KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF HEALTH SCIENCES

FACULTY OF PHARMACY AND PHARMACEUTICAL SCIENCES

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

STABILITY STUDIES ON FLUCLOXACILLIN IN CAPSULE DOSAGE FORMS AND THE DESIGN OF AN HPLC MEHOD FOR THE ASSAY OF FLUCLOXACILLIN IN CAPSULE DOSAGE FORMS

BY

MICHAEL WORLAKO KLU

AUGUST, 2012

STABILITY STUDIES ON FLUCLOXACILLIN IN CAPSULE DOSAGE FORMS AND THE DESIGN OF AN HPLC MEHOD FOR THE ASSAY OF FLUCLOXACILLIN IN CAPSULE DOSAGE FORMS

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF

MASTER OF PHILOSOPHY IN PHARMACEUTICAL CHEMISTRY

In the Department of Pharmaceutical Chemistry

Faculty of Pharmacy and Pharmaceutical Sciences

By

MICHAEL WORLAKO KLU

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,

KUMASI

AUGUST, 2012

DECLARATION

I declare that this work was done by me at the Department of Pharmaceutical Chemistry,

KNUST and that any reference material consulted has been duly acknowledged.

This work has not been submitted anywhere for the award of any degree.

HEAD OF DEPARTMENT

....

DATE: 15-4-13

PROF. R.K. ADOSRAKU

SUPERVISOR

.....

DATE: 15/4/2013

PROF. JOHN S.K. AYIM

CANDIDATE

.....

DATE: 15/04/13

MICHAEL WORLAKO KLU

ABSTRACT

Stability studies on flucloxacillin sodium for capsule formulations were done. Fixed amounts of flucloxacillin sodium were mixed with varying amounts of dried starch, undried starch and sodium carboxymethylcellulose (sodium cmc). The mixtures were exposed in a room for three months to humidity and iodimetry was used to monitor the amounts of flucloxacillin in the mixtures over the three months.

Five capsule brands; A1, B, C, D and E were also sampled from the market and subjected to the same study. Their contents: A1 (115.0%), B (87.15%), C (92.25%), D (86.59%) and E (55.23%) were seen to reduce with time upon exposure to moisture whereas the unexposed samples showed no significant reduction in their contents.

It was noticed after the three month period that the mixtures with the dried starch experienced the least breakdown, followed by those with the undried starch while those with sodium cmc saw the most breakdown.

A reversed phase high performance liquid chromatography (RP- HPLC) method was also designed to assay flucloxacillin. The mobile phase used was methanol and potassium dihydrogen orthophosphate adjusted to a pH of 5 with dilute sodium hydroxide solution in the ratio (60: 40 v/v) on a Phenomenex ® Bondclone 10 C18 column (300×3.9mm I.D., 5µm particle). The method was validated and linearity, specificity, precision, robustness and accuracy were confirmed.

Key words: flucloxacillin sodium, sodium cmc, starch (dried and undried), iodimetry and RP-HPLC.

DEDICATION

I dedicate this work to my dear parents, Victor Klu and Beatrice Zowada, my siblings and niece, Angela Bedjirah.

ACKNOWLEDGEMENT

I would like to thank the Almighty God for seeing me through this programme successfully.

My sincere gratitude also goes to my supervisor, Prof. J.S.K Ayim for his patience, understanding and invaluable support in the course of the project.

My heartfelt gratitude also goes to my friends and course mates who assisted and supported me in diverse ways.

I would also like to acknowledge my family for their unceasing prayers and support for me particularly during the period of my study.

Finally, I would also like to thank in a special way all the staff and technicians especially Mr. Rashid for their assistance and support during my study.

TABLE OF CONTENTS

Content

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1 -INTRODUCTION AND LITERATURE REVIEW

1.0 INTRODUCTION

Antibiotics are naturally occurring substances that are produced by bacteria and fungi and are able to inhibit the growth of other bacteria and fungi. Penicillin is one of the first antibiotics to be discovered by man. Its discovery was made by chance by Sir Alexander Fleming in 1928 when he noticed zones of inhibition around blue-green moulds that were found in a medium he had inoculated with *Staphylococcus aureus.* **(Rosenberg)**

After its discovery, penicillin has seen a lot of development from its isolation as powder through mass production and usage in World War II to structural development. Penicillin can be classified into four types namely:

- Amino-penicillin e.g. ampicillin
- Naturally occurring penicillin e.g. benzyl penicillin
- Penicillinase resistant penicillin e.g. flucloxacillin
- Antipseudomonal penicillin e.g. carbenicillin etc **("Penicillin Antibiotics (Classification, Side Effects and Uses)")**

All these penicillins act on bacteria to kill them by inhibiting their cell wall synthesis.

Flucloxacillin is an isoxazolyl penicillin whose bulky side chain makes it resistant to beta lactamases produced by susceptible bacteria. It can be used in the treatment of boils among other infections and can cause muscle pains as one of its side effects. Its use is contraindicated in persons who react to penicillins.

Flucloxacillin as a drug comes in various forms; capsules, suspensions and injections. Flucloxacillin is an unstable drug which can be broken down by moisture, acids and bases. This implies that formulations of the drug ought to be kept away from these. It is however difficult to

totally keep formulations away from moisture because of the high humidity that persists in our environment.

The experiment is therefore aimed at investigating excipients which when formulated with flucloxacillin will slow down its breakdown by moisture.

Flucloxacillin can be analyzed by iodimetry which is somewhat tedious and by high performance liquid chromatography (HPLC) and ultraviolet- visible spectroscopy both of which employ expensive reagents. The investigation also includes the development of an HPLC method which is inexpensive and rapid.

1.2 JUSTIFICATION

One major problem associated with the use of flucloxacillin like other penicillin antibiotics is the hypersensitivity reactions some people have when they use them. Degradation products such as penicilloic acids have been implicated in these reactions. The penicilloic acid among other breakdown products comes about when the drug is exposed to acids, bases, alcohols, enzymes and moisture. It is much easier to keep the drug away from all these factors compared to keeping it away from moisture as it is present everywhere owing to the very humid nature of our atmosphere. Thus flucloxacillin still breaks down when kept in airtight packaging materials.

Another problem with flucloxacillin use is the development of resistant strains of bacteria towards it. Failure of patients to take complete courses of antibiotics is a major cause. Another major cause is the issue of under-dosage because if a patient took a complete course of a low dose medication, levels attained in the body would be well below therapeutic levels to kill off any bacteria. This low dosage can come about when one takes a medication in which a significant amount of the drug has broken down even prior to the expiration of the product.

It is therefore very important that stability studies be conducted on flucloxacillin to investigate the effects of excipients on it and possibly find out which excipient and in which amounts with the active drug will render it most stable.

Millpharm and Arrow generics, a pharmaceutical company based in the UK through the agency of Medicines and Health Regulatory Agency (MHRA) in the UK which is similar in function to the Food and Drugs Board (FDB) in Ghana, recalled flucloxacillin capsules on 21st August 2007 due to stability concerns of the finished product. **(MHRA, 2007)** This can have very serious financial implications on companies. This could occur in Ghana and also since our humidity is higher than in the UK it is only prudent to formulate a very stable product.

1.3 OBJECTIVES

The objectives of the research work are:

- To determine the order of reaction for the breakdown of flucloxacillin by moisture.
- To investigate the effects of some excipients on the stability of flucloxacillin.
- To determine which of the excipients used and in which proportion is most compatible with flucloxacillin as regards its stability.
- To show that flucloxacillin can breakdown when exposed to moisture.
- To develop a simple HPLC method for the assay of flucloxacillin in capsules.

1.4 LITERATURE REVIEW

1.4.1 HISTORY OF PENICILLIN

Penicillin is one of the earliest antibiotics discovered by man.

Antibiotics are naturally occurring substances produced by some bacteria and fungi and are capable of inhibiting the growth of other bacteria and fungi.

Sir Alexander Fleming observed in 1928 that *Staphylococcus aureus* colonies were being inhibited by *Penicillium notatum* and this showed that an inhibitory agent was present. It happened when Fleming accidentally exposed a Petri dish inoculated with *Staphylococcus aureus* for a few days and noticed that it was contaminated by a blue green mould which grew visibly in the medium. There was a clear ring around the mould where there was no bacterial growth and Fleming inferred from this observation that the mould produced substances that inhibited the growth of the bacterium and was also later found to be lethal to a wide range of other bacteria which are harmful. The mould was discovered to be and became known as *Penicillium notatum* after he had grown a pure culture and consulted with mycologist C.J. La Touche.

Fleming then conducted several experiments to investigate the antimicrobial activity of this mould. The result was that the mould destroyed the harmful bacteria used by Fleming in his study and upon further experiments he proved that the mould was non toxic. Fleming, since he was not a chemist, could not isolate penicillin for keeping to be used by humans. In 1929, he therefore published his findings on his novel project but this did not arouse interest from the scientific community.

Fleming's discovery did not really show to be of relevance at the time simply because penicillin usage had not gained popularity until in the 1940s when it was isolated as a brown powder by the Australian Howard Florey and German refugee Ernst Chain. The brown powder was found to

retain its antibacterial activity for some few days. They did some studies on the powder and proved that it was safe to be used in humans.

Mass production of penicillin was then started to save many lives that would have been lost during the Second World War due to bacterial infestation of small cuts and wounds.

Fleming is credited for the discovery of penicillin and Florey and Chain rendered the antibacterial agent usable. As a result, all three were awarded a Nobel Prize in Physiology or Medicine in 1945. Fleming's discovery led the way in the discovery of other antibiotics that killed bacteria. **(Rosenberg)**

1.4.1.1 Developments from Penicillin

Penicillin's narrow spectrum of activity coupled with the reduced oral activity of phenoxymethylpenicillin stimulated a search for derivatives that would demonstrate a broad spectrum of activity against bacteria. The isolation of the penicillin nucleus, 6- aminopenicillanic acid (6- APA), made it possible for the production of semi-synthetic derivatives that were superior to benzyl penicillin in terms of bioavailability, spectrum of activity, stability and tolerance by patients.

Ampicillin was the first major development and it was shown to have a wider spectrum of activity than those earlier discovered as such it is used to treat infections caused by gram positive and gram negative bacteria. Modification of ampicillin led to amoxicillin that has a longer duration of action.

