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SPECTROPHOTOMETRIC DETERMINATION OF NATURAL AND SUBSTITUTED INDOLES BY COMPLEXING WITH MALEIC ANHYDRIDE

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ABSTRACT

A Spectrophotometric method has been developed for the quantitative determination of natural and ring-substituted bidder by complains with malefic anhydride. The coloured complex exhibits absorbance maxima in the region 490.530 nm. The RSD of the method is 26%. The method is specific and simple and thus ideal for the routine analysis of indole compounds by absorbance measurements in the visible region. The analysis of each indole complex can be carried out within 15 minutes.

INTRODUCTION

Considerable interest has been shown in the past to study the electron donor properties of indoles in charge transfer complexes (Szent-Gyorgi and Isenberg, 1960; Millie *et al*, 1968). Many natural biologically important compounds possess the indole nucleus and the charge transfer phenomena has been implied in quantitative determination of these compounds in the form of coloured complexes (Hutzinger, 1969). Information about the identification of these complexes has been gathered using ultraviolet and visible spectrophotometric methods (Sundberg, 1970; Andrews and Keefer, 1964). A satisfactory explanation of the properties of these complexes has been provided by Mulliken's charge transfer theory (Milliken, 1952). Most of the relevant literature in the field has previously been cited (Manzar, 1980-1982).

Several electron acceptors are available for the quantitative determination of natural and ring substituted indoles (Manzar, 1981, 1982; Manzar and Fasihullah, 1988 Manzar and Alam, 1992). These colour reagents are sensitive and specific and give a variety of colours with different indoles (Manzar and Koss, 1980; Hutzinger and Hearcock, 1972).

In this work a new electron acceptor, maleic anhydride, is employed for the quantitative determination of the natural and substituted indoles by spectrophotometric method. This new electron acceptor forms stable complexes with indoles. These coloured complexes are suitable for the analytical work. The natural indoles mentioned in this study have not been photometrically detected before in the form at coloured complexes with maleic anhydride.

MATERIALS AND METHODS

The following natural indoles and ring-substituted indoles were used in this investigation:

Gramin, serotonin, 3-indole acetic acid, 3-indole acetaldehyde and 5-hydroxy-3-indole acetic acid.

Indoles used in this study were commercial samples purchased from Aldrich Pharmaceuticals, USA. The electron acceptor, maleic anhydride, was obtained from Anechemia Co.

All solvents and reagents used were analytical grade or of the purest form available from BDH/Merck.

Reagent solution

A 10^{-4}M solution of maleic anhydride was prepared in acetone.

Indole solution

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

Colour development

Indoles dissolved in methanol or isopropyl alcohol were treated with the solution of the complexing agent, maleic anhydride in acetone.

Method of analysis

Solutions of the 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 the freshly prepared solution of maleic anhydride was added to each flask and kept for a few minutes at laboratory temperature (28° - 30°C) until a stable colour was obtained. The volume of the solution was made up with the solvent (acetone or methanol) and the absorbance of each coloured sample was measured at the respective maximum on EISS Spectrophotometer in a 1.0 cm cuvette using the reagent solution as blank. A calibration curve was constructed for each natural indole solution and the derivative and the validity of Beer's law was confirmed in the region. The concentration of each indole complex in the solution was calculated at the wavelengths of absorption maxima; gramin, 510 nm; serotonin, 560 nm; 3-indole acetic acid, 500 um; 3-indole acetaldehyde, 490 nm and 5-hydroxy-3-indole acetic acid, 530 nm.

RESULTS AND DISCUSSION

A new electron acceptor maleic anhydride has been used for the quantitative determination of naturally occuring indoles and ring-substituted indole derivatives by spectrophotometric method. The indoles gramin and serotomin have previously been determined spectrophotometrically with other strong electron acceptors such as chioranil (Mannar and Fasihullah, 1988) and 1,3,5-trinitrobenzene (Manzar and Alam, 1992).

The ring substituted indole derivatives, 3-indole acetic acid, 3-indole acetaldehyde and 5-hydroxy-3-indole acetic acid have not until now been photometrically determined in the form of acceptor-donor complexes with maleic anhydride. The colours obtained from natural indoles and ring-substituted indole derivatives after reaction with maleic anhydride are shown in Table 1.

All colours were produced immediately on mixing the solutions and were stable over the period of measurement (Table 1).

