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SPECTROPHOTOMETRIC DETERMINATION OF NATURAL INDOLES BY COMPLEXING WITH 1,3,5-TRINITROBENZENE

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ABSTRACT

A spectrophotometric method has been developed for the quantitative determination of some natural indoles by complexing with the electron acceptor 1,3,5-trinitrobenzene and measuring the absorbance in the region 460-529nm. The RSD of the method is 2.5%. It is simple; rapid and convenient for the routine analysis of indoles compounds.

Introduction

The indoles nucleus has been known to act as a good electron donor in charge transfer complexes (Millie *et al,* 1968; Fujimore, 1959; Foster and Hanson, 1964). Considerable amount of data involving charge transfer properties of indoles is available because several biologically important compounds possess the indoles ring system and charge transfer phenomena have been implied in explaining their mode of action (Hotzinger, 1969). The pronounced electron donor ability of indoles has also been utilised for quantitative determination of these compounds (Manzar and Kost, 1980; Manzar, 1981). Most of the relevant literature in the field has previously been cited (Manzar, 1981, 1982).

Intense colour are usually associated with charge transfer complexes in the solid state (Janak, 1964; Peurifoy and Nager 1960; Holzinger, 1969) as well as in solution (Bullock, 1967; Szent-Gyorgy, 1960). This property has been used as a basis of the quantitative determination of natural indoles.

In this paper we report the use of a complexing agent for quantitative determination of natural indoles which are biologically important.

Material and Methods

Material

The natural indoles gramin, tryptophol, tryptophan, tryptoamine and serotonin were commercial samples purchased from Aldrich/Eastman. The electron acceptor, 1,3,5-trinitrobenzene, was also obtained from commercial source.

Reagent solution

A 10^{-4}M solution of 1,3,5-trinitrobenzene was prepared in acetone (AR quality, Merck).

Indole solution

A 10^{-4}M solution of the individual indole was prepared in methanol or acetone.

Colour development

Indoles dissolved in methanol or acetone (50 ml) were treated with the solution of the complexing agent 1,3,5-trinitrobenzene in acetone (4.0 ml).

Method of analysis

Solutions of individual indoles were made by dissolving appropriate quantity of the substance in a 50 ml volumetric flask. 1-6 ml of the indole solution was pipetted out and placed into 10 ml volumetric flasks separately. 4.0 ml of freshly prepared solution of 1,3,5-trinitrobenzene was added to each flask and kept for a few minutes at laboratory temperature (25-28°C) until a stable colour was obtained. The volume was made up with the solvent (acetone or methanol) and the absorbance of each coloured sample was measured at the respective absorption maximum on EISS spectrophotometer in a 1.0 cm cuvette using the reagent solution as blank. Calibration curve was constructed for each natural indole solution and the validity of Beer's law confirmed. Isomolecular series method was used to determine the composition of complexes (Job, 1969; Voburch and Cooper, 1941).

Results and Discussion

A new electron acceptor 1,3,5-trinitrobenzene has been used for quantitative determination of naturally occuring indoles by spectrophotometric method. These indoles have recently been determined spectrophotometrically with another strong electron acceptor, chloranil (Manzar and Fasihullah,1988).

The natural indoles studied are structurally similar with the same substitution pattern. The colours obtained from the various indoles with 1,3,5-trinitrobenzene are shown in Table 1. All colours were produced immediatley on mixing the solutions and were stable over the period of measurement. The colours are ascribed to charge-transfer transitions between the acceptor and the donor complexes (Hammond and Burkardt, 1970). Structurally similar indoles gave similar colour with 1,3,5-

trinitrobenzene. The colours formed with substituted indoles showed a clear and consistent correlation with the electron donor-acceptor properties of the substituent (Hutzinger, 1969). The increase in the stability of the complex with increasing electron-donor properties of the indole is reflected in the intensity of the colour changes (Berg and Lam, 1964). This has been shown very clearly in the case of serotonin. Results show that the present method is suitable for all those substituted indoles which form stable coloured complexes with a strong electron acceptor like 1,3,5-trinitrobenzene. The immediate appearance of colour is a characteristic feature of these charge-transfer complexes (Mulliken, 1950). The stability of coloured complexes is not less than 20 minutes which is good enough for analytical purposes.

The absorption characteristics of the complexed indoles (Table 3) provide useful information from the qualitative and quantitative analytical point of view and also with regard to the basic understanding of parameters influencing complexation (Hutzinger, 1964).

The absorption spectra of all natural indoles were found to absorb between the wavelengths of 460-520nm. Thus the complexes are characterised by intense absorption in the visible region which is in fact the most prominent feature of 1:1 molar complexes (Both, 1960). A satisfactory explanation of this property is given by Mulliken's (1950) charge-transfer theory. In case of serotonin and tryptamine the maximum absorption was markedly increased. For serotonin, which is 5-hydroxy-tryptoamine, the absorption is more prominent. Serotonin is a good donor as compared with the other natural indoles (Kerreman *et al.*, 1959) so it gives better results with the reagent and also forms a more stable complex

The composition of the coloured complexes of natural indoles with 1,3,5-trinitrobenzene was determined by isomolecular series method (Job, 1969; Voburoch and Cooper, 1941) and was found to be in the ratio of 1:1. Using the Beer's law relation the concentration of each indole complex in the solution was calculated. Results of the quantitative determination of each indole by this method are given in Table 2 and 3. The optical characteristics and precision data obtained by regression analysis are reported in Table 3.

Statistical values clearly show that the results of the analysis of indoles with 1,35-trinitrobenzene are quite satisfactory. The proposed method of analysis may be recommended for the quantitative determination of natural indoles by complexing with 1,3,5 dinitrobenzene. The method is simple and economical. The relative standard deviation in each case is within 25%. The time period requ[i]red for each analysis is about 20 minutes.

Table 1

Characteristics of coloured indole complexes with 1,3,5- trinitrobenzene.

Indole	Colour	Appearance of colour (mins)	Stability of colour (mins)
Gramin	Orange	Immediate	20
Tryptophol	Orange	Immediate	30
Tryptophan	Light orange	Immediate	55
Tryptoamine	Orange	Immediate	90
Serotonin	Purple	Immediate	120

Table 2

Quantitative determination of indoles with 1,3,5-trinitrobenzene

Indoles	Added $Mx10^4$	Found $Mx10^4$	SD	RSD
Gramin	5.51	5.49	0.091	0.0167
Tryptophol	2.40	2.45	0.032	0.0133
Tryptopan	4.00	4.06	0.098	0.0242
Tryptoamine	3.00	3.04	0.078	0.0259
Serotonin	5.64	5.71	0.082	0.0143

Table 3

Optical characteristics and precision data obtained by regression analysis.

Indoles	λ_{max}(nm)	Concentration range, $Mx10^4$	Molar Absorptivity	Intercept	RSD %
Gramin	500	1.0-8.0	430	0.002	1.67
Tryptophol	460	1.0-8.0	650	0.001	1.33
Tryptopan	490	1.0-8.0	1420	0.0005	2.42
Tryptoamine	510	1.0-8.0	1270	0.002	2.59
Serotonin	520	1.0-8.0	770	0.0005	1.43

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