Diazo-8-Quinolinol Coupling and Spectrophotometric Determination of Aromatic amino Compounds

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Summary: Sulphamethoxazole and Sulphadiazine have been estimated in different concentration ranges, based on diazo-coupling with 8-Quinolinol followed by Spectrophotometric determination. The method is found to obey the Beer's Lambert law for the concentration as low at $1.3x10^{-2}$ mg/ml.

Introduction

The usefulness of the coupling reaction of the diazonium salts with phenols and aromatic amines to form coloured azo compounds in which the two aromatic nuclei are linked through the azo grouping, -N=N-, has already been demonstrated for the preparation of pigment [1], azo dyes [2,3,4]. There is also described coupling of benzene diazonium tetrafluoroborate [5] with various primary automatic amines and phenols at the ortho positions, using different solvent systems. The determinations of diazotizable substances [6] of pharmaceutical interest were also carried out using flow injection spectrophotometric (FIS) method in a sodium dodecyl sulphate (SDS) micellar solution.

Although the diazo coupling reaction is increasingly used for preparation of azo dyes, pigments, and other reactions, the technique has not been much exploited for quantitative purpose except FIS. Problems encountered in achieving accurate amino compound quantitation using FIS include poor injection reproducibility detection depending on UV absorption, electrochemical or fluorescene (Which are not easily adaptable) and the reaction time lagging.

The primary goal of this paper is to study the possibilities of coupling of diazotized compound with 8-hydroxyquinoline (8HQ), also called 8-Quinolinol, followed by spectrophotometric measurement. This is the basis to quantify the aromatic amino compounds including the compounds of pharmaceutical interest. The authors observed that the simple diazo coupled spectrophotometric method is superior in terms of simplicity and reproducibility. The results are compared with those obtained by diazotization alone.

Results and Discussion

Dependence of coupling on pH of the medium

The 8-hydroxyquinoline reacted with diazotized compound and was found to be pH dependent. Absorbance of sulphamethexazole-8-quinolinol derivatives varied from 0.50 to 0.76. The results are presented in Table-1 which indicated that the absorbance was maximum at 475 nm for pH 8.0. It decreased as the pH was raised.

Mole ratio of the diazotized amino compound to 8-Quinolinol

In order to determine the mole ratio, the coupling reaction was carried out with diazotized sulphamethoxazole (SMOZ) from Septran tablets.

Table-1: Effect of pH on the absorbance of diazo-8-quinolinol coupled derivatives

Volume of SMOZ* (0.001M) or 1mg/ml (ml)	Volume of 8HQ (0.001M) (ml)	pH	Absorbance (at 475 nm.)
1.8	1.8	7	0.56
1.8	1.8	8	0.76
1.8	1.8	9	0.68
1.8	1.8	10	0.62
1.8	1.8	11	0.50
1.8	1.8	12	0.63

The quantity of the active principle was changed from 0.5 to 2.5 ml of SMOZ (0.001M) while

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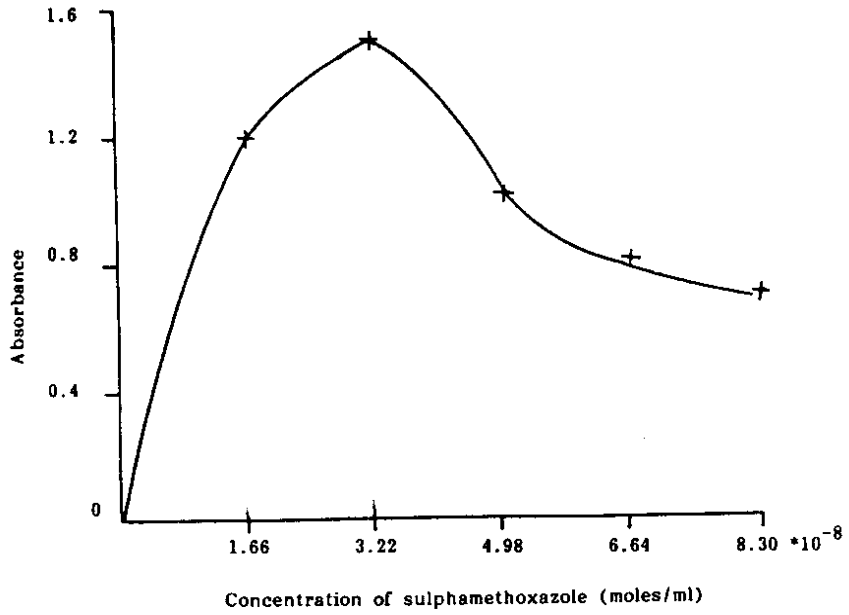

Fig. 1: Mole ratio of diazotized sulphamethoxa-zole to 8-Quinolinol (8-Quinol kept fixed to 8.30×10^{-8} moles/ml).

keeping the quantity of 8-Quinolinol (8-HQ) fixed to 2.5 ml (0.001M). By measuring the absorbance it was found that it was maximum for 1:2.5 mole ratio of diazotized SMOZ to 8-HQ. The results are produced in Table-2. Fig. 1. The absorbance spectrum (Fig. 1) clearly indicated that there was no change as the above desired mole ratio was reached; confirmed that the diazo-coupling reaction was complete. This mole ratio was maintained during the studies unless stated otherwise.

