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SPECTROPHOTOMETRIC DETERMINATION OF TETRACYCLINES USING p-N,N-DIMETHYLPHENYLENEDIAMINE AND SODIUM METAPERIODATE

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ABSTRACT

The oxidative coupling of the proposed method is simple, rapid and sensitive with reasonable precision and accuracy. The precision of the method was found by analyzing a set of eight solutions, each containing a final concentration value approximately in the middle of the Beer's law range. The percent relative standard deviation in method is presented in Table-2. The accuracy of the method was determined by taking different known amounts (within Beer's law limits) of the drug and analyzing them by proposed method. The results are given in Table – 3. In the determination of tetracyclines the excipients usually present in formulations (glucose, starch, sodium hexaphosphate and some vitamins) and the other antibiotics such as cycloserline, streptomycin lidocaine or penicillins did not interfere.

Keywords: Tetracyclines, spectrophotometer, p-N,N-dimethylphenylenediamine, sodium meta periodate, p-or o-benzoquinoneimine.

INTRODUCTION

There are three classes of components which are important to the colour development process of oxidative hair dyeing or colour photography. These are the primary intermediates (o-or p-phenylenediamine and o- or p-N-methylaminophenol), the oxidant (potassium dichromate, potassium ferricyanide, molecular oxygen, H_2O_2 – peroxidase, sodium hypoclorite etc.) and couplers (unoxidised primary intermediate, nucleosubstituted amino[1-3] or phenol preferable [4-13]. Most authors appear to have assumed that since the reaction is general, the choice of diamine or coupler to be used in a particular analytical method[17-18] is not particularly critical. Further more none of the previous workers have referred (except Corbett) to the possibility of hydrolysis of the intermediate reactive species (p-or o-benzoquinoneimine). In fact, these factors, together with the pH and reactant concentration at which the colour is developed, are vital factors in determining the *oxidative coupling to the assay [19-28] of particular diamine or cou*pler. Coupling takes place in the para position (or ortho position if para position is not free) to the phenolic hydroxyl group. In continuation to the studies on the use of DMPD (p-N,Ndimethyl phenylene diamine) and oxidant in the amino compounds [1-3] and cysteine through hydrogen sulphide[5] the author has carried out for the first time micro determination [29-37] of tetracycline using DMPD-IO_4^-

EXPERIMENTAL

Preparation of Reagents

(i) Tetracyclines (1mg/ml)

This solution was prepared by dissolving 100 mg of the I.P. grade tetracycline (tetracycline hydrochloride,chlortetracycline hydrochloride,xytetracycline, hydrochloride or doxycycline hydrochloride) in 100 ml. of distilled water.

Working solutions were further diluted to 200 µg/ml. with distilled water.

(ii) DMPD solution (0.05%)

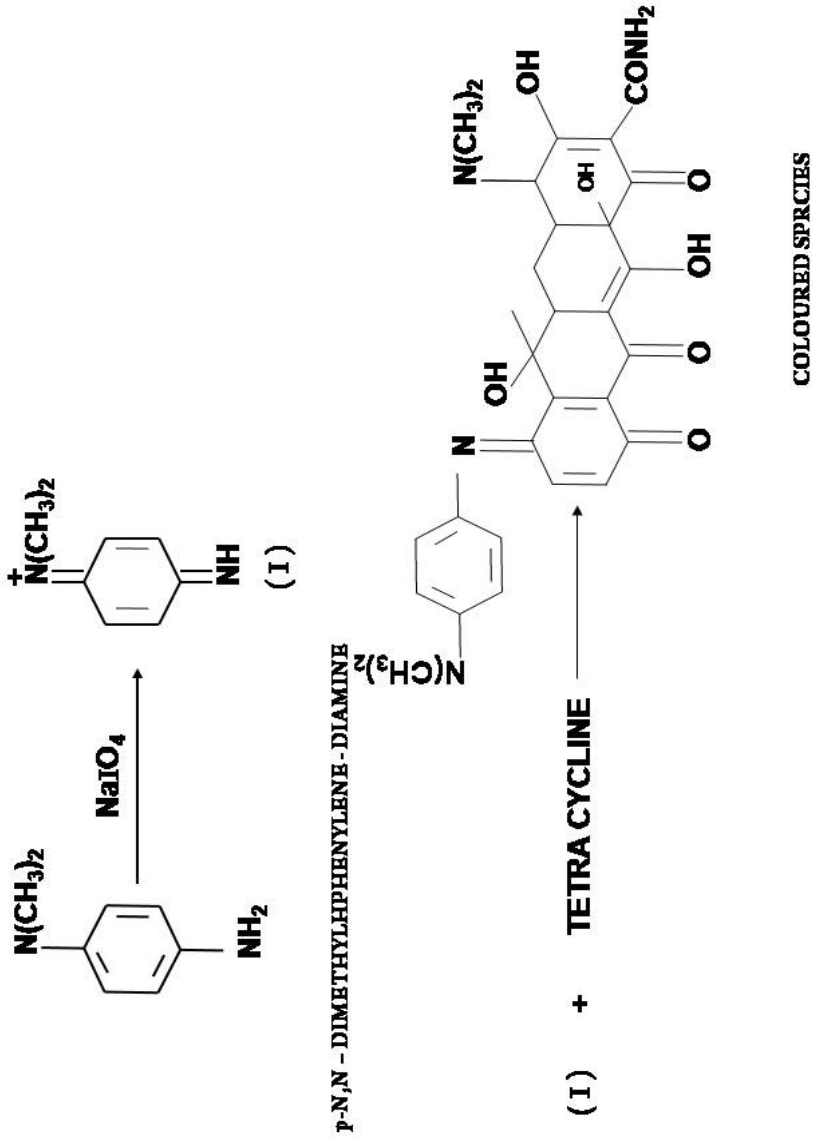
DMPD solution was freshly prepared by dissolving 50 mg of the analytical grade substance in 100 ml of distilled water.

