

# Spectrophotometric Determination of Boron with Chlorotetracycline\*

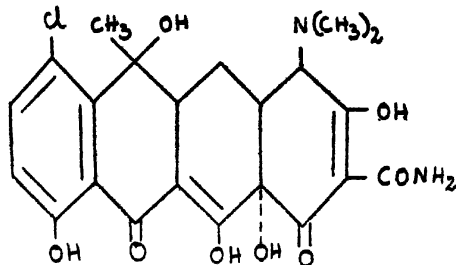
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A spectrophotometric method for the determination of boron at trace levels is presented with chlorotetracycline as a new analytical reagent in concentrated sulphuric acid. The absorption maxima of the reagent was a 410 nm and of the boron-chlorotetracycline complex was found to be at 570 nm. The coloured system conformed to Beer's law between 2.1 and 12.6  $\mu\text{g}$  of boron. The spectrophotometric sensitivity of the method is  $1.8 \times 10^{-3} \mu\text{g}/\text{cm}^2$  and the molar absorptivity is 5,800. The mole ratio of boron to chlorotetracycline in the coloured complex was found to be 1:1.

**T**HE naturally occurring tetracyclines are complex organic molecules with a large number of functional groups. So it is not surprising that they form complexes with a large number of metal ions. The behaviour of tetracycline and its analogs towards metal ions has been the subject of numerous investigations. Albert<sup>1</sup> from a preliminary investigation with chlorotetracycline reported the formation of complexes first a 1:1 and latter as the pH rose 2:1 ligand to metal ion. The metal ions were  $\text{Fe}^{3+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Mn}^{2+}$ . In the same year Oxford<sup>2</sup> reported the formation of stable yellow complexes in alkaline solution between Mg, Ca, Ni, Cu, Co, and Sr and chlorotetracycline. The same author determined chlorotetracycline colorimetrically by extraction of its calcium complex into *n*-butanol and also proposed its use in analytical chemistry for the detection of calcium in the presence of magnesium and of cobalt in the presence of nickel.



Ishidate and Sakaguchi<sup>3</sup> investigated the complex formation of chlorotetracycline (and anhydrochlorotetracycline) with various metal ions. In acetate buffer of pH 4.6 chlorotetracycline and Zr(IV) formed a 1:2 metal to ligand complex. With thorium, aluminium and uranyl ions 1:1 complexes

were reported. With anhydrochlorotetracycline orange chelates were formed with the metal ions, Zr, Th, Al, and  $\text{UO}_2^{2+}$ , and spectrophotometric curves were reported for these complexes. Chlorotetracycline was determined colorimetrically as its thorium(IV) complex by Sakaguchi and Taguchi<sup>4</sup>. Sakaguchi<sup>5</sup> determined chlorotetracycline as its boron complex in sulphuric acid. Sakaguchi and Hanaki<sup>6</sup> used the above method for the determination of chlorotetracycline in urine. The same authors<sup>7</sup> reported the formation of two complexes with boric acid, depending on the concentration of sulphuric acid, chlorotetracycline and boric acid.

Chlorotetracycline hydrochloride is commercially available under the trade name, Aureomycin, which was used in the present investigations. The antibiotic dissolved in concentrated sulphuric acid with a bluish green colour and a red fluorescence, which rapidly turned to yellowish green. On the addition of boric acid it turned reddish orange. With a reagent concentration of 200  $\mu\text{g}$  in 5 ml sulphuric acid, the colour was violet and the limit of identification was less than 1  $\mu\text{g}$  of boron. Using chlorotetracycline as a colour forming reagent with boric acid, a new spectrophotometric method for the determination of boron at trace levels in concentrated sulphuric acid is presented. The reagent in sulphuric acid had absorption maximum at 410 nm and that of the boron-chlorotetracycline complex was at 570 nm, which was used for the analytical determinations. The colour reaction followed Beer's law between 2.1 and 12.6  $\mu\text{g}$  of boron for the amount of reagent used. The spectrophotometric sensitivity of the method as defined by Sandell is  $1.8 \times 10^{-3} \mu\text{g}$  of boron per  $\text{cm}^2$  at 570 nm. The molar absorptivity of the coloured product calculated on the basis of boron was 5,800 litre mole<sup>-1</sup>cm<sup>-1</sup>.

## Experimental

**Reagent solution:** The chlorotetracycline hydrochloride, Aureomycin, capsule was cut and the

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contents used for the preparation of the reagent solution. Appropriate amounts of the powder was weighed and dissolved in a definite volume of concentrated sulphuric acid. Lower concentrations were prepared from this solution. The reagent solutions were prepared on each day.

98 per cent sulphuric acid ( $d = 1.84$ ), AnalaR, B.D.H., Boric acid, Pro-analysi, Merck, were used in the determinations.

**Stock solution of Boron:** 3.026 g of boric acid was dissolved in sulphuric acid and made upto volume in a 250 ml standard flask (1 ml = 2.114 mg of boron). Lower concentrations of boron were prepared by successive dilution of the stock solution with sulphuric acid.

**Apparatus:** Hilger U.V. Speck Spectrophotometer with 1.00 cm matched quartz cells were used for the absorption measurements. Systronix photoelectric colorimeter type 101/111 was used in the interference study due to various metal ions. Ground glass stoppered test tubes of about 30 ml capacity were used for the development of colour. To minimize contamination from the glassware the test tubes were soaked for eighteen days in concentrated hydrochloric acid as recommended by Jackson<sup>8</sup>. These tubes were washed thoroughly with tap water and with deionized water. In subsequent work the washings were done with washing soda, thoroughly with tap water and with deionized water.

