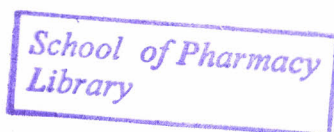


MALTOL METHOD FOR THE ASSAY
OF STREPTOMYCIN:AN
INVESTIGATION OF THE STABILITY OF
THE MALTOL COMPLEX WITH TIME.

A Dissertation By
KITENG'E GEORGE NDAKA
U29/2059/2006



Submitted in partial fulfillment of the requirements for the award of Bachelor of Pharmacy Degree of The University Of Nairobi.



SUPERVISOR,

DR.OGETO,

DEPT. OF PHARMACEUTICAL CHEMISTRY,

SCHOOL OF PHARMACY.

TABLE OF CONTENTS

❖ Declaration.....	ii
❖ Dedication.....	iii
❖ Acknowledgements.....	iv
❖ Abstract.....	v
❖ Introduction.....	3,4
❖ Literature review.....	5
❖ Objective.....	6
❖ Justifications.....	7
❖ Materials and apparatus.....	8
❖ Method.....	9,10
❖ References.....	11
❖ Work plan.....	11

DECLARATION

I solemnly declare that this is my original work and has not been submitted to any other university.

Signed..........Date..17/03/2010.....

Kiteng'e George Ndaka

(U29/2059/2006)

I declare that I have supervised and approved this work.

Signed.....Date.....

Supervisor:

Dr.J.O Ogeto.

Senior lecturer,

Dept.of Pharmaceutical Chemistry,

University of Nairobi.

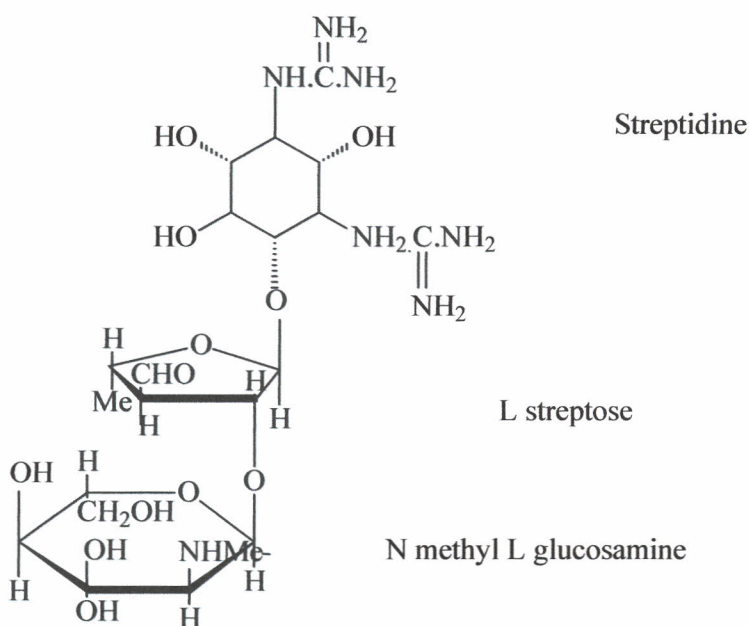
Introduction

Streptomycin is an antibacterial agent that is produced by growth of *Streptomyces griseus*, a fungus. It is an aminoglycoside antibiotic composed of three sugars namely;

L-Streptose

- N-methyl-l-glucosamine
- Streptidine (an aminocyclitol)

These three sugars are glycosidically linked as shown:



PHYSICAL DESCRIPTION:

Appearance: White powder or almost white powder; hygroscopic; odourless or with a slight odour.

Very soluble in water; practically insoluble in absolute ethanol, in chloroform and in ether.

Molecular formula: (C₂₁H₃₉N₇O₁₂)₂·3H₂SO₄

Molecular weight: 1457.4

Optical rotation: -77 to -79° (c=1% in H₂O, 25°C)₂

Structure: Aminoglycoside antibiotic in which streptidine is linked glycosidically to the disaccharide streptobiosamine.

Streptomycin is an antibiotic which inhibits initiation, elongation and termination of protein synthesis in prokaryotes. It is most active in a slightly alkaline medium against *Klebsiella spp*, *Salmonella spp* and *M. tuberculosis*. It inhibits protein biosynthesis on 70S ribosomes. In its action it's bound to the 23S

core protein of the ribosomal subunit. This induces misreading of mRNA which causes incorporation of incorrect amino acids into the peptide, resulting in formation of non-functional monosomes.

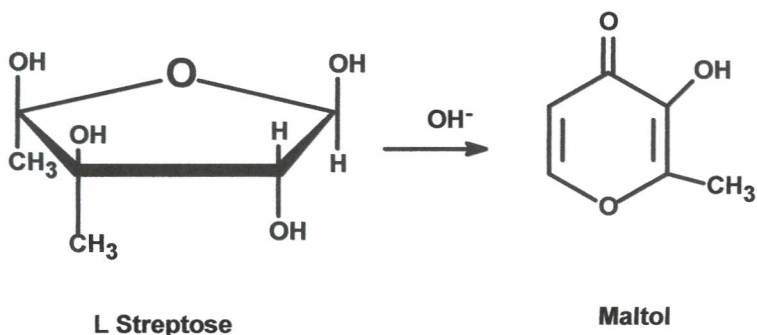
Streptomycin has gained wide clinical use in the management of tuberculosis whose incidence has escalated partly as a result of increase in HIV infection. Other diseases managed by streptomycin in combination with other drugs include brucellosis, plaque and tularemia.

However, widespread emergence of resistant strains has significantly reduced its effectiveness against common Gram-negative aerobes. The resistant is thought to be due to mutation of the ribosomal site of the antibiotic. Streptomycin sulphate is poorly absorbed and irregularly absorbed from the GIT. It's readily absorbed after intramuscular administration and widely distributed through out the body.

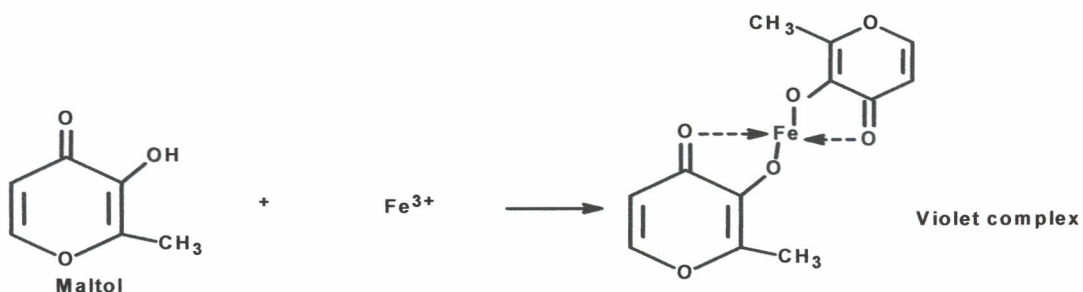
This project will involve;

- ✓ Estimation of streptomycin in a multiproduct containing streptomycin as an active ingredient in an alkaline medium by the maltol method
- ✓ Stability of the maltol complex on exposure to 40°C for 1 hour.

The streptose sugar of streptomycin undergoes degradation in dilute alkaline conditions to maltol.



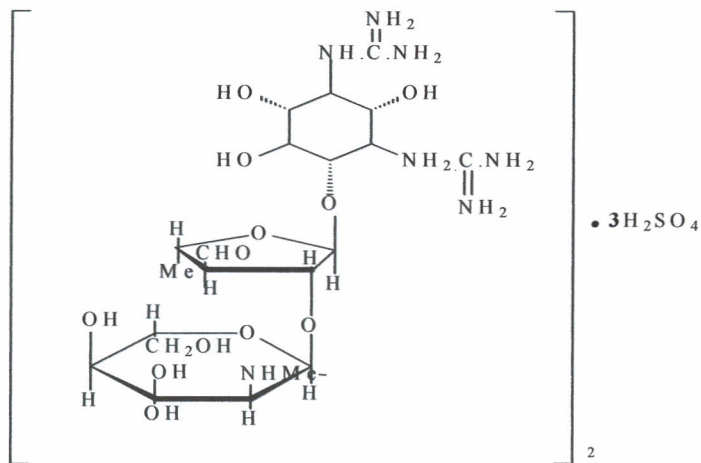
If the aldehyde group is reduced to the alcohol, dihydrostreptomycin is formed. Dihydrostreptomycin does not show maltol formation because it is more stable in alkaline conditions. The aldehyde group is responsible for the above instability. A maltol method of assay was developed by Schenk and Spielman on the observation that heating streptomycin in dilute alkaline solutions causes formation of maltol. Maltol, being a phenol, is complexed with ferric ions (Fe^{3+}), to form a violet colored complex of maltol with ferric ions as shown below



The reaction proceeds quantitatively and can be used to estimate streptomycin in a multiproduct containing streptomycin as an active ingredient. The absorbance of the complex is determined spectrophotometrically at 540nm.

Formulation and administration

Streptomycin is a water-soluble aminoglycoside. It is marketed as the sulfate salt of streptomycin. The chemical name of streptomycin sulfate is D-Streptamine, O-deoxy-2(methyl amino)- α -L-glucopyranosyl- (1 \rightarrow 2) -O-5-deoxy-3-C-formyl- α -L-xylofuranosyl-(1 4)-N,N'-bis(aminoiminoethyl)-sulfate. The empirical formula for Streptomycin Sulfate is $(C_{21}H_{39}N_7O_{12})_3 H_2SO_4$



PHARMACOLOGY

Streptomycin is an antibiotic used in combination with other drugs to treat tuberculosis. The standard regimen for new smear positive pulmonary TB is 2 months of rifampicin, Isoniazid, Pyrazinamide, Ethambutol followed by 6 months of Ethambutol and Isoniazid under direct observed therapy. Patients with recurrent smear positive TB or those returning for treatment after default, failure or relapse, the regimen include streptomycin, rifampicin, ethambutol, pyrazinamide and isoniazid in the initiation phase of therapy. Though streptomycin is no longer considered a first line treatment for TB, some strains of multi-drug resistant TB (MDRTB) are susceptible to the drug.

Mechanism of action

Streptomycin is bactericidal. It penetrates the bacterial cell membrane by oxygen dependent active transport via a polyamine carrier system. The drug binds onto the 30S subunit of ribosome by displacing Mg^{2+} normally associated with ribosomes leading to mistranslation of mRNA resulting in insertion of wrong amino acids with resultant nonsense proteins. This upsets permeability of the membrane leading to the influx of bacterial cell contents, electrolytes and water hence death.

Resistance

There is widespread emergence of resistant strains to streptomycin. This is mainly associated with R-factor mediated enzymes that modify the drug by N-acylation, O-phosphorylation O-adenylation or decreased drug permeability across the bacterial cell membrane.

Antibacterial Spectrum

Mycobacterium tuberculosis, *Pasteurella pestis* (plague), *Francisella tularemia* (tularemia), *Brucella*, *Calymmatobacterium granulomatis* (donovanosis, granuloma inguinale), *H. ducreyi*, *H. influenzae* (in respiratory, endocardial and meningeal infections), *K. pneumoniae*, *E. coli*, *proteus*, *A. aerogenes*, *K. pneumoniae* and *Enterococcus faecalis* in urinary tract infections, *Streptococcus viridians*, *Enterococcus faecalis* (in endocardial infections concomitantly with penicillin) and Gram-negative bacillary in combination with another antibacterial agent.

Pharmacokinetics

Following intramuscular injection, peak serum level is reached within 1 hour with gradual diminishing to about 50% after 5 to 6 hours.

Appreciable concentrations are found in all organ tissues except the brain. Significant amounts have been found in pleural fluid and tubercles cavities. Streptomycin passes through the placenta with serum levels in the cord blood similar to maternal levels.

Small amounts are excreted in milk, saliva, and sweat. Streptomycin is mainly excreted by glomerular filtration. Any reduction in kidney function results in decreased excretion of the drug and concurrent rise in serum and tissue levels.

Contra-indications

Streptomycin is contra-indicated in those patients who have shown previous toxic or hypersensitivity reactions.

Adverse drug effects

The following side effects are commonly associated with streptomycin

- Ototoxicity
- Nausea and vomiting
- Paraesthesia of face, rash and fever
- Urticaria, angioneurotic edema and eosinophilia

The following reactions are less frequent; deafness, muscular weakness, exfoliative dermatitis, anaphylaxis, leucopenia, pancytopenia and thrombocytopenia.

Vestibular dysfunction resulting from the parenteral administration of streptomycin is cumulative and dose dependent. When 1.8 to 2.0 g/day are given, symptoms are likely to develop in the large percentage of patients especially in the elderly or those with impaired renal function within four weeks of administration.

Its therefore recommended that caloric and audiometric tests be done before, during, and following streptomycin therapy in order to facilitate detection of any vestibular impairment and/or dysfunction which may occur. Clinical judgment as to termination of therapy must be exercised when side effects occur.

Mild neuromuscular blockade has also been reported.

Literature review.

The biological inactivation of streptomycin in acid and alkaline solutions was first noted by Waksman and his coworkers. Folkers *et al* and Carter *et al* have shown that, on acid hydrolysis streptomycin is cleaved into two basic fractions; streptidine 1, 3-diguanido-2, 4, 5, 6 tetrahydroxycyclohexane and streptobiosamine. On acid hydrolysis the latter compound yields N-methyl-1-glucosamine and on alkaline hydrolysis of streptidine yields streptomine 1,3-diamino 2,4,5,6 tetrahydroxycyclohexane^{1,2,4,7}.

In 1945, Schenk and Spielman found that streptomycin is hydrolyzed by NaOH at 100°C for 3 mins to yield maltol. Maltol (a phenol) yielded a brilliant violet color with ferric chloride. The formation of maltol by alkaline hydrolysis of streptomycin was found to be quantitative¹².

The rapid inactivation of streptomycin in NaOH was indicated by Carter *et al*. When the hydrolysis of streptomycin chloride was carried out in NaOH at 100 or for a longer period at 40 a substance was isolated and characterized as maltol by absorbance^{3,9}.

Roux assayed streptomycin colorimetrically using sodium nitroprusside to form a colored complex with maltol⁹.

Heding and Henrie used paper chromatography method to identify streptomycin A from its derivatives at one time. The method was based on formation of bis (2-ethyl-hexyl) phosphate salts with streptomycin⁵

Duda *et al* assayed streptomycin using the maltol method and compared the results with a corresponding biological assay. The maltol method was found to be effective only for samples containing more than 0.5mg streptomycin base¹⁰

Boxer *et al* formed maltol by reaction of streptomycin with NaOH AT 100 C for 3mins. maltol was then reacted with ferric ions producing a purple colored complex and with Folin coicateau's phenol reagent producing a blue complex. The absorbance was then determined photometrically⁶

Sato and Ikada separated streptomycin and its dihydroxy derivative using thin layer chromatography (TLC) and visualizing the plates using sodium nitroprusside and potassium permanganate¹¹

Marshal *et al* developed a semiquantitative method based on a colored semicarbazone compound formed from streptose sugar⁸

Objectives

General objective.

This project aims at determining the stability of streptomycin complex formed between maltol and ferric ions on exposure to different temperatures for 1 hour.

Specific objectives

1. To estimate the presence of streptomycin in a multiproduct in an alkaline medium and read the absorbance of the maltol complex formed.

2. To determine the stability of streptomycin maltol ferric complex on exposure to 40°C for 1 hour

Justification

Streptomycin has activity against Gram-negative bacteria e.g. E.coli, klebsiella, and some species of salmonella and proteus. However, its main indication is in tuberculosis management. It's used in combination with isoniazid, rifampicin, pyrazinamide in TB therapy. It has also been used in treatment of plague and tularemia.

Considering the range of conditions that streptomycin can manage, it is a very useful drug in developing countries. Due to increased incidence of HIV/AIDS cases of TB have escalated. This therefore calls for provision of high quality drugs to save lives and hence the determination of its stability is crucial in quality ascertainment.

STORAGE / STABILITY AS SUPPLIED:

The hygroscopic powder should be stored at 2-8°C with desiccant.

Purity by TLC was unchanged after 2.5 years. Supplier's suggested shelf-life is three years.

Streptomycin salts are stable when dry for at least two years at room temperature but some loss in potency occurs at higher temperatures. The rate of deterioration is unaffected by light and air.

SOLUBILITY / SOLUTION STABILITY:

Streptomycin sulfate is soluble in water at 50 mg/mL, obtaining a clear solution ranging from colorless to light yellow.

. Additional solubilities reported (as mg/mL at 28°C): 5 methanol 0.85; ethanol 0.30; isopropanol 0.01; petroleum ether 0.015; carbon tetrachloride 0.035; ether 0.035.

Streptomycin at 1 mg/mL in water can be kept refrigerated for 30 days. Solutions at pH 3-7 retain potency for several weeks at room temperature, though some discoloration may occur. Changes in color that occur when solutions are exposed to light are not necessarily accompanied by loss of activity, but solutions should preferably be stored in the dark. Streptomycin is inactivated by acids, alkalis, oxidizing and reducing agents, urea and other carbonyl-containing compounds, as well as by cysteine and other sulfhydryl-containing substances

Estimation determination of streptomycin in a multiproduct helps the manufacturer/user be sure of the intended purpose since the presence of the active ingredient is assured.

By determining the stability of streptomycin sulphate its potency and efficacy is assured on prescription and administration.

Stability determination also helps the manufacturer and retailers to ascertain the best storage conditions to prolong their shelf life

Materials and apparatus

Materials and reagents

Name	Manufacturer	Grade
1.Streptomycin sulphate standard		
2.Streptomycin containing multiproduct solution	Norbrook Laboratories Ltd	
3.Sodium hydroxide pellets		Laboratory grade
4.Conc.sulphuric acid		Analar grade
5.Ferric ammonium sulphate		Laboratory grade

Apparatus

Name	Capacity	Quantity
1.Bulb and Graduated pipettes	2ml,5ml,20ml	2, 3,2
2.Thermometers		2
3.Ice bath		1
4.Volumetric flasks	50ml,100ml	3,3
5.Test tubes and Beakers	100ml	14,8

Instruments

Name	Make
1.Analytical weighing balance, stopwatch and Thermostatic water bath	
2. Oven	
3.UV/VIS Spectrophotometer and 10mm cuvettes	T90 SR NO:18-1901-01-0245

Methodology

Preparation of reagents.

A) Preparation of 2.5 M sulphuric acid

*6.75ml of 18.8 M sulphuric acid was pipetted

*20ml of distilled water was put into a 50ml volumetric flask and placed in a cold water bath

*The pipette sulphuric acid was added into the water in bits allowing to cool each time (reaction is exothermic) and the solution made to volume (50ml) with distilled water

*The resulting solution was approximately 2.5 M H_2SO_4

B) Preparation of 0.5 M H_2SO_4

*1.35ml of 18.8M sulphuric acid was dissolved in 20ml of distilled water in bits in a 50ml volumetric flask placed in a cold water bath and the solution made to volume using distilled water

C) Preparation of 3M NaOH

*12g of NaOH pellets were weighed

*90ml of distilled water was pipetted and put in 100ml volumetric flask. The NaOH pellets were then added to the water in bits with continuous swirling. The flask was held under running cool water since this reaction is exothermic

*The resulting solution was then transferred into a 100ml volumetric flask then topped to 100ml.

*The resulting solution was approximately 3M NaOH.

D) Preparation of 1% Ferric ammonium sulphate solution in sulphuric acid

*1g of ferric ammonium sulphate was weighed accurately and dissolved in 50ml of 0.5M H_2SO_4 IN A 100ml volumetric flask.

*The solution was then made to volume using distilled water

*The resulting solution was then filtered through a filter paper to remove insoluble particles.

*The resulting solution was about 1% ferric ammonium sulphate and is pale yellow in color.

E) Preparation of the standard solution

*A primary solution of streptomycin (20mg/ml) was prepared by weighing streptomycin powder equivalent to 1g streptomycin base and dissolving in 50ml distilled water in a 50ml volumetric flask

*2.5ml of the primary solution was pipette and put into a 100ml volumetric flask and made to volume. The flask was shaken to ensure even mixing. This was the standard solution.

EXPERIMENTAL

a) Streptomycin determination in a multiproduct containing streptomycin as an active ingredient

*20ml of the multiproduct solution was transferred into a 100ml beaker followed by 20ml of the 3M NaOH

*Ten 5ml portions of the resulting solution were put into different test tubes and immersed in a water bath at 60⁰C for 5 minutes and then placed into an ice bath.

(Placed in the ice bath to stop the conversion of streptose to maltol)

*To each of the test tubes 4ml of the 2.5M H₂SO₄ was added followed by 2ml of 1% ferric ammonium sulphate

*the solutions were then withdrawn from the ice bath and left to equilibrate for ten mins at room temp and the absorbance of the solutions taken at 540nm.

*The above procedure was repeated with ten test tubes of the standard and a comparison of the absorbances made.

b) Determination of the stability of the maltol complex with time in an oven at 40⁰C

*60ml of the standard solution was pipetted into a beaker and then 60ml of 3M NaOH added. Then 20ml of the resulting solution was put into a beaker and placed in an oven set at 40⁰C for 1one hour.

*The beaker was then removed and placed in an ice bath. This was followed by addition of 12ml of 2.5M sulfuric acid and 6ml of 1% ferric ammonium sulfate.

*The absorbances of the resulting solution were scanned in the wavelength of 540nm at 24- hour intervals for two days. (A freshly prepared sample was made each day and its absorbance read at 540nm and compared with those of the maltol complex of that day.)

Work plan

Activity	Time
1.Proposal writing	March
2.Identification and preparation of reagents	March
3.Stability at different temperatures	April
4.Stability in alkaline medium	April
5.Results and data compilation and analysis	May
6.Dissertation writing	June and July
7.Dissertation submission and presentation	July or August

References

- 1 *Martindale: The Extra Pharmacopoeia*, 31st ed., J.E.F. Reynolds, Ed. (Royal Pharmaceutical Society)
2. *Martindale: The Extra Pharmacopoeia*, 28th ed. (Pharmaceutical Press, 1982), p.1213.
3. *Merck Index*, 12th ed., #8983 (1996ty, 1996), p. 275.
4. *United States Pharmacopeia, XXII*, p. 1490
5. Heding, Henric, chem. Abstracts (59120, 1970)
6. Boxer G.E Jenelik, V .C Leghorn P.M Biochem 169,153(1947)
7. Textbook of clinical pharmacology
8. Marshall, E.K JR Blanchard K.C and Buhler E.L.J Pharmacology
9. The internet (Google scholar, PubMed)
10. Duda E.Martin.L.Kissin; Biol Rescent Eng chem. Abstract 1649, 79(1976)15(B)
11. Sato & Ikada HSCI, pap.Istphs.chem Roes (Tokyo) 59,159 1965
12. Shenk.J. & Spielman, M.A.J, Amer.ChemSoc.67, 227b (1945)
13. Dr.K.O Abuga, Lecture notes on Aminoglycosides.SOP (UoN).2005.
14. Okaru A.O (Bpharm IV 2006) Maltol method for the assay of streptomycin: An investigation of the absorbance-concentration profile.
15. Okubasu E.T (Bpharm IV 2004) Maltol method: Determination of absorbance time and suitability of the method for samples kept over 24 hours.