Table 1
Characteristics of coloured indole complexes with maleic anhydride

Indole	Colour	Appearance of colour	Stability of colour (mins.)
Gramin	Light yellow	Immediate	25
Serotomin	Brown-grayish	Immediate	60
3-Indole acetic acid	Brown	Immediate	5-25
3-Indole acetaldelyde	Yellowish	Immediate	50
5-Hydroxy-3-indole acetic acid	Brown	Immediate	120

Structurally similar compounds give similar colours with the complexing agent. The colour formed with ring-substituted indoles shows a clear and consistent correlation with the electron donor-acceptor properties of the substituent (Cooper *et al.*, 1966). The colour varies from yellow to brown when the substituent is

changed from electron donating group in position 5 of the indole ring. As the substituent is changed by the strong electron donating hydroxyl group, the colour becomes more intense and the stability is increased. The stability of the complex increases with increasing electron donor properties of the indole as reflected in these colour complexes (Griegleb, 1961). The influence of 5-hydroxy substituent on complex formation is illustrated by serotonin and 5-hydroxy indole acetic acid. The mechanism of colour formation varies with different types of substituents, the colours formed with indoles and maleic anhydride are undoubdtedly due to complex formation (Foster and Hanson, 1964). The immediate appearance of colour is a characteristic feature of charge transfer complexes (Manzar, 1981). The stability of coloured complexes is not less than 20 minutes which is quite satisfactory for analytical purposes.

The results show that the proposed method is suitable for all those substituted indole derivatives and natural indole which form stable coloured complexes with a good acceptor like malefic anhydride.

The absorption of all natural indoles occurs between the wavelengths of 490-560 um. The absorption spectrum of the complexed indoles provides useful information for qualitative and quantitative analysis, and also with regard to basic understanding of the parameters influencing complexation (Holzinger, 1972). The influence of the substituent on complex formation is illustrated by absorption spectra of 5-substituted indole complexes. As the substituent is changed with the strong electron donating hydroxyl group, the maxima change to higher wavelengths and the band becomes broader and more intense. Gramin, 3-indole acetic acid and 3-indole acetaldehyde exhibit absorption between the wavelengths of 490-530 nm In case of serotonin and 5-hydroxy-3- indole acetic acid where the OH-group is introduced into the ring, the absorption was markedly increased and a bathochromic shift occured from 530 to 560 nm. Thus complexes are characterized by an intense absorption spectra in the visible region which is the most prominent feature of 1:1 molar complexes (Manzar, 1985). A satisfactory explanation of this property is given by the Mulliken's charge transfer theory (Mulliken, 1952). The composition of the coloured complexes of natural indoles and ring-substituted indoles with malefic anhydride has been determined by isomolecular series method (Job, 1928, Vusburgh and Cooper, 1941) and is found to be in the ratio of 1:1 which is most suitable for quantitative analysis. This ratio allowed a complete quantitation of natural indoles and ring-substituted indoles with maleic anhydride in the form of charge transfer complexes.

The optical characteristics of the complexes and precision data obtained by regression analysis are reported in Table 2.

Table 2
Optical characteristics and precision data obtained by regression analysis

Indole	Concentration range, $Mx10^4$	Intercept	λ_{max} (nm)	Molar Absorptivity	RSD %
Gramin	1.0-7.0	0.0005	510	963	2.62
Serotonin	1.0-7.0	0.002	560	1292	1.33
3-Indole acetic acid	1.0-7.0	0.002	500	940	2.59
3-Indole acetaldelyde	1.0-7.0	0.002	490	1902	1.91
5-Hydroxy-3-indole acetic acid	1.0-7.0	0.001	530	2942	1.20

Results of the quantitative determination of each indole by this method have been calculated and the relative standard deviation of each set of six determinations are given in Table 3. The mean relative standard deviation is within 1-2.6%.

Table 3
Quantitative Determination of Indoles with Maleic Anhydride

Indoles	Added $Mx10^4$	Found $Mx10^4$	SD	RSD
Gramin	2.40	2.49	0.065	0.0262
Serotonin	2.40	2.45	0.032	0.0133
3-Indole acetic acid	3.00	3.04	0.078	0.0259
3-Indole acetaldehyde	1.53	1.57	0.030	0.0191
5-Hydroxy-3-indole acetic acid	1.20	1.26	0.015	0.0120

Statistical values dearly show that the results of the analysis of natural and ring-substituted indole derivatives with maleic anhydride in the form of coloured complexes are quite satisfactory with an RSD of 2.6%. The time period requ[i]red for each measurement is about 15 minutes. Thus the method is precise, rapid, simple and economical and may be recommended for the quantitative determination of natural and ring-substituted indole derivatives.

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