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INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES
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# ANALYTICAL STUDY OF OXYTETRACYCLINE HYDRACHLORIDE

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**Abstract**
The potency of antibiotic content in the sample has been determined by biological means. An assay has also been made based on biological test to determine its ability to kill or inhibit the growth of living microrganisms. Turbidometric assay of oxytetracycline hydrochloride has been found to be having assay value of 101.92% which is well within the reported limit (98% -105%).

Key-words: Oxytetracycline, Turbidometric assay, Therapeutics potency

## Introduction

The term antibiotics was put forward by vuillemin [1] in 1889. Later on Benedict and Langly [2] coined a more general and acceptable definition of antibiotics which states that a chemical compound derived from living organism capable in small concentration to inhibit the life process of micro-organism. Oxytetracycline Hydrochloride classified under the tetracycline's category with four cyclic rings is obtained from soil actinomycetes. In addition to gram-positive and gram-negative bacteria, tetracycline also inhibits the growth of other micro-organisms like *Ricbettisea, chlamydie, mycoplasma* and some protozoa [3]. It was first found near Pfizer laboratories in soil samples yielding the soil actinomyceteand streptomyces rimosus by finlay and coworkers. Robert B Woodward determined the chemical structure of oxytetracycline to enable to synthesize the drug under the tradename, terramycin[4]. Oxytetracycline hydrochloride has four fused Six membered rings. The classical tetracyclines were derived from *streptomyces* species. It is amphoteric in nature and forms salts with both strong acids and bases. It may exist as salts of sodium or chloride [5].

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Oxytetracycline hydrochloride is an antibiotic, bright yellow in colour, possessing potent antimicrobial activity. Its opthalmic ointment is indicated for the prophylxis and local treatment of superficial occular infection. It is generally well tolerated after acute overdoses and can be given more than 400 mg/kg/day orally without any demonstration in toxicity. [6]

## Material and methods

The inhibition of microbial growth under standardized conditions is utilized for demonstrating the therapeutic efficiency of the drug. Potency of the drug has been determined by turbidometric method. This method is based on inhibition of growth of test micro organism in a liquid medium containing uniform concentration of antibiotics. [7, 8].

**Table 1: Specification of microorganism used**

| | |
|---|---|
| Name of Organism | Staphylococcus Awreus |
| ATCC Number | 1 NHCl |
| Name of Media | antibiotic assay medium |
| Incubation time | 3-4 hrs. |
| Incubation temperature | 37$^{o}$C |

**Standard preparation**

Accurately weighed working standard equivalent to 200mg of oxytetracycline was transfered to a 100ml volumetric flask with approximately 50ml of 0.1N HCl and sonicated for 20min. volume was then made up to 100ml.1ml aliquot of this solution was further diluted to 100ml with sterile purified water, Further, 5ml aliquot of this solution is diluted to 100ml with sterile purified water. The resulting solution contains 1mcg/ml of oxytetracycline.

**Sample preparation**

For bulk feed supplement (powders) study, accurately weighed amount of sample equivalent to 200 mg of oxytetracycline activity was transfered to a 100ml volumetric flask with 50ml of 0.1N HCL solution it was sonicated for 20 minutes. the volume was made upto 100ml using with 0.1N HCl and shaken well.It was allowed to settle for 20 minutes.The supernatant liquid was dilutes to 100ml with sterile purified water to give a final concentration of 1mcg/ml of oxytetracycline.

**Test procedure**

Six different standard solutions were prepared for plotting the standard curve by diluting 1mcg/ml of the standard preparation with sterile purified water as given in table 2.

Table 2: Dillutions for test.

| Standard preparation (1mL) | Final volume with sterile purified water(1mL) | Standard concentration (µg/mL) |
|---|---|---|
| 7.5 | 10 | 0.75 |
| 5.0 | 10 | 0.5 |
| 4.0 | 10 | 0.4 |
| 3.0 | 10 | 0.3 |
| 2.0 | 10 | 0.2 |
| 1.0 | 10 | 0.1 |

Arbitrarily two samples of median concentrations were also prepared by diluting the solution of the "sample preparation "with the purified water as shown in table 3.

**Table 3: Median concentration of sample**

| Sample preparation | Final volume. | Sample concentration |
|---|---|---|
| 4.0ml | 10ml | 0.4 mcg/ml |
| 3.0ml | 10ml | 0.3mcg/ml |

1ml of each concentration of the standard as well assolution and sample solutions was taken in test tubes in duplicate. To these test tubes, 9ml of test medium previously seeded with the test organism was added to 2 control tubes one containing the inoculated culture medium and the other containing uninoculated culture medium were subjected to incubation at $37^0c \pm 0.1^0c$ for 3-4 hours.

Subsequently, 0.5 ml of formalin was added to each test tube.the samples, thus,prepared were used for absorbance studies at 530 nm in spectronic-20 colorimeter using inoculated sample as a reference.the results have been plotted in figure 1 as absorbance (AU) versus concentration (µg/ml) to get the dose response curve at wavelength of 530nm by UV-Vis spectrophotometer(id no 004).

The potency factors were calculated using expression:

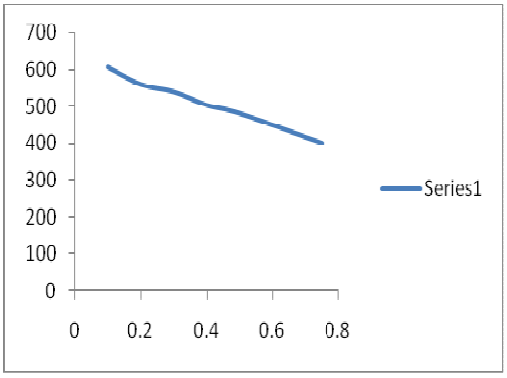
$$\textbf{Potency factor} = \frac{\text{std dil. x conversion factor}}{\text{Sample dilution}}$$

And assay value by the expression:

$$\textbf{Assay value} = \frac{\text{\% potency x potency factor}}{100}$$

## Conclusion

Attempts have been made to assess the efficiency of drug and its antimicrobial activity. Microbial assay study has been carried out using turbidometric method. This is due to the matter of fact that the observed turbidity does not interfere with the drug efficiency. It is based on comparison of the inhibition of growth of micro-organism. This has been accomplished by measuring the concentration of antibiotics under study using the reference. The inhibition of growth of test micro-organism in a standard solution of antibiotics has been determined by measuring the light transmittance using Spectrophotometer. The potency of drug has been found to be 101.95% which falls well within the reported range of 95%-105%. It is obvious that the test drug does inhibit the growth of micro-organism. The drug is also reported to exhibit its activity against the gluconate oxidation by *Esherichia Ecoli.*[7, 10] it is clearly seen from standard curve that the test drug absorbs the radiation corresponding to .500AU which reflects its ability to inhibit the growth of micro-organism.as a consequence turbidity of test samples found to decrease as per expectation.




The studies indicate that the oxytetracycline hydrochloride is capable of inhibiting the growth of micro-organism and potency of the drug is found to be 101.926 which is well within prescribed limit (98%-105%).

## Acknowledgements

The author acknowledge the help of Dr. B.K Mehta H.O.D, Department of Chemistry, Vikram University, Ujjain in inspiring the study. The work

was carried out in Alpa laboratories, Indore as part of my M.Phill desertation.

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