Further advancements led to the production of penicillins that are beta-lactamase resistant like flucloxacillin, cloxacillin, dicloxacillin and methicillin. These showed activity against bacteria that produced the enzyme, beta-lactamase, that destroys penicillin but are inactive against methicillin resistant *Staphylococcus aureus* which developed with time.

Antipseudomonal penicillins like ticarcillin, carbenicillin and piperacillin were also later discovered and they showed activity against gram negative bacteria. **("Penicillin Development")**

1.4.1.2 Classification of penicillins

The penicillins are classified based on their ability to kill various kinds of bacteria. From narrow to broad spectrum of antibacterial activity they have been classified as:

- Natural Penicillins: They include penicillin G and penicillin V. They are active against gram positive bacteria like streptococci, staphylococci and some gram negative bacteria like meningococcus.
- Penicillinase- resistant penicillins: Penicillins in this class include cloxacillin, dicloxacillin, oxacillin and nafcillin. They are very active against those strains of bacteria like staphylococcus that produce penicillinase.
- Aminopenicillins: Drugs like ampicillin, amoxicillin, bacampicillin come under this class. They were the first penicillins discovered to kill gram- negative bacteria like *Escherichia coli* and are also acid stable.
- Extended spectrum or anti- pseudomonal penicillins: Alpha- carboxypenicillins like carbenicillin and ticarcillin and acylaminopenicillins such as piperacillin and azlocillin belong here. Their spectrum of activity is comparable to that of the aminopenicillins but have an additional advantage in that they are also active against many gram negative organisms like *Pseudomonas aeruginosa*. They can be inactivated by beta- lactamases. **("Penicillin Antibiotics (Classification, Side Effects and Uses)")**

1.4.2 FLUCLOXACILLIN

Flucloxacillin is an isoxazolyl penicillin antibiotic and like the other penicillins has a beta-lactam ring. It belongs in the same class as cloxacillin, dicloxacillin, oxacillin and nafcillin. Its spectrum of activity is narrow and it is very active against those bacteria e.g. *Staphylococcus aureus* that produce beta- lactamases; those enzymes that inactivate penicillins. It is however inactive against Methicillin resistant *Staphylococcus aureus.* It is also very stable to gastric acid.

Mode of action

The bactericidal action of flucloxacillin like the other penicillins is by the inhibition of cell wall synthesis of susceptible bacteria by blocking the cross linkage of the peptidoglycan polymer. **(Brunton et al, 2006)**

Indications for flucloxacillin

1. Skin and soft tissue infections: cellulitis, boils, furunculosis
2. Infected skin conditions: acne and eczema
3. Respiratory tract infections: pneumonia, empyema and lung abscesses.
4. Other infections like osteomyelitis and urinary tract infections and those caused by flucloxacillin sensitive organisms. **(British National Formulary, 2009)**

Side effects/ adverse effects

1. Hepato-biliary disorders

- Cholestatic jaundice and hepatitis

2 .Immune system disorders

- Hypersensitive reactions —
- Very rarely anaphylactic shock often with oral administration and angioneurotic edema.

3. Gastrointestinal disorders

- Nausea , diarrhoea
- Sore mouth or tongue and very rarely a black hairy tongue
- Pseudomembraneous colitis

4. Blood and lymphatic system disorders

- Neutropenia which may include agranulocytosis
- Thrombocytopenia

5. Skin and subcutaneous tissue disorders

- Urticaria
- Rash
- Purpura
- Rarely Stephen Johnson's syndrome, erythema multiforme and toxic epidermal necrolysis

6. Musculoskeletal and connective tissue disorders

- Arthralgia
- Myalgia

7. Renal and urinary system disorders

- Interstitial nephritis (**"con014961.pdf,"**)

Contraindication

1. Penicillin hypersensitivity **(British National Formulary, 2009)**

Precautions and special warnings

1. Renal impairment

2. Hepatitis and cholestatic jaundice

During prolonged treatments there should be regular monitoring of renal and hepatic functions

1.4.2.1 Pharmacokinetic profile of flucloxacillin sodium

- **Absorption**

Flucloxacillin is administered by the intramuscular route or by the oral route since it is acid stable. The levels of the drug in blood serum after one hour are as follows:

1. When 250mg was administered to fasting subjects via the oral route the level obtained in the blood serum was about 8.8mg/l.

2. When 500mg was administered to fasting subjects via the oral route the level obtained in the blood serum was about 14.5mg/l.

3. When 500mg was administered intramuscularly the amount in the blood serum was about 16.5mg/l.

The total amount of the drug absorbed after oral administration was about 79% of the amount of drug administered.

- **Distribution**

Flucloxacillin diffuses well into body tissues. Amounts that have been respectively recovered in spongy bone and compact bone are 11.6mg/l and 15.6mg/l with a mean level of 8.9mg/l in serum.

Flucloxacillin is excreted in minimal amounts in breast milk and only small quantities diffuse into the cerebrospinal fluid of subjects whose meninges are not inflamed.

- **Metabolism**

Approximately 10% of flucloxacillin is metabolized to penicilloic acid when administered by the oral route to normal subjects. It has an elimination half- life of 53 minutes.

- **Excretion**

Excretion is mainly renal with about 65.5% and 76.1% of the dose administered remaining unchanged in urine within 8 hours respectively by the oral and parenteral routes. A small amount is however excreted in the bile. There is therefore a reduced clearance of flucloxacillin in cases of renal impairment.

- **Protein binding**

About 95% of the drug is bound to proteins following administration. **("con2033471.pdf,")**

1.4.2.2 Drug interactions

- Probenecid, aspirin and anti-inflammatory agents compete for renal excretion of flucloxacillin and other penicillins thereby leading to toxicity.
- Flucloxacillin like other penicillins reduces the effectiveness of oral contraceptives.
- There is also reduced excretion of cytotoxic drugs like methotrexate by flucloxacillin.

("con2033471.pdf,")

1.4.2.3 Formulations available

- Capsules
- Injection
- Oral solution
- Suspensions

1.4.2.4 Storage

Flucloxacillin sodium should be kept airtight and at a temperature not exceeding 25°C.

Reconstituted suspensions must be kept refrigerated at temperatures between 2-8°C.
("con2033471.pdf,")

1.4.2.5 Stability of flucloxacillin

The penicillins have a very unique molecule; having a beta lactam ring which is fused to a thiazolidine ring. The strained beta lactam ring can be cleaved by nucleophiles, acids, bases, moisture, alcohol and enzymes. Flucloxacillin, though a penicillin, is not liable to enzymatic cleavage because of its bulky isoxazolyl side chain that hinders this form of cleavage.

Flucloxacillin is hydrolyzed in aqueous solutions by degrading the beta lactam ring. The hydrolysis is promoted by an increase in temperature or in acids or bases. The breakdown products include penillic, penicillenic acid and penicilloic acids which reduce the pH and further promote the degradation process. Minimal amounts of penicillamine have also been detected. This degradation process is insignificant at a pH of about 6.8 and the inclusion of a buffer like citrate buffer retards the breakdown. **(Sweetman, 2009)**

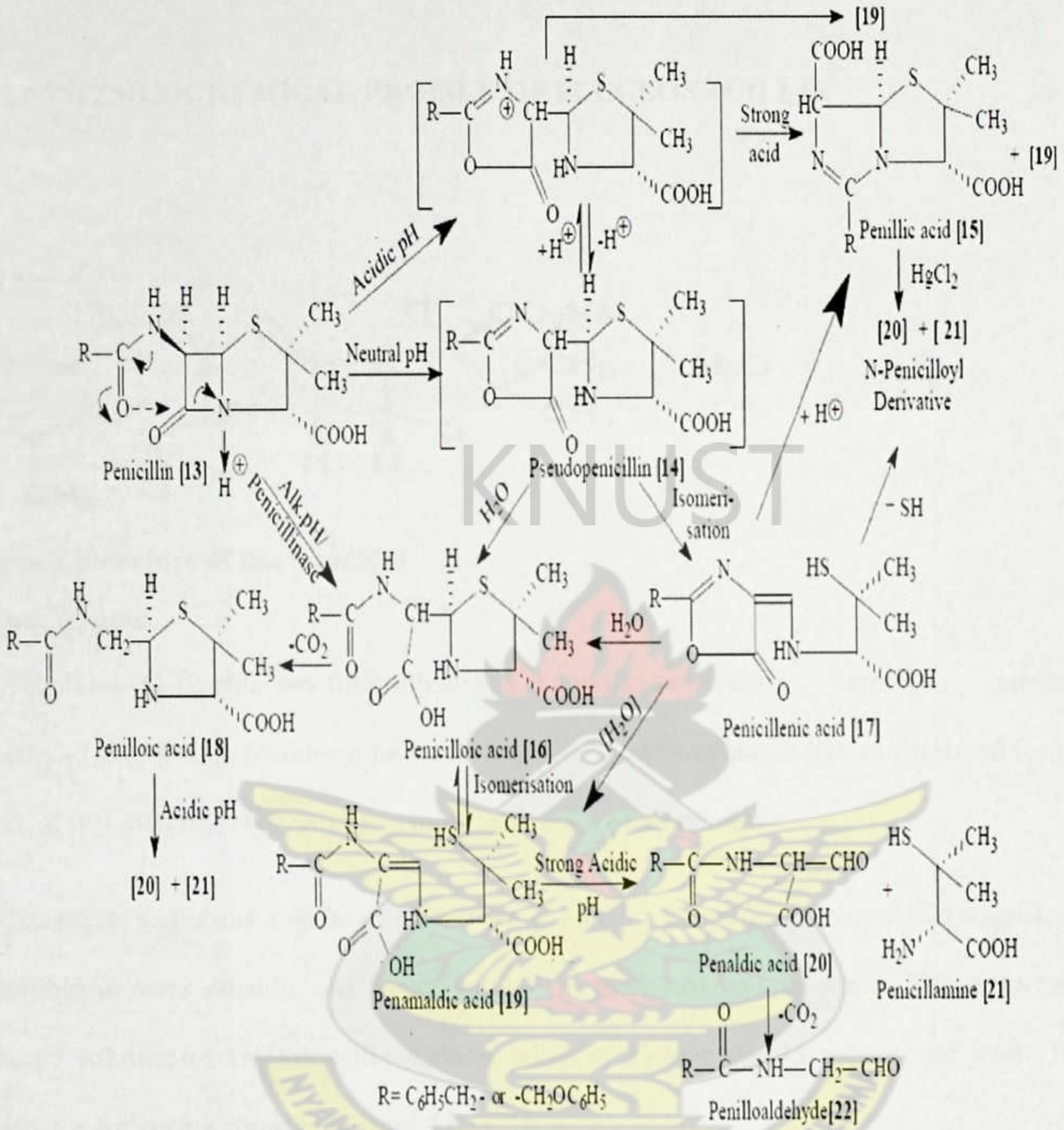


Figure 1 Scheme for the breakdown of penicillins in different media

(Deshpande et al, 2004)