Table-2: Mole ratio of diazotized sulphamethoxa-zole to 8-Quinolinol.

Volume of SMOZ* (0.001M or 1mg/ml) (ml)	Volume of 8HQ (0.001M) (ml)	Absorbance/	Wavelength (nm)
0.5	2.5	1.2/475	1.3/486
1.0	2.5	1.5/475	1.5/476
1.5	2.5	1.0/475	1.0/476.5
2.0	2.5	0.8/475	0.9/462
2.5	2.5	0.7/475	0.9/466

*SMOZ : Sulphamethoxazole

Linearity

Sulphamethoxazole (SMOZ) (0.001M) from tablets in varying amounts from 0.4 to 2.0 ml in diazotized state was coupled with 8-hydroxyquinoline. The absorbance measured is presented in Table-3. There was found a gradual increase in the absorbance values as the quantity of the active principle increased. However, the concentration versus absorbance linear relationship was obtained for lower concentrations varying from 0.4 to 1.2 mg/ml of SMOZ and beyond which plateau was obtained, shown in Fig. 2.

Table-3: Concentration of the active principle versus absorbance relationship

Active principle(s)	Volume of active principle(s) (1 mg/ml) (ml)	Volume of 8-QH (0.001M) (ml)	Absorbance (475 nm.)
Sulphamethoxazole	0.4	1.0	0.61
	0.8	2.0	1.30
	1.2	3.0	1.80
	1.6	4.0	1.95
	2.0	5.0	2.00
Sulphadiazine	0.4	1.0	0.50
	0.8	2.0	0.99
	1.2	3.0	1.45
	1.6	4.0	1.50
	2.0	5.0	1.59

Sulphamethoxazole in a suspension prepa-ration was also determined which gave similar

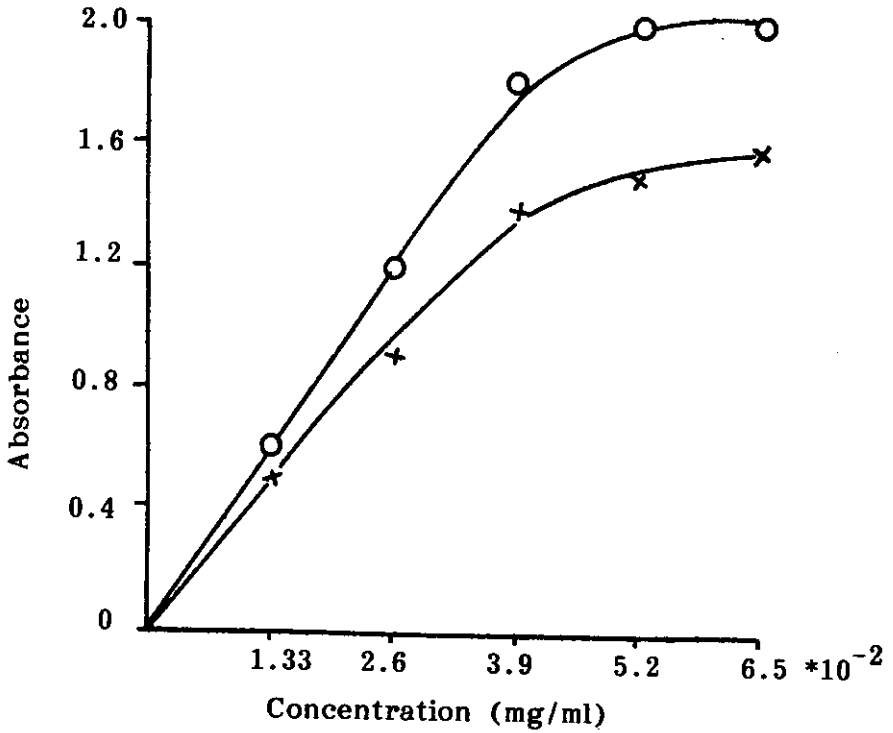

Fig. 2: Concentration vs Absorbance relationship; O = Sulphamethoxazole; X = Sulphadiazine

results as that of the tablets. The diazo-coupling reaction followed by the spectrophotometric measurement was equally suitable for estimation of sulphadiazine in tablets. These determinations also indicated the concentration versus absorbance linearity relationship was valid for lower concentration of the active principle similar to that indicated above, shown in Fig. 2. Table-3.

Comparative studies

The diazotized coupling spectrophotometric technique was compared with the analytical method based on diazotization and detection using external indicator. Sulphadiazine in tablets and sulphamethoxazole both in suspension and tablets were analysed by this way and it was found that there is a percentage error of 3-7 by the process of diazotization alone. This is higher than the author's method which centered maximally around 3%. The percentage error due the present method is lower. This is because the diazotization reaction is carried out in excess of sodium nitrite and does not require the detection of end point as that of the diazotization alone method.