The absorption spectra of chlorotetracycline and its boron complexes in concentrated sulphuric acid after keeping for eighteen hours are shown in Fig. 1. The absorption maxima of the reagent was at 410 nm, whereas that of the boron complex with excess of reagent was at 570 nm.

**Procedure:** The volume of an aliquot of boron as boric acid (upto 12.6  $\mu\text{g}$  of boron) in concentrated sulphuric acid was adjusted to 9 ml with the acid and 1.0 ml of chlorotetracycline hydrochloride (= 1.0 mg) in sulphuric acid was added. The solution was mixed, the tube stoppered and allowed to stand for 18 hours at room temperature. The absorbance measurements were made at 570 nm in the spectrophotometer against the reagent blank prepared at the time of the test.

## Results and Discussion

**Lambert-Beer Law:** Varying concentrations of boron, as boric acid, were taken into test tubes, the colour was developed and measured as in the procedure. Beer's law was obeyed over the range 2.1 to 12.6  $\mu\text{g}$  of boron in 10 ml solution for the amount of reagent used.

**Interference due to Foreign Ions:** Wherever possible the solution used for the interference study contained 5.0 mg of the cation or anion in 1.0 ml sulphuric acid, so that when 2.0 ml was used, the amount of the ion was approximately 1000 fold over the amount of boron taken. The solubilities of the salts were an important limitation. In other cases saturated solutions of the salts in concentrated

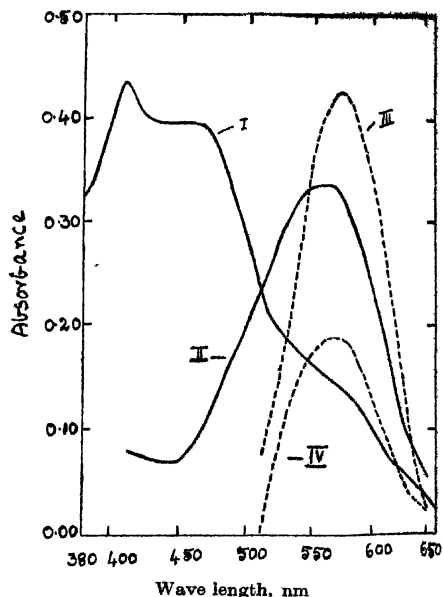


Fig. 1

- Curve I. Absorption Spectra of Chlorotetracycline Hydrochloride (250  $\mu\text{g}$  in 10 ml).
- Curve II. Absorption Spectra of Boron-reagent Complex (250  $\mu\text{g}$  reagent + 1.21 mg boric acid in 10 ml). Absorbances for Curves I and II are against concentrated Sulphuric acid.
- Curve III. Absorption Spectra of boron-reagent Complex with excess of reagent (80  $\mu\text{g}$  boric acid + 1.0 mg Reagent in 10 ml).
- Curve IV. Absorption Spectra of boron-reagent Complex with excess of boron (solution of Curve II used). Absorbancies for Curves II and IV are against their respective reagent blanks.

sulphuric acid were used. In the case of the sulphates of sodium, potassium and ammonium 200 mg of the salt was weighed directly into the tubes containing 9.0 ml of the solution of boron (= 10.5  $\mu\text{g}$ ). Interference due to the various ions were made on 10.5  $\mu\text{g}$  of boron as reference. Saturated solutions (2.0 ml) of  $\text{Mg}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Be}^{2+}$ ,  $\text{Hg}^{2+}$ , did not interfere. Ten milligrams each of  $\text{Ca}^{2+}$ ,  $\text{Cd}^{2+}$ , and  $\text{Pb}^{2+}$  did not interfere. 200 mg of each of sodium sulphate, potassium sulphate and ammonium sulphate caused no interference. 10 mg quantities of the acidic radicals, arsenate, acetate, oxalate, phosphate, ferrocyanide and tungstate did not interfere. Interference was caused by oxidizing agents, fluoride and nitrate.

The spectrophotometric sensitivities of hydroxy-anthraquinone derivatives which also require a high concentration of sulphuric acid and are widely used for the determination of boron, are given below for comparison with the new reagent.

Quinalizarin <sup>9</sup>	...	$3.0 \times 10^{-3} \mu\text{g}/\text{cm}^2$
Carminic acid <sup>10</sup>	...	$2.0 \times 10^{-3} \mu\text{g}/\text{cm}^2$
Diaminochryszazin <sup>11</sup>	...	$2.2 \times 10^{-3} \mu\text{g}/\text{cm}^2$
Diaminoanthrarufin <sup>11</sup>	...	$2.5 \times 10^{-3} \mu\text{g}/\text{cm}^2$
Chlorotetracycline	...	$1.8 \times 10^{-3} \mu\text{g}/\text{cm}^2$

*Composition of the Complex* : The mole ratio of boron to chlorotetracycline in the complex was investigated using Job's method<sup>12</sup> of continuous variation with equimolar solutions ( $1.96 \times 10^{-4} M$ ). After keeping the solutions containing the complex for 18 hours the absorbancies were measured against their respective blanks at 570 nm. The mole ratio was found to be 1 : 1 in the complex.

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