1.4.2.6 PHYSICOCHEMICAL PROFILE OF FLUCLOXACILLIN

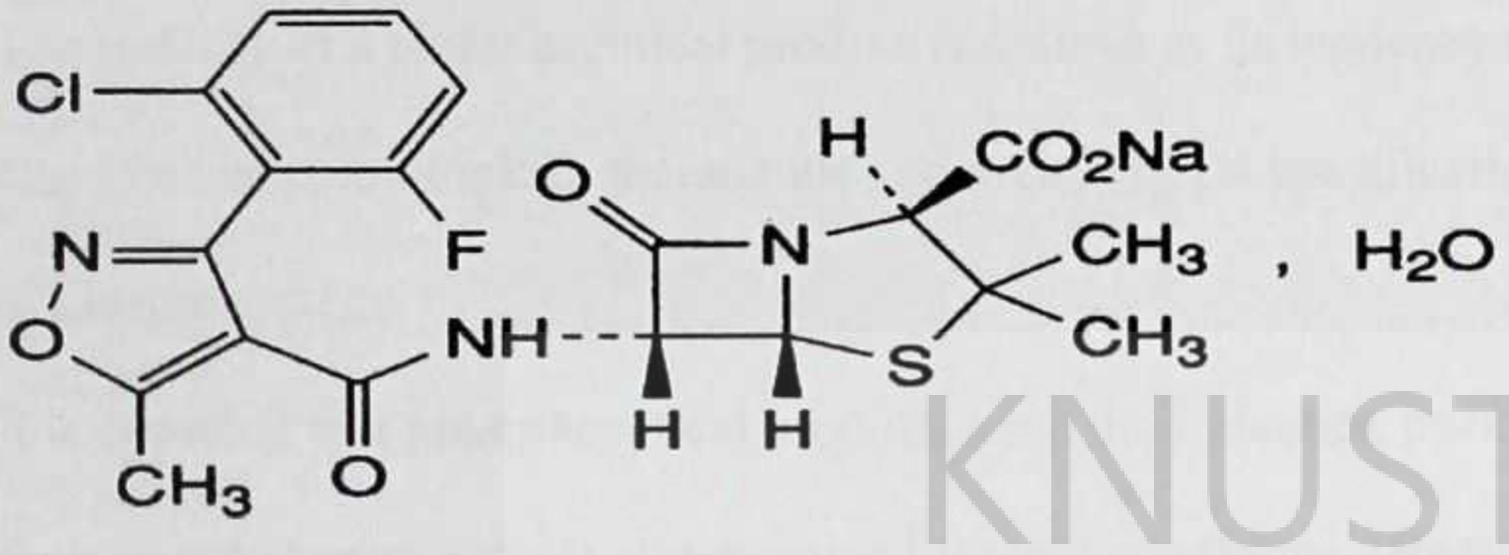


Figure 2 Structure of flucloxacillin

Chemical name

(2S,5R,6R)-6-((3-(2-chloro-6-fluorophenyl)-5-methylisoxazol-4-yl) carbonyl) amino)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo(3.2.0) heptanes-2-carboxylate. It has an empirical formula of $C_{19}H_{16}ClFN_3NaO_5S$, H_20 and has a molecular mass of 493g/mol.

Flucloxacillin sodium is a white or almost white powder crystalline in nature and hygroscopic. It is soluble in 96% ethanol and freely soluble in methanol and in water. The absorbance of 10%w/v solution of flucloxacillin sodium taken at 430nm should not exceed 0.04. The pH obtainable for such a solution is from 5.0 to 7.0.

The specific optieal rotation of 1% w/v solution determined using the anhydrous substance as a reference is +158 to +168.

Flucloxacillin can be assayed by high performance liquid chromatography (HPLC), iodimetric titration and ultraviolet-visible spectroscopy and the content of the drug must not be below 95.0% and should not exceed 101.0%.

Flucloxacillin should be kept in an airtight container and its storage temperature should not exceed 25°C. **(BP 2007)**

1.4.3 STABILITY OF PHARMACEUTICAL PRODUCTS

The stability of a pharmaceutical product is defined as its tendency to keep its physical, chemical, microbiological, therapeutic and toxicological specifications in a particular container or closure system.

It is expected that pharmaceutical products keep their identity, purity, quality and strength during their stated storage periods at determined storage conditions. **(Remington, 2005)**

The stability of a pharmaceutical product is influenced by:

- Stability of the active ingredient
- Potential interaction between active ingredients and excipients
- The manufacturing process
- The dosage form
- The container-liner closure system (packaging material)
- The environmental conditions faced during shipment, storage and handling
- The time period between manufacture and usage. **(Olaniyi, 2010)**

1.4.3.1 Types of stability

- **Chemical stability**

The product keeps it chemical integrity and labelled strength within limits that have been specified.

- **Physical stability**

Appearance, uniformity, suspendability, dissolution and palatability of the pharmaceutical product are retained.

- **Microbial stability**

Here, microorganisms are unable to thrive in the product. The antimicrobial agents used in the product keep the product's effectiveness within specified limits.

- **Therapeutic stability**

The drug's effectiveness for its purpose remains the same.

- **Toxicological stability**

The drug doesn't show any appreciable increase in toxicity.

Product stability has been subtly divided in two: chemical and physical stability.

These two forms are nearly inseparable as they go together. For instance physical factors like heat, light and moisture are needed to trigger chemical reactions that cause degradation of drugs. Physical stability data can be obtained during the preformulation stage of a drug and knowledge of these is necessary for three main reasons:

- A pharmaceutical product must appear appealing to the consumer as changes in physical appearance like colour fading and cloudiness could cause the consumer to lose confidence in the product.
- Some products are packaged in multi-dose containers and as a consequence dosage uniformity is a necessity. Non- uniform dosage can be a direct result of physical breakdown. For example, a broken down emulsion will lead to non uniform doses being removed when administered.
- The active ingredient must retain its integrity during the expected shelf life of the drug as any form of breakdown can lead to the formation of an altered substance that could be harmful to the user. **(Remington ,2005)**

1.4.3.2 Stability problems some pharmaceutical dosage forms present with.

- **Tablets**

A tablet that is stable should keep its shape, size and its original colour during its shelf life.

Cracks, chips or powder deposition at the bottom of their containers are indications of physical instability.

- **Capsules**

There are two types, hard and soft gelatin capsules. The former is normally used by manufacturers. Gelatin can absorb moisture when put in an environment of high humidity and cause capsule shells to swell and have a distorted shape. When kept in very dry environments they easily crack when subjected to slight pressures.

- **Suspensions**

A stable suspension upon moderate shaking disperses homogenously. A major instability indication is when particles of a suspension settle and form hardened masses that do not redistribute readily upon shaking.

Ointments

A stable ointment is one that remains homogenous during its shelf life. Problems associated with these products are changes in consistency and separation of oils unto the surface of the product.

(Remington, 2005)

1.4.4 KINETICS OF DRUG DECOMPOSITION

Kinetics deals with the study of the rate at which changes whether chemical or physical occur.

The study of kinetics enables the prediction of shelf life of drugs and the amount of change that occurs after a certain given time.

Decomposition of drugs can occur by a first order reaction, second order reaction and a zero order reaction.

The derived equation for a first order reaction is given as In c = In c_0-kt

The derived equation for a second order reaction is given as $1/c = 1/c_0 + kt$

The zero order equation is written as $c = c_0 - kt$

Where c= concentration after a certain period of time, t, c_0 = initial concentration and k = rate constant. **(Aulton, 2002)**

1.4.5 THIN LAYER CHROMATOFGRAPHY (TLC) OF SOME PENICILLINS

A mixture of penicillamine, ampicillin, amoxicillin and benzyl penicillin have been successfully separated using various mobile phase systems like acetonitrile/ water and butanol/ acetic acid/ water respectively in ratios of 3:5 and 4:1:1. Other mobile phase systems were also used successfully. A cation exchange stannic arsenate cellulose support material was used. Iodine vapour was employed to aid spot visualization. **(Nabi et al, 2006)**

Amoxicillin, ampicillin, cloxacillin, cephalexin, streptomycin, tetracycline, gentamicin, erythromycin and co-trimoxazole have been separated in a mixture on a titanic silicate inorganic ion exchanger successfully. Erythromycin and tetracycline were well separated from the other antibiotics when a solvent system of chloroform/ methanol/ ammonium hydroxide in the ratio of 1:1:1 was used as mobile phase. Several other systems of solvent as mobile phases gave other separations. A 1% w/v ninhydrin in ethanol was used to locate amoxicillin, ampicillin, cloxacillin, cephalexin, gentamicin and co-trimoxazole spots while 5% (w/v) potassium dichromate in concentrated sulphuric acid was used in locating streptomycin, erythromycin, tetracycline and doxycycline spots. **(Husain et al, 2004)**

Penicillin G and cloxacillin have been detected and quantitated in milk using TLC. The milk was treated to remove proteins with 2 volumes of acetonitrile and then shaken with buffers and organic solvents; first with a buffer of pH 2.2 and then with a buffer of pH 7. The antibiotics were extracted into methylene chloride and spotted on silica gel plates and developed with chloroform/ acetone/ glacial acetic acid in the ratio of 10:9:1. Spraying the plates with 1N HCl and starch and final exposure to iodine vapour aided the location of the spots which appeared blue- black. **(Moats, 1983)**

Flucloxacillin and cloxacillin have the same method of identification outlined in the **BP (2007).** They have the same reference solutions made of equal amounts of cloxacillin, dicloxacillin and flucloxacillin. The mobile phase used was 30 volumes of acetone and 70 volumes of 154g/l ammonium acetate adjusted to a pH of 5 with glacial acetic acid. The support material was silica gel and iodine vapour was used for spot visualization.

Amoxicillin trihydrate has been identified using a mixture of equal amounts of amoxicillin trihydrate and ampicillin trihydrate as reference and a mobile phase made of 10 volume of acetone and 90 volume of 154g/l solution of ammonium acetate adjusted to a pH of 5 with glacial acetic acid. The support material was silica gel and iodine vapour again was used for spot visualization**. (BP, 2007)**

1.4.6 IODINE TITRATIONS OF PENICILLINS

Penicillins can be assayed by iodimetry because the penicilloic acids they produce when they are broken down in acid or alkaline media are able to take up iodine which aids their quantification. Penicillins have been shown to be quantifiable in culture fluids. The penicillin was extracted from the culture fluid using amyl acetate at a pH of 2 from the aqueous phase followed by a re-extraction from the amyl acetate using a known volume of phosphate buffer of pH 7. The

penicillin in the phosphate buffer was then assayed using standard solutions of sodium thiosulphate and iodine using sodium starch glycollate as indicator. A correction factor was introduced for those substances that were extracted with the penicillin from the culture fluid and took up iodine during the assay. **(Beloff-Chain & D'accadia, 1952)**

The sodium and potassium salts of benzyl penicillin have been assayed by iodimetry. A solution of the drug of either the sodium or potassium salt was hydrolyzed by 1M sodium hydroxide to give the penicilloic acid which was stabilized in acetate buffer which also created a desired pH for the titration. The excess hydroxide solution was neutralized with 1M hydrochloric acid so as not to alter the pH of the medium. A standard solution of iodine of concentration 0.01M was added to the mixture and the vessel stoppered and kept away from light. After twenty minutes the excess iodine was titrated against a standard solution of thiosulphate using starch mucilage as indicator. To effectively quantify the amount of benzyl penicillin present, other impurities that could absorb penicillin in addition to the penicilloic acids generated were ruled out by carrying out a blank titration where the drug was not hydrolyzed by sodium hydroxide. The blank determination proceeded with an addition of acetate buffer and standard iodine solution to a solution of the drug. The vessel was stoppered, kept away from light and titrated against a standard solution of thiosulphate using starch mucilage as indicator after twenty minutes. The difference between the two determinations aided in the quantification of benzyl penicillin. **(BP 1980)**

The sodium and potassium salts of phenoxymethyl penicillin have also been assayed like benzyl penicillin with the only difference being the preparation of the samples. Aliquots for the assay were obtained from the resulting solution obtained by: adding an amount of phenoxymethyl penicillin to a saturated solution of sodium hydrogen carbonate, followed by

addition of some water and 1M hydrochloric acid with occasional shaking and adjustment to the desired volume again with water. **(BP 1980)**

Phenoxymethyl penicillin has been assayed using the method described above in body fluids during an in vitro study on the pharmaceutical equivalence of phenoxymethyl penicillin in tablet formulations available in Bangladesh. **(Apu et al., 2011)**

1.4.7 HPLC METHOD VALIDATION

Method validation is a way of ensuring through studies carried out in the laboratory that an analytical method's performance characteristics meet the requirements for the intended application. Method validation is necessary to ensure that a particular procedure when followed with a particular instrument or equipment by different analysts in the same or different laboratories will give reliable and reproducible results or data. **("HPLC validation PE.pdf,")**

The following are the parameters considered when validating an analytical procedure:

- **Precision**

Precision gives an insight into the extent to which individual test results agree when the analytical procedure is applied to samples of a homogenous material. Precision therefore in effect measures how reproducible the analytical procedure is under ordinary circumstances of operation and it is assessed on three levels; repeatability, intermediate precision and reproducibility. Precision is determined by using the method to assay the sample severally to obtain about six to ten results which are valid statistically and is often expressed as relative standard deviation.

- **Accuracy**

Accuracy is a measure of how close a test result obtained from an analytical procedure is to a true value. It can be determined by assaying a material to which a substance of known purity has been added. The assay or analysis is checked against a blank and test solutions to rule out any interferences. The accuracy is then calculated as percentage of analyte recovered from the test by the assay **("HPLC validation PE.pdf")**. It can also be determined alternatively by comparing the results from the designed analytical procedure with that of a second well approved, recognized and characterized procedure. **("Validation of analytical procedures: text and methodology," 2005)**

- **Linearity**

This is the tendency of the designed method to give responses or results that are either directly or after mathematical computations within a particular range proportional to the concentrations of the substance or analyte.

- **Range**

The range is simply the interval between the upper and lower levels of an analyte that has already demonstrated linearity, precision and accuracy. It can be determined on a curve that may be linear or non-linear.

- **Ruggedness**

Is the ability of an analytical procedure to give reproducible results for the same material or sample under varying conditions: different analysts or operators, instruments, reagents, assay temperatures, slight changes in mobile phases and so on.

- **Limit of detection**

Is the lowest concentration of an analyte that may be detected in a sample and may or may not be quantifiable. This parameter is very important when it comes to tests for impurities and assays of formulations that have low concentrations of drug substances. **("HPLC validation PE.pdf")** This parameter can be calculated based on signal to noise ratio, standard deviation of the response and the slope, standard deviation of the blank and on the calibration curve. **("Validation of analytical procedures: text and methodology," 2005)**

- **Limit of quantification**

Is the lowest concentration of an analyte that may be determined in a sample with an acceptable level of precision and accuracy. **("HPLC validation PE.pdf")** This parameter can also be calculated based on signal to noise ratio, standard deviation of the response and the slope, standard deviation of the blank and on the calibration curve. **("Validation of analytical procedures: text and methodology," 2005)**

- **Selectivity and Specificity**

Selectivity is the method's tendency to quantitate accurately and specifically the compound or analyte in the presence of other components which may be found in the same matrix as the analyte.

Specificity ensures that any signal obtained is due to the analyte and any excipients, degradation products and impurities present do not cause any interference.

- **System suitability tests**

Once a method is validated it becomes the responsibility of the analyst to routinely ensure that the method is operational within the set parameters that were established in the validation process.

One simple way to do this for an HPLC system is obtain a chromatogram for a sample and compare it with a standard one. This permits comparisons of peak shape, peak width and baseline resolution.

Alternatively for an HPLC method or system the following can be determined experimentally: tailing factor, capacity factor, resolution, number of theoretical plates and relative standard deviation. **("HPLC validation PE.pdf")**

1.4.8 EXCIPIENTS FOR PHARMACEUTICALS

Excipients are inert or inactive substances that are included in pharmaceutical formulations. They serve as carriers for medications, aides in pharmaceutical processing as they can help the absorption of drugs when administered; and also help in bulking up medications of very potent drugs to make it easier for their administration thus enabling a convenient and accurate dosage. Based on the sort of excipient used in a formulation, they can act as binders, disintegrants, preservatives, for coating etc. **("Excipients for pharmaceuticals.pdf,")**

1.4.8.1 Starch

Starch is a carbohydrate substance or a polysaccharide made of a large number of glucose units linked by glycosidic bonds. Starch is produced by green plants and is the major energy or food reserve of plants being stored in stems, seeds and roots. It is therefore contained in large amounts in cassava, rice, potato, maize and wheat.

Starch in the pure form is a white, amorphous, tasteless and ordourless powder. It is insoluble in organic solvents like ethanol, ether, acetone and water. It turns blue- black on reaction with iodine solution.

Starch is one of the earliest excipients used in the production of pharmaceuticals like tablets and capsules. It is used as a diluent, binder and a disintegrating agent. Starch is hygroscopic and absorbs about 10% to 17% of water. **(Musa et al, 2011)**

Starches recommended by the British Pharmacopoeia and are used in the pharmaceutical industries include:

- Maize starch obtained from the caryopsis of *Zea mays* L.
- Rice starch obtained from the caryopsis of *Oryza sativa* L.
- Potato starch obtained from the tuber of *Solanum tuberosum* L.
- Wheat starch obtained from the caryopsis of *Triticum aestivum* L.
- Tapioca starch obtained from the rhizomes of *Manihot utilissima* Pohl **(BP 2007)**

Some non- pharmaceutical uses of starch

- Used as adhesives and glue for making corrugated paperboards.
- Used as binders in agrochemicals like fertilizers.
- Used in textiles as constituent of weaving production lines and printing cloth plastics.
- Used together with vegetable oils to make bio-lubricants.
- Used to make body powder in the cosmetic industry. **("newsletter19.pdf (application/pdf Object)," 2003)**

1.4.8.2 Sodium carboxymethylcellulose

Sodium carboxymethyl cellulose (Na CMC) is a derivative of cellulose with carboxymethyl groups linked to some of the hydroxyl groups found in the glucopyranose moieties that constitute the backbone of cellulose. **("Codex Alimentarius," 2009)**

The synthesis of Na CMC from cellulose involves two steps. The first step involves introducing cellulose into an alkaline solution to open up the tightly held cellulose chains allowing water to permeate it and to render it soluble. The cellulose is then reacted with sodium monochloroacetate to yield Na CMC which is water soluble. **(Hoefler)**

Na CMC is white or slightly yellowish and almost ordourless hygroscopic powder or granules. It dissolves in water to form a colloidal solution which is viscous but it is not soluble in ethanol. It is identified by one of three methods:

- Shaking vigorously a 0.5% solution of Na CMC no layer of foam should be formed.
- The formation of a precipitate when 5ml of 5% copper sulphate or aluminium sulphate is added to 5ml of 0.5% Na CMC solution.
- By adding 1ml of water to 1ml of a 1% solution in a small test tube plus 5 drops of 1-naphthol and tilting tube followed by the addition of 2ml of sulphuric acid down the side of the test tube, a red colour should be produced at the interface.

Na CMC is used as an excipient in the pharmaceutical industry and serves as a binder, suspending agent, stabilizer and a thickening agent. **("Sodium carboxymethylcellulose- FAO," 1984)**

A non -pharmaceutical use of Na CMC is as a food additive in cakes, dairy products and animal feed. **(Hoefler)**

1.4.9 REVIEW OF OTHER ANALYTICAL METHODS USED TO ASSAY FLUCLOXACILLIN

It is necessary that drug products not only contain the actual active ingredients that are stated on them but also in their right quantities so as to make them desirable to be used by humans and sometimes animals. The rates at which substandard products are released unto the markets and their usage have led to a lot of problems. Some people have had their health problems worsened as a result of this. There is also the development of resistant strains of bacteria when poor antibiotic products are used.

To avoid this, analysts have come out with a lot of analytical procedures to assay drugs in formulations, body matrices and tissues.

Flucloxacillin have been assayed using titrimetry, HPLC and ultraviolet- visible spectroscopy and these can be found in the British Pharmacopoeia (BP) and articles that have been published.

1.4.9.1 Assay of flucloxacillin using titrimetry

This method was described for cloxacillin in **BP (1968) and BP (1973).** The procedure simply involved hydrolyzing the drug in a neutralized solution with sodium hydroxide and the excess alkaline solution was titrated with a standard solution of hydrochloric acid. A blank was performed and the difference in readings represented the amount of sodium hydroxide required. The theory behind the titration makes it applicable to flucloxacillin as it will react in like manner as cloxacillin to yield penicilloic acid upon hydrolysis with sodium hydroxide. The procedure is satisfactory for pure flucloxacillin powder, capsules and injections.

1.4.9.2 Assay of flucloxacillin using UV- visible spectroscopy

Direct UV analysis of flucloxacillin like the other penicillins may yield results that may be lacking in accuracy and precision because penicilloic acids and other degradation products interfere with the results **(Jun et al, 1985)**. To overcome this problem, **BP (1993)** employed the use of a derivatizing agent, imidazole- mercury reagent. This reagent was warmed with the drug at 60°C for 25minutes to give a product, penicillenic acid- mercury (II) mercaptide that absorbed more strongly and at a longer wavelength of detection of 364nm where all interferences due to penicilloic acid and other substances were eliminated.

A similar method by **Jun et al (1985)** used 1, 2, 4 - triazole as the derivatizing agent but this had a shorter reaction time of 10 minutes when warmed at 60°C with the drug and the detection wavelength ranged from 323-346nm.

A UV method has also been described where iodine solution was added to methanolic solutions of the drug and the absorbances measured. The method was reported to be based on charge complexation reaction of iodine with flucloxacillin **(Gujral et al 2009)**.

The methods are suitable for flucloxacillin pure powder, capsules, injections, oral solutions and suspensions.

1.4.9.3 Assay of flucloxacillin by HPLC

Several HPLC methods have been outlined for the assay of flucloxacillin with UV detection. A method has been developed and validated for the determination of the drug in plasma with dicloxacillin as internal standard in the reverse phase mode with a C-18 column. The mobile phase was composed of potassium dihydrogen phosphate and acetonitrile (64.5% and 35.5% respectively). **(Zhou et al 2007)**

The **BP 2007** and **BP 2009** describe a method in the reverse phase mode. The mobile phase system was made of 25 volumes of acetonitrile and 75 volumes of 2.7g/l of potassium dihydrogen phosphate adjusted to pH 5 with sodium hydroxide. The flow rate was 1ml/min and detection wavelength was 225nm.

Flucloxacillin has also been assayed in the presence of amoxicillin also in the reversed phase using different compositions of the mobile phase system mentioned above **(Nikam et al 2009)**.

A variation by **Shanmogasundram et al (2009)** for a similar assay employed a buffer and acetonitrile on strong cation exchange column.

CHAPTER TWO – EXPERIMENTAL

2.1 INSTRUMENTS AND MATERIALS

- Erweka tablet disintegration apparatus
- Buchi heating bath B-419
- Buchi rota vapor R- 210
- Buchi distillation chiller B- 741
- Hanna instruments pH 211 microprocessor pH meter
- Adam – analytical weighing balance, WA 210; 210/ 0.0001g
- Volumetric flasks (1000ml, 500ml, 250ml, 100ml, 25ml)
- Delivery pipettes (10ml, 20ml and 25ml)
- Graduated pipettes (1ml and 5ml)
- Measuring cylinder
- Burette
- Conical flasks
- Boiling tubes
- Clean white sheets of A4 paper
- Beakers (25ml, 250ml, 1000ml)
- Plastic funnel
- Petri dishes
- Spatula
- No. 1 Whatmann filter papers
- Drying oven
- Syringe

- Kontron instruments HPLC pump 422
- Power Chrom integrator
- Perkin Elmer UV/ visible detector
- Phenomenex ® Bondclone 10 C18 300×3.9mm column
- Eutech instruments pH meter
- Fisher Scientific FS28H Sonicator

2.2 REAGENTS AND SAMPLES USED

- Sulphuric acid (98% w/w) (BDH, Poole, England)
- Hydrochloric acid (36% w/w) (Philip Harris plc, Shaneson, England)
- Sodium hydroxide (99%) (BDH, Poole, England)
- Potassium iodide (Finkem Laboratory Reagents)
- Potassium iodate (Fissons Scientific Equipment)
- Sodium acetate (BDH, Poole, England)
- Glacial acetic acid (Philip Harris plc, Shaneson, England)
- Iodine (Breckland Scientific Supplies, UK)
- Sodium thiosulphate (BDH, Poole, England)
- Disodium hydrogen orthophosphate (BDH, Poole, England)
- Potassium dihydrogen orthophosphate (BDH, Poole, England)
- Starch mucilage powder (Finkem Laboratory Reagents)
- Distilled water
- Chloroform (BDH, Poole, England)
- Formaldehyde (BDH, Poole, England)

- Pure flucloxacillin powder (assay: 96.0%)
- Pure Amoxicillin powder (assay: 98.80%)
- Capsule excipients (starch and sodium carboxymethyl cellulose)
- Methanol
- Copper sulphate penta- hydrate (Fissons Scientific Equipment)
- Capsule brands from different manufacturing companies

Flucloxacillin brands used

Table 1 Data on the brands of flucloxacillin used

Brand code	Batch no.	Manufacturing date	Expiry date	Country of origin
A1	6811L	Nov- 2011	Nov- 2014	Ghana
B	FL2S	Oct- 2011	Oct- 2013	Ghana
C	11032	Nov- 2011	Nov- 2014	Ghana
D	0230191	Dec- 2011	Nov- 2012	Ghana
E	FL110307	Mar- 2011	Feb- 2013	India

Flucloxacillin powder, amoxicillin powder and excipients used

Table 2 Data on pure samples used.

Material	Source	Batch number	Manufacturing date	Expiry date
Starch	Letap	Stex1103558	Mar- 2011	Feb- 2014
Sodium CMC	Letap	19202	May- 2011	May-2012
Flucloxacillin powder	Letap	VCLFCLXL	Nov- 2010	Oct- 2013
Amoxicillin powder	Ernest Chemists Ltd.	Amx 11070991	July- 2011	June- 2015

2.3 METHODS

2.3.1 PREPARATION OF SOLUTIONS

- **Preparation of primary standard, KIO_3 solution, to standardize 0.01M $Na_2S_2O_3 .H_2O$**

About 0.1783g of potassium iodate crystals was weighed in a small beaker on an analytical balance and transferred quantitatively into 500ml volumetric flask using distilled water. The flask was stoppered, shaken vigorously to ensure complete dissolution and homogeneity. The solution was further adjusted to the 500ml mark with distilled water, stoppered, shaken and labelled accordingly.

- **Preparation of 2M H_2SO_4**

About 11ml of the stock sulphuric acid was measured with a measuring cylinder and poured in a thin stream into a beaker containing about 50ml of distilled water. The mixture was stirred and allowed cool after which it was quantitatively transferred into 100ml volumetric flask and made to the mark with distilled water. The flask was stoppered, shaken and labelled appropriately.

- **Preparation of starch mucilage**

About 5g of starch mucilage powder was mixed with about 100ml of water in a beaker. This was gently heated over a Bunsen flame with stirring for about five minutes to give the starch mucilage.

- **Preparation and standardization of 250ml of 0.01M $Na_2S_2O_3 .H_2O$**

0.6208g of sodium thiosulphate was weighed in a small beaker on an analytical balance and transferred quantitatively using distilled water into 250ml volumetric flask. The flask was

stoppered, shaken vigorously to ensure complete dissolution and homogeneity. The solution was further adjusted to the 250ml mark with distilled water, stoppered, shaken and labelled accordingly.

The prepared thiosulphate solution was filled into a clean burette. 20ml of the primary standard was pipetted into a conical flask. About 2g of potassium iodide and 5ml of 2M sulphuric acid were added and titrated against the titrant until the analyte turned pale brown. 5ml of starch mucilage added and titration continued until the blue- black colour is discharged. The factor of the primary standard and hence the thiosulphate were calculated.

- **Preparation of 0.01M iodine, I_2 solution**

About 5g of potassium iodide was dissolved in about 20ml of water in a glass mortar. 2.5380g of iodine was added and allowed to dissolve. The undissolved portion was triturated with a glass pestle to yield a homogenous solution. This was transferred quantitatively into a 1000ml flask and made to the mark with distilled water. The flask was stoppered, shaken and labelled accordingly.

- **Preparation of mixed phosphate buffer pH 4**

About 1.008g of disodium hydrogen orthophosphate and 0.6020g of potassium dihydrogen orthophosphate were dissolved in sufficient distilled water to produce 200ml and pH was adjusted with drops of glacial acetic acid to 4.**(BP 1988)**

- **Preparation of mixed phosphate buffer pH 7, 0.2M**

About 0.1000g of anhydrous disodium hydrogen orthophosphate and 0.0602g of potassium dihydrogen orthophosphate were dissolved in sufficient water to produce 200ml.**(BP 1988)**

- **Preparation of formaldehyde- sulphuric acid solution**

2ml of formaldehyde solution was mixed with 100ml of sulphuric acid (96%w/w).**(BP 2007)**

- **Preparation of 1M NaOH**

4.0590g of sodium hydroxide pellets was weighed in a small beaker using an analytical balance. The pellets were then transferred quantitatively with distilled water into a bigger beaker containing about 50ml of water. The solution was stirred with a stirrer to cause complete dissolution and allowed to cool. This was then transferred quantitatively into a 100 ml volumetric flask and adjusted to the mark with distilled water. The flask was stoppered, shaken and labelled appropriately.

- **Preparation of 1M HCl**

About 9ml of the stock hydrochloric acid was measured with a measuring cylinder and poured in a thin stream into a beaker containing about 50ml of distilled water. The mixture was stirred and allowed cool after which it was quantitatively transferred into 100ml volumetric flask and made to the mark with distilled water. The flask was stoppered, shaken and labelled appropriately.