More importantly, the coupling with 8HQ is done in the mole ratio of 1:2.5 for the active principle to the 8HQ where the quantity of 8HQ is in excess and thus it guarantees for completion of the reaction and the method is fool proof, free from any interference.

Experimental

Chemicals

8-Hydroxyquinoline; hydrochloric acid; zinc chloride, citric acid; potassium dihydrogen phosphate (E.Merck); sodium nitrite; methanol; boric acid (BDH); sulphadiazine (Zafa); Septran (Wellcome); diethyl barbituric acid; potassium iodide (Fluka) and Potato starch (Difco) were used.

Reagents

8-Hydroxyquinoline (8HQ) (0.001M)

A quantity of 0.145g of 8HQ was dissolved in 8 ml of methanol. Then added a few millilitre of 3M sodium hydroxide and the volume made upto one litre with distilled water

Sodium nitrite solution (0.01M)

A quantity of 0.69g sodium nitrite was dissolved in water and volume made upto 100ml using water. This was then diluted 10 times with water to obtain 0.001M solution.

Starch-iodide paste

A quantity of 0.75g of potassium iodide (dissolved in 5 ml of water) was first added to 2.0 g zinc chloride solution in 10 ml of distilled water. Both were mixed and the volume was made upto 100ml with distilled water. A suspension of potato startch, separately prepared from 5 g in 135 ml of water, was then added slowly to the warm solution of mixed KI-$ZnCl_2$. This was further boiled for 2 minutes and cooled under tap water.

Buffer solution (pH 8.0)

This was prepared by mixing 6.008 g citric acid, 3.893 g potassium dihydrogen phosphate, 1.76 g boric acid and 5.266g pure diethylbarbituric acid in distilled water, making up the volume to one litre. From this stock solution 100 ml was taken in a 250 ml beaker and sodium hydroxide (1M) was added dropwise till the pH reached to 8.0.

Sample preparation

Sulphadiazine

Two tablets of sulphadiazine (500 mg sulphadiazine each) were crushed to powder form. 5 ml of methanol and a few ml of concentrated hydrochloric acid were added in it. The solution was filtered and the filtrate was diluted to one litre with distilled water in a volumetric flask. Each ml of solution contains 1 mg of sulphadiazine.

Sulphamethexazole

One tablet of Septran (400 mg) was crushed and 5 ml of methanol were added in it, followed by a few ml of concentrated hydrochloric acid. The solution was filtered and the residue washed several times with small portions of water. The filtrate and the washings were diluted to 400 ml with distilled water. Each ml contains one millgram of sulphamethoxazole.

The same procedure (crushing not involved) was followed for preparing sample from Septran suspension containing sulphamethexazole. Each ml of the solution contains one milligram of the active principle.

Standard solution of aromatic amines and sulphadiazine

Solution of aromatic amines and sulphadiazine (both of known purity) were prepared in the same way as described above. Each ml of the solution(s) contained one milligram of the active principle(s).

Instrumentation

Hitachi model U-2000 double beam spectrophotometer and Bausch and Lomb Spectronic-20 were used to measure the absorbances.

General procedures

a. Diazotization

An aliquots each of 0.4-2.0 ml of the aromatic amino compound solutions was taken in five different titration flasks. These were heated gently on naked flame and then cooled to 0°C, using ice-bath. Then titrated against sodium nitrite solution (0.01M) taking care that the temperature did not exceed 5°C. The completion of diazotization was tested by the appearance of blue colour with starch-iodide paste as an external indicator. An excess of sodium nitrite solution was added to ensure completion of the diazotization process.

b. Coupling, detection and spectrophotometric measurement

The above diazotized solutions were kept on ice-bath and soon proceeded for coupling reaction with 8-hydroxyquinoline (previously cooled to 5°C). Varying amounts of 8HQ were added slowly to each flask such that 2-3 minutes time elapsed. After coupling the solutions in different flasks were diluted to 30 ml each with buffer solution of pH 8.0. The absorbance were then measured at 475 n.m. against reference blank (containing only the diazotized solution obtained from the same fraction of volume of the active principle(s) as the sample and diluted to 30 ml with buffer).

The absorbances were plotted against the ml (or concentration) of 8-hydroxyquinoline. The quantity of 8-HQ giving the maximum absorbance was taken as the end point. The maximum absorbance was then used to determine the quantity of the active principle.

Alternatively, the diazotized compound was coupled with 8-HQ, using its known quantity but a little excess over the mole ratio of 1:2.5 for amino compound to 8-HQ. The diazo-coupled compound was then diluted as before to 30 ml with a buffer solution of pH 8.0 and its absorbance was measured against stated reference blank at 475 nm. The measured absorbance was then co-related with the amount of the amino compound by comparison with the standard.

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