(iii) Sodium meta periodate

This solution was prepared by dissolving solution(0.2%) 200mg of the analytical grade reagent
In 100ml of distilled water

(iv) Buffer solutions

It was prepared by mixing 6.1ml potassium dihydrogen phosphate and 38.8 ml of disodium hydrogen phosphate.All the other chemical reagents were of analytical grade.



Scheme-1

Instrumentation

Spectral and absorbance measurements were made on Shimadzu double beam spectrophotometer UV – 140 with matched 1 cm quartz cells pH measurements were carried out using Systronics pH meter 335.

In order to obtain the optimum conditions for the determination of mentioned antibiotics with the reagent DMPD –IO_4^-, the effects of possible variables were studied and the results are summarised below.

Absorption Spectra

The absorption spectrum of the coloured species formed in mixing antibiotic with DMPD– IO_4^-, in appropriate pH medium exhibiting maximum absorbance was scanned in the wave length region 400 – 700 nm against reagent blank and the results are graphically represented in (Scheme- 1). The λmax was 625 nm for tetracyclines Under the experimental conditions, IO_4^- with DMPD or tetracycline has practically no or low absorbance in this region.

Procedure

Into a series of 100 ml separating funnels, different volumes of the drug solution (0.3 – 4.0 ml) were pipetted out. To each funnel 15 ml of phosphate buffer (pH 7.0), 1.0 ml ofDMPD, 2.0 ml of $NaIO_4$ and requisite volume of distilled water so as to make total volume 25.0 ml were added. After 20 min, 10.0 ml of n-butanol was added. The funnels were shaken gently for 2 min and the n-butanol layer was separated. A blank experiment was carried out omitting the drug. The absorbance of the coloured extract was measured λmax at 625 nm during the stability period (30 min – 2 hrs.) against a reagent blank. The mean value of three experiments was taken and the extracted amount of the drug was obtained from a standard calibration curve prepared with the same drug under identical conditions.

For dosage forms

Twenty tablets were weighed and powdered. A quantity of tablet or capsule powder equivalent to 100 mg of antibiotic was dissolved in and filtered. The filtrate was made upto 100 ml with distilled water. Aliquots of the solution were analyzed by following the procedure given above for bulk samples.

RESULTS AND DISCUSSION

Comparison of the results incorporated in Tables 1 –4 reveal that the proposed method is simple, rapid and sensitive with reasonable precision and accuracy. In order to find out the suitability of the proposed method, the pharmaceutical preparations were analysed by the proposed and reported methods [18-27]. Further, recovery experiments were conducted by adding a known amount of the drug to previously analysed formulations, unfed human urine and sheep blood and determining the total content of the drug by the proposed method. The results are given in Table –4

Table-1: Optical Characteristics

Parameter	Tetracycline hydrochloride	Chlortetracycline hydrochloride	Oxytetracycline hydrochloride	Doxycycline hydrochloride
Concentration (C)(µg /ml)	5-50	5-50	5-50	5-50
Regression equation*	A=0.0010+0.0137	A=0.0099+0.0121	A=0.0097+0.0134	A=0.0010+0.0131
Correlation coefficient	0.9992	0.9996	0.9992	0.9991
Molar absorptivity($1.Mole^{-1}cm^{-1}$)	6.12×10^3	6.1×10^3	5.72×10^3	5.46×10^3
Sandell's sensitivity (µg/$cm^2$0.001 absorbance unit)	0.072	0.074	0.080	0.080
Optimum photometric range (µg /ml)	9.8- 44.2	9.8- 44.2	12.2- 43.4	12.2 - 43.4

*Found in this work; It must be determined independently by users of the method.

Table-2: Accuracy of the Method

Antibiotic	% RSD	Percent range of error confidence limit	
		0.05 level	0.01 level
Tetracycline hydrochloride	1.80	± 1.92	± 2.79
Chlortetracycline hydrochloride	1.59	± 1.74	± 2.54
Oxytetracycline hydrochloride	2.2	± 2.32	± 3.47
Doxycycline hydrochloride	1.98	± 2.15	± 2.91

Table-3: Precision of The Method

Antibiotic	Amount of antibiotic (µg)		
	Taken	Found	% error
Tetracycline hydrochloride	400	395.1	1.225
Chlortetracycline hydrochloride	400	396.8	0.8
Oxytetracycline hydrochloride	400	394.0	1.5
Doxycycline hydrochloride	400	394.4	1.4

Table-4: assay of Formulations and % Recovery data*

Sample	Labelled amount (mg)	Amount found (mg) in method**		% Recovery* (proposed method)
		Proposed	Reported	
Tetracycline hydrochloride (Capsule)	250	247.2	245.8	98.9
Tetracycline hydrochloride (Tablet)	500	492.7	493.9	98.5
Oxytetracycline hydrochloride(Capsule)	250	248.3	250.1	99.3
Doxycycline hydrochloride (Capsule)	100	98.4	99.1	98.8

***** After adding 5mg of the drug ** Each result is the average of three determinations.

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