Given 10^{-4} M/1	Observed 10^{-4} M/1	S*	S_r**
Gramine	3.45	3.40	0.0751	0.0220
Melatonin	8.64	8.50	0.0509	0,0059
Serotonin	9.08	9.04	0.0170	0.00188
Tryptophan	7.35	7,30	0.0576	0.00789
Tryptamine	6.24	6.28	0.0404	0.00643

*S = Standard deviation
**S_R = Relative Standard deviation

length: gamine, λ_{max} = 520 nm. serotonin, λ_{max} = 500 nm. tryptoamine, λ_{max} 490 nm, tryptophan, λ_{max} = 480 um, serotonin, λ_{max} = 520 nm. Results of the quantitative determination of each natural indole are given in Table 3.

Results (Table 3) show that the statistical values are satisfactory for analytical purpose. The proposed method may be recommended for the quantitative determination of natural indole which form coloured complexes with a strong acceptor such as chloranil, The method of analysis is precise, cheap and simple. The method is used to deter mine up to 10^{-4} Mil of coloured complex solution with a relative standard error S_r =-0.0. The total time period of each analysis is 20 minutes.

Experimental

Materials:

Following natural indoles were used in this study: (1) granine, (2) melatonine, (3) serotonin, (4) tryptophan, (5) tryptamine.

The natural indoles used in this investigation were commercial samples purchased from the following companies: Aldrich, Regis and Sigma. The electron acceptor chloranil was also obtained from commercial source.

Solution:

(i) Reagent solution was prepared in acetone (AR quality, Merck) 10^{-4} M/1.
(ii) Indole solution was prepared in 50 ml ethanol (pure) 10^{-4} M/1.

Colour development:

Conditions for the formation of coloured complexes are shown in Table 2. Indoles (10-25 mg) in alcoholic solution with 100 mg chloranil in acetone in 50 ml flask.

Method of analysis:

Dissolve 10 mg indole derivatives in ethanol in 50 ml volumetric flask. Pipette out 1, 2, 3, 4, 5, 6 ml solution from the 50 ml flask into 10 ml volumetric flask separately. Add 4 ml reagent solution in each 10 ml volumetric flask shake each flask until the colour is developed at room temperature keeping the solution for few minutes at laboratory atmosphere until a stable coloured solution is obtained. Make up the volume with the solvent and then determined the optical density in a EISS spectrophotometer in a 1 cm curvette. Reagent solution was used as a reference. Calibration curve were determined for each natural indole solution. Isomolecular series method was used to determine the complex composition (Job, 1928; Voburoch and Cooper, 1941).

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