- **Preparation of 2.40% w/v glacial acetic acid**

About 5.80ml of the stock glacial acetic acid was measured with a measuring cylinder and poured in a thin stream into a beaker containing about 50ml of distilled water. The mixture was stirred and allowed cool after which it was quantitatively transferred into 250ml volumetric flask

and made to the mark with distilled water. The flask was stoppered, shaken and labeled appropriately.

- **Preparation of 5.44% w/v sodium acetate buffer**

13.6000g was weighed accurately with a small beaker and quantitatively transferred into a 250ml volumetric flask with the 2.40% w/v glacial acetic acid. More of the glacial acetic acid was added, stoppering and shaking the flask upon every addition for thorough dissolution until the volume of the solution reached the 250ml mark.

- **Preparation of primary standard, KIO_3 solution, to standardize 0.02M $Na_2S_2O_3.H_2O$**

About 0.1783g of potassium iodate crystals was weighed in a small beaker on an analytical balance and transferred quantitatively into 250ml volumetric flask using distilled water. The flask was stoppered, shaken vigorously to ensure complete dissolution and homogeneity. The solution was further adjusted to the 250ml mark with distilled water, stoppered, shaken and labelled accordingly.

- **Preparation and standardization of 0.02M $Na_2S_2O_3.5H_20$**

About 4.9636g of sodium thiosulphate heptahydrate was weighed in a small beaker on an analytical balance and transferred quantitatively using distilled water into 1000ml volumetric flask. The flask was stoppered, shaken vigorously to ensure complete dissolution and homogeneity. The solution was further adjusted to the 1000ml mark with distilled water, stoppered, shaken and labeled accordingly.

The prepared thiosulphate solution was filled into a clean burette. 20ml of the primary standard was pipetted into a conical flask. About 2g of potassium iodide and 5ml of 2M sulphuric acid

were added and titrated against the titrant until the analyte turned pale brown. 5ml of starch mucilage added and titration continued until the blue- black colour is discharged. The factor of the primary standard and hence the thiosulphate were calculated.

2.3.2 PREPARATIONS OF PURE FLUCLOXACILLIN AND DRIED STARCH MIXTURES

- **250mg flucloxacillin and 200mg dried starch**

2.0146g of pure flucloxacillin powder and 1.6117g of dried starch were weighed into a glass mortar and triturated to obtain a homogenous mixture and transferred into clean Petri dish and the dish labelled. The Petri dish was exposed for 3 months to the atmosphere in a room and samples drawn and analyzed or assayed periodically.

- **250mg flucloxacillin and 100mg dried starch**

2.0048g of pure flucloxacillin powder and 0.8020g of dried starch were used as described above.

- **250mg flucloxacillin and 50mg dried starch**

2.0003g of pure flucloxacillin powder and 0.4005g of dried starch were used as described above.

- **Control – Petri dish + dried starch only**

An amount of the dried starch was put into a previously weighed Petri dish and the new weight of the dish taken. The dish was exposed in a room and the weight taken periodically on same days of analyses of the flucloxacillin-dried starch samples.

- **Preparation of dry starch and its moisture content determination**

A known weight of starch powder was put into a pre-weighed Petri dish. The weight of the starch and Petri dish was taken after which the dish with the starch was heated in an oven set to a temperature of about 130°C for 90 minutes. The weight of the Petri dish and the starch was again

taken after the 90th minute and the loss in weight expressed as a percentage gave the moisture content of the starch. The starch obtained thereafter was the dried starch.

2.3.3 PREPARATIONS OF PURE FLUCLOXACILLIN AND UNDRIED STARCH MIXTURES

- **250mg flucloxacillin and 200mg undried starch**

2.0029g of pure flucloxacillin powder and 1.6026g of undried starch powder were used.

- **250mg flucloxacillin and 100mg undried starch**

2.0017g of pure flucloxacillin powder and 0.8007g of undried starch powder were used.

- **250mg flucloxacillin and 50mg undried starch**

2.0034g of pure flucloxacillin powder and 0.4006g of undried starch powder were used.

The various combinations of flucloxacillin and undried starch were made as was done for dried starch and flucloxacillin.

- **Control – Petri dish + undried starch only**

To show whether or not starch absorbs moisture from air, an amount of the undried starch was put into a previously weighed Petri dish and the new weight of the dish taken. The dish was exposed in a room and the weight taken periodically on same days of analyses of the flucloxacillin- undried starch samples.

2.3.4 PREPARATIONS OF PURE FLUCLOXACILLIN AND SODIUM CMC MIXTURES

- **250mg flucloxacillin and 200mg sodium cmc**

2.0077g of flucloxacillin powder and 1.6062g of sodium cmc were used.

- **250mg flucloxacillin and 100mg sodium cmc**

2.0034g of flucloxacillin powder and 0.8013g sodium cmc were used.

- **250mg flucloxacillin and 50mg sodium cmc**

2.0014g of pure flucloxacillin powder and 0.4003g sodium cmc were used.

The various combinations of flucloxacillin and sodium cmc were made as was done for dried starch and flucloxacillin.

- **Control – Petri dish + sodium cmc**

An amount of the sodium cmc was put into a previously weighed Petri dish and the new weight of the dish taken. The dish was exposed in a room and the weight taken periodically on same days of analyses of the flucloxacillin- sodium cmc samples.

2.3.5 PHARMACOPOEIAL TESTS

2.3.5.1 Uniformity of content for capsules

20 capsules were randomly selected from a batch of each brand and collectively weighed. The capsules were then individually weighed, opened, emptied into an airtight container and the weight of the shells of each capsule taken. This was done for all twenty capsules and the weight of the shells for the twenty capsules collectively taken. The average weight of the capsules was calculated and the weight of the content of each capsule realized by the difference between the weight of the capsule and its shells. The deviation and hence percentage deviation were calculated for each capsule. **(BP 2007)**

2.3.5.2 Disintegration test for capsules

The disintegration apparatus was filled with distilled water to a level such that the basket rack assembly was at least 15mm below the surface of the water on the upward stroke and not less than 25mm from the bottom of the vessel on the downward stroke. The thermostatic device was turned on to keep the water at 37°C. When the temperature was attained six capsules were

selected from a particular brand and each capsule was put in each of the six tubes of basket and the apparatus started and simultaneously a stopwatch was also started. The time taken for all the capsules to disintegrate was taken. This was done for all the brands. **(BP 2007)**

2.3.6 IDENTIFICATION TESTS

- **For flucloxacillin**

A small amount of the pure powder was placed in a clean test- tube with the aid of a spatula. The sample was moistened with a small amount of distilled water after which 2ml of sulphuric acid – formaldehyde reagent was added and heated in a water-bath for a minute. The colour of the solution turned yellowish. For the capsules, an amount of the contents was taken and mixed with water, filtered and the filtrate was treated as described above. **(BP 2009)**

- **For Amoxicillin**

The pure amoxicillin powder was identified as for pure flucloxacillin.

- **For starch**

To a small amount of the starch in a boiling tube, a small amount of the iodine solution was added and the tube swirled. The development of a blue- black colour confirmed the sample to be starch. It was also tested for in the capsules by taking a small amount of the capsule content and adding a small amount of distilled followed by a small amount of iodine solution. **(BP 2009)**

- **For sodium CMC**

A 0.5%w/v solution of sodium cmc was made by dissolving about 0.2500g in distilled water and making to 50ml in a volumetric flask with distilled water. A 5% solution of copper sulphate penta-hydrate was also made by dissolving 2.5000g and making to 50ml in a volumetric flask with distilled water. 5ml of both solutions were mixed in a boiling tube and the formation of a

precipitate indicated the presence of sodium cmc. **("Sodium carboxymethylcellulose- FAO," 1984)**

- **Test for Magnesium stearate**

About 0.1000g of the capsule content was taken and mixed with about 10ml of distilled water in a boiling tube and thoroughly stirred to effect homogeneity. Similar volume of a 1M NaOH was then added to the suspension obtained. Formation of a white precipitate of magnesium hydroxide indicates the presence of magnesium stearate. **(Vogel, 1979)**

2.3.7 DETERMINATION OF IODINE ABSORBING IMPURITIES

0.1250g of pure flucloxacillin powder was dissolved in sufficient mixed phosphate buffer pH 7.0 to produce 25ml. To 10ml, 10ml of mixed phosphate buffer pH 4.0 and 10ml of 0.01M iodine were added and titrated immediately with 0.01M sodium thiosulphate using starch mucilage, added towards the end of the titration, as indicator. The titration was repeated without the substance being examined. The difference between the titrations represented the amount of iodine- absorbing substances present. Each ml of 0.01M sodium thiosulphate VS is equivalent to 0.524mg of iodine- absorbing substances. **(BP 1993)**

For the capsules the weight of powder equivalent to 0.1250g flucloxacillin was taken and extracted with the buffer (pH =7) and the filtrate made to 25ml and assayed as described above.

2.3.8 ASSAY OF FLUCLOXACILLIN

0.100g of flucloxacillin powder was dissolved in sufficient water to produce 100ml. 10ml was transferred to a stoppered flask; 5ml of 1M NaOH was added and allowed to stand for 20 minutes. 20ml of freshly prepared buffer solution containing 5.44 per cent w/v of sodium acetate and 2.40 per cent w/v of glacial acetic acid, 5ml of 1M HCl hydrochloric acid and 25ml of

0.01M iodine were added. The flask was stoppered and allowed to stand for 20minutes protected from light and then titrated with 0.02M sodium thiosulphate using starch solution added towards the end of the titration, as indicator.

To a further 10ml of the initial solution, 20ml of the buffer solution and 25ml of iodine were added and allowed to stand for 20minutes protected from light and then titrated with 0.02M sodium thiosulphate using starch solution added towards the end of the titration as indicator.

The difference between the titrations represented the volume of 0.01M iodine equivalent to the total flucloxacillin present. **(BP 1980)**

For the capsules and starch (dried and undried)/ flucloxacillin mixture, the weight of powder equivalent to 0.100g of flucloxacillin was used. The drug was extracted with distilled water and the filtrate was made to 100ml and assayed as described above.

Since sodium cmc is water soluble, the weight of powder equivalent to 0.100g of flucloxacillin was dissolved in distilled water, made to 100ml and assayed as described water.

2.3.9 HPLC METHOD DEVELOPMENT AND VALIDATION

2.3.9.1 Chromatographic conditions

- Column : Phenomenex ® Bondclone 10 C18 300×3.9mm
- Flow rate: 1.00ml/ min
- Mobile phase: methanol : KH_2PO_4 buffer (60: 40)
- Wavelength of detection : 225nm
- Temperature : ambient
- Injection volume: 100µl

2.3.9.2 Preparation of the mobile phase

The potassium dihydrogen orthophosphate (KH_2PO_4) buffer was made by weighing 1.3500g of KH_2PO_4 and quantitatively transferring it into a 500ml volumetric flask and making to volume with distilled water. The flask was shaken to ensure complete dissolution of the salt and a homogenous solution. The solution was thus adjusted to pH 5 with a 1M sodium hydroxide solution. 200ml of this solution was taken with a measuring cylinder and mixed thoroughly with 300ml of distilled methanol to obtain 500ml of the mobile phase.

2.3.9.3 Column conditioning and equilibration

Before samples were injected, a water- methanol mixture in a ratio of 50:50 was pumped through the column for about an hour followed by the mobile phase for about 30 minutes to ensure that good results were obtained.

2.3.9.4 Method development

The choice of mobile phase was guided by extensive reading of a myriad of published works on the assay of flucloxacillin. Several of those methods employed the aforementioned buffer and varied combinations of acetonitrile. A few however used completely different systems of solvent. Several combinations of methanol and KH_2PO_4 buffer at pH 5 were tried beginning with a 50: 50 combination until a ratio of methanol to KH_2PO_4 buffer of 60: 40 was settled on as it gave a neat chromatogram and an ideal retention time.

2.3.9.5 Method validation

- **Linearity**

A concentration of 0.10% w/v of flucloxacillin sodium was prepared by weighing 10mg and transferring quantitatively into a 10ml volumetric flask and making to volume with the mobile phase. From this concentration, serial dilutions were made to obtain concentrations of 0.08%,

0.05%, 0.03% and 0.01%. Each of these concentrations was injected and their peak areas plotted against their respective concentrations to obtain a calibration curve. The coefficient of correlation, **the limits of detection** and **quantification** were all calculated from the calibration curve.

- **Selectivity**

A solution of amoxicillin tri-hydrate in the mobile phase was made and filtered as the drug was sparingly soluble. The filtrate was injected and its retention time was noted. A solution of flucloxacillin was made and spiked with a solution of amoxicillin and mixed thoroughly. A sample of the resulting solution was injected three times and their retention times noted.

- **Precision**

A concentration of 0.08% was made from 0.10% and samples injected to obtain six determinations for the intra-day precision. For the inter-day precision samples of the same concentration were injected on three consecutive days to obtain six different determinations for each of the three days. The relative standard deviation was thus calculated.

- **Robustness**

Using a concentration of 0.08%, injections were made at varying flow rates while holding other parameters constant. The detection wavelength was also varied while keeping other parameters constant.

CHAPTER 3- RESULTS

3.1 I DENTIFICATION TESTS

3.1.1 FOR FLUCLOXACILLIN IN SAMPLES USED

Table 3 Results for flucloxacillin identification in capsule brands

SAMPLE	OBSERVATION	INFERENCE
Flucloxacillin powder	An intense yellow coloration was formed	Sample passed
A1	An intense yellow coloration was formed	Sample passed
B	An intense yellow coloration was formed	Sample passed
C	An intense yellow coloration was formed	Sample passed
D	An intense yellow coloration was formed	Sample passed
E	An intense yellow coloration was formed	Sample passed

**** All capsules of various brands employed in experiment were well sealed in their blisters, clean and intact and had no physical defects upon a thorough examination.***

3.1.2 TEST FOR AMOXICILLIN AND POWDERED EXCIPIENTS USED

Table 4 Results for tests for amoxicillin and excipients

SAMPLE	OBSERVATION	INFERENCE
Starch powder	Blue – black color was seen	Sample passed
Sodium CMC powder	A precipitate was formed	Sample passed
Amoxicillin powder	An intense yellow coloration was formed	Sample passed

3.1.3 TEST FOR SOME EXCIPIENTS IN CAPSULE BRANDS

Table 5 Results for the test of some excipients in capsule brands

SAMPLES	APPEARANCE OF CONTENT	TEST FOR STARCH	TEST FOR MAGNESIUM STEARATE	TEST FOR SODIUM CMC
A1	Content appeared white and powdery.	Intense blue black coloration	No white precipitate formed	No precipitate was formed
B	Powdery white content with a very faint yellowish tinge.	Specks of blue black particles were seen.	No white precipitate formed	No precipitate was formed
C	Powdery white content	No blue-black coloration.	No white precipitate formed	No precipitate was formed
D	Powdery white content	No blue-black coloration	No white precipitate formed	No precipitate was formed
E	Powdery white content but had a slight yellowish tinge and had some granules.	Specks of blue black particles were seen.	No white precipitate formed	No precipitate was formed

3.2 PHARMACOPOEIAL TESTS

3.2.1 Uniformity of content for capsules

Table 6 Results for uniformity of content for capsule brands.

SAMPLES	RESULTS
A1	Failed.
B	Passed
C	Failed
D	Failed
E	Failed

3.2.2 Disintegration test for capsule brands

Table 7 Results for disintegration test

SAMPLE	DISINTEGRATION TIME/ MINUTES	RESULT
A1	12	Passed
B	15	Passed
C	17	Passed
D	53	Failed
E	25	Passed

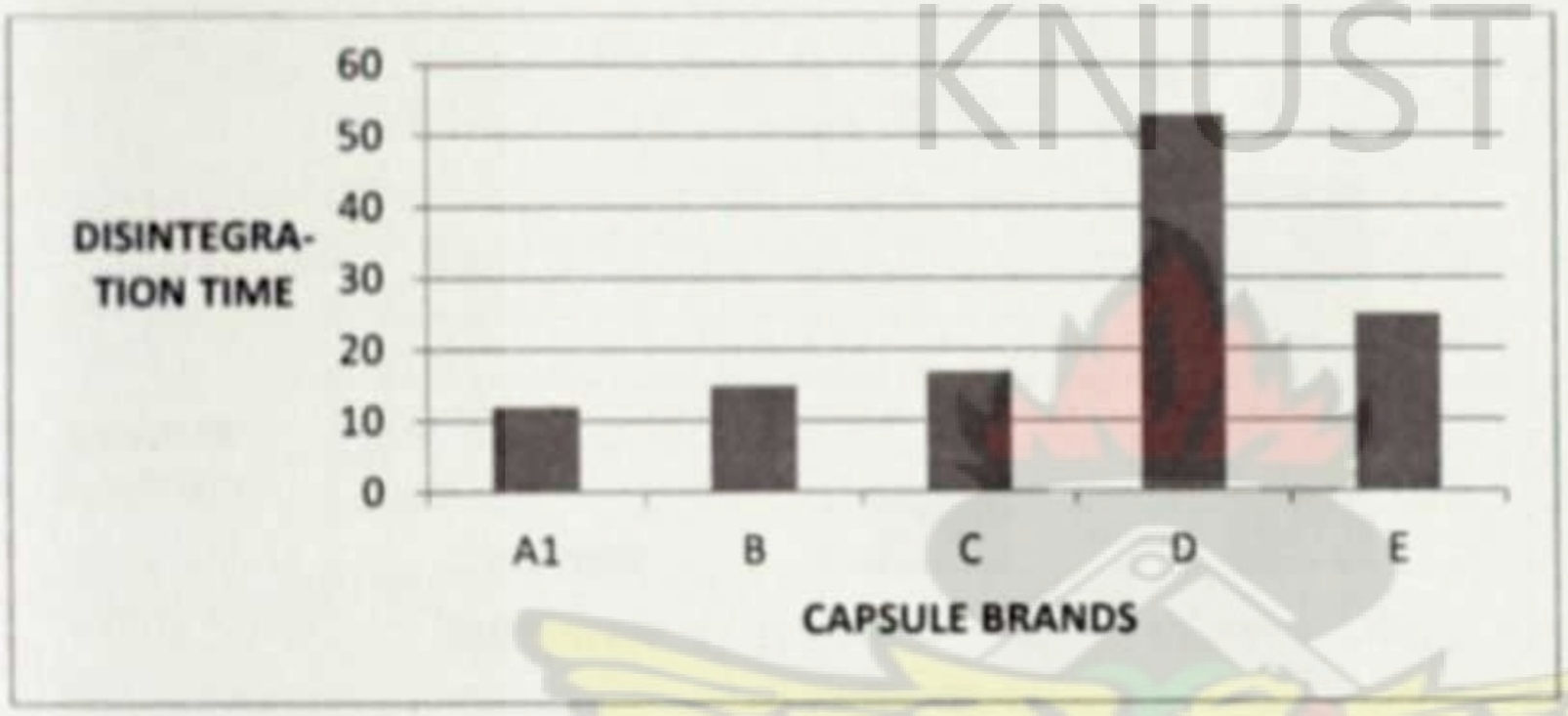


Figure 3 Chart showing disintegration time for capsule brands

3.3 DETERMINATION OF AMOUNT OF MOISTURE

3.3.1 Moisture content determination in excipients after the three month period

Table 8 Varying weights for the various excipients after moisture uptake for three months

TIME/WEEK	WT OF PETRI DISH + SODIUM CMC/g	WT OF PETRI DISH + DRIED STARCH/g	WT OF PETRI DISH + UNDRIED STARCH/g
0	53.2063	46.2450	84.7382
1	53.3190	46.2473	84.8201
2	53.4113	46.2539	84.8227
4	53.4370	46.2652	84.8341
8	53.5184	46.2667	84.8358
12	53.6202	46.2689	84.8609

Table 9 %w/w of moisture taken up by the excipients after three months

Excipient	% w/w moisture
Sodium CMC	12.50
Dried starch	2.82
Undried starch	0.84

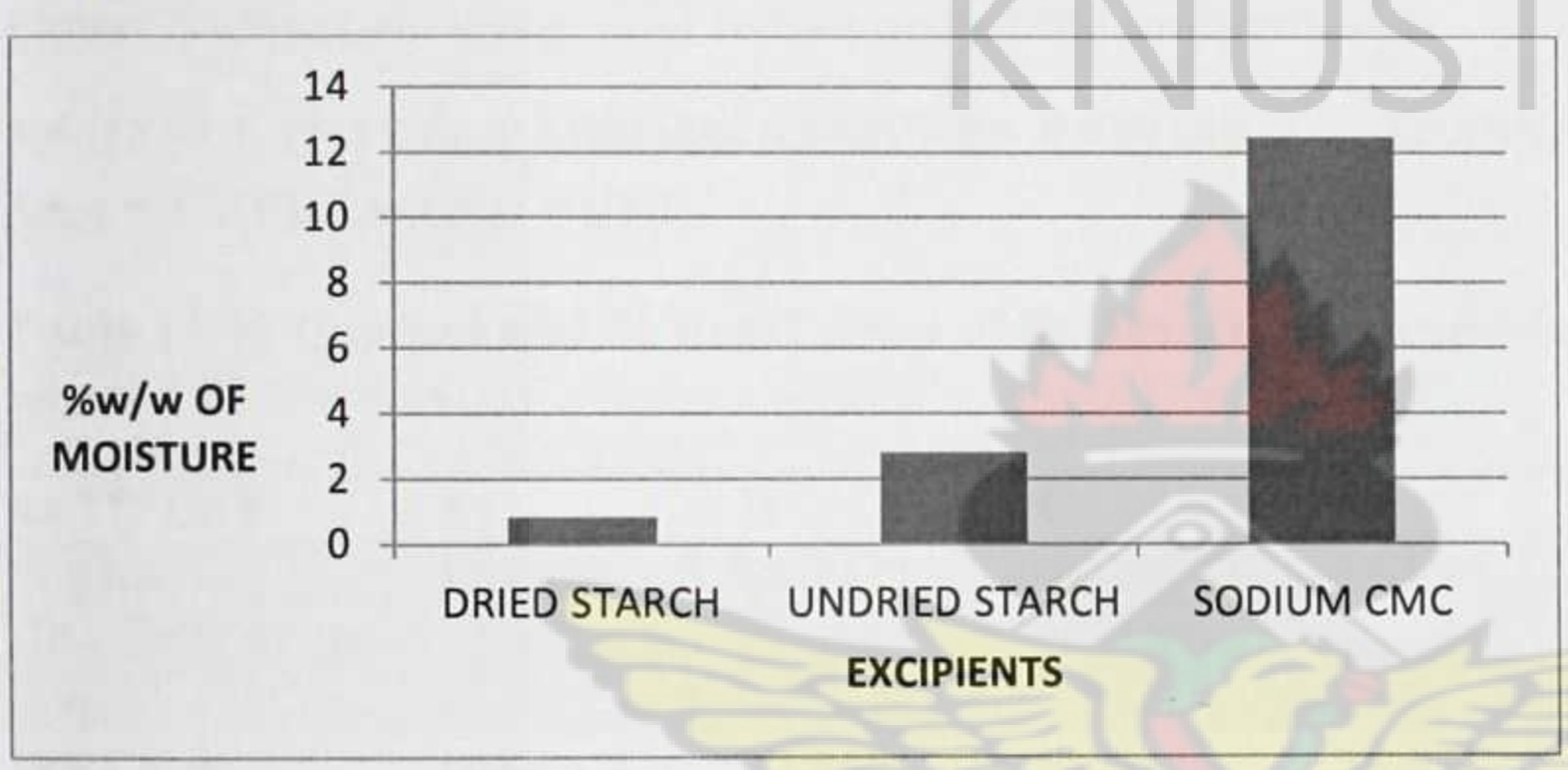


Figure 4 Chart showing the amount of moisture absorbed

3.4 IODINE ABSORBONG IMPURITIES IN PURE AND SAMPLES

Table 10 Contents of I_2 absorbing substances in capsule brands.

SAMPLE	IODINE ABSORBING IMPURITIES (% w/w)
A1	3.21
B	4.59
C	2.50
D	1.88
E	14.61
PURE POWDER	3.86

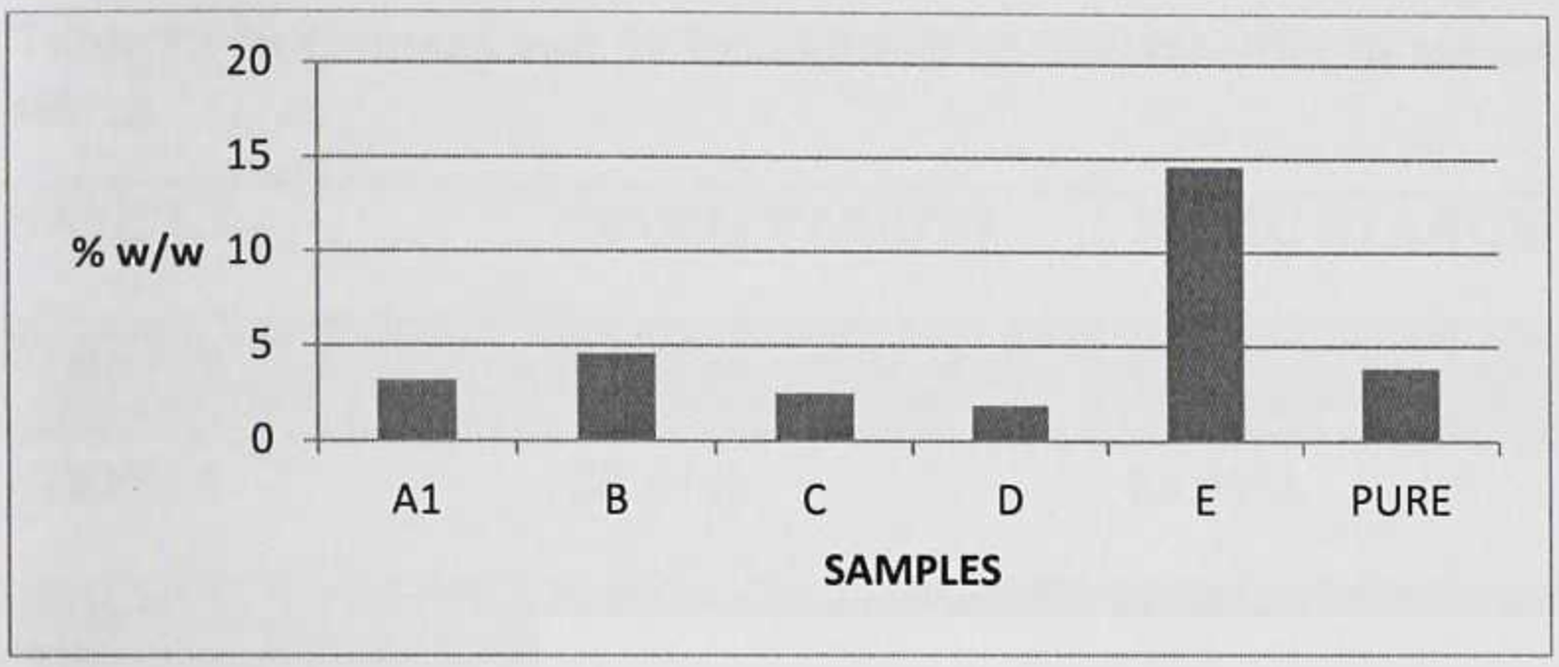


Figure 5 Chart showing % of iodine absorbing impurities

3.5 RESULTS FOR VARIOUS SAMPLES EXPOSED AND ASSAYED FOR 12 WEEKS

3.5.1 SAMPLES AND PURE

Table 11 % Content and % breakdown of flucloxacillin in capsule brands over three months

SAMPLES	A1	B	C	D	E	PURE
WEEK O	115.0%	87.15%	92.25%	86.59%	55.23%	91.14%
WEEK 1	105.66%	77.86%	88.31%	78.97%	44.53%	82.04%
% BREAKDOWN	8.12%	10.66%	4.27%	8.80%	19.34%	9.98%
WEEK 3	99.63%	64.58%	73.19%	78.60%	37.64%	76.88%
% BREAKDOWN	13.37%	25.90%	20.66%	9.23%	31.84%	15.65%
WEEK 4	95.24%	60.76%	61.25%	71.46%	31.73%	74.17%
% BREAKDOWN	17.18%	30.28%	33.60%	17.47%	42.55%	18.62%
WEEK 8	84.75%	15.71%	7.73%	19.64%	9.82%	20.87%
% BREAKDOWN	26.30%	81.97%	91.62%	77.32%	82.22%	77.10%
WEEK 12	47.68%	6.48%	0.00%	0.73%	3.30%	5.13%
% BREAKDOWN	58.54%	92.56%	100%	99.16%	94.02%	94.37%
AIRTIGHT SAMPLES	114.52%	86.87%	91.74%	86.50%	54.82%	

3.5.2 DRIED STARCH AND 250MG FLUCLOXACILLIN COMBINATIONS

Table 12 % Content and % breakdown of flucloxacillin in various proportions with dried starch

SAMPLE	200MG STARCH	100MG STARCH	50MG STARCH
WEEK 0	91.64%	91.51%	91.81%
WEEK 1	80.44%	84.99%	85.36%
% BREAKDOWN	12.22%	7.12%	7.03%
WEEK 2	80.41%	79.58%	79.81%
% BREAKDOWN	12.25%	13.04%	13.07%
WEEK 4	73.19%	72.32%	70.73%
% BREAKDOWN	20.13%	20.97%	22.96%
WEEK 8	51.05%	42.44%	38.25%
% BREAKDOWN	44.29%	53.62%	58.33%
WEEK 12	32.35%	22.63%	16.48%
% BREAKDOWN	64.70%	75.27%	82.05%

3.5.3 "UNDRIED" STARCH AND 250MG FLUCLOXACILLIN COMBINATIONS

Table 13 % Content and % breakdown of flucloxacillin in various proportions with undried starch

SAMPLE	200MG STARCH	100MG STARCH	50MG STARCH
WEEK 0	97.20%	99.44%	100.29%
WEEK 1	89.22%	90.05%	89.22%
% BREAKDOWN	8.21%	9.44%	11.04%
WEEK 2	84.75%	86.01%	87.82%
% BREAKDOWN	12.81%	13.51%	12.43%
WEEK 4	73.80%	75.40%	72.20%
% BREAKDOWN	24.07%	25.19%	28.01%
WEEK 8	55.60%	50.68%	44.03%
% BREAKDOWN	42.80%	49.03%	56.10%
WEEK 12	36.78%	26.57%	18.45%
% BREAKDOWN	62.12%	73.28%	81.60%

3.5.4 SODIUM CMC AND 250MG FLUCLOXACILLIN COMBINATIONS

Table 14 % Content and % breakdown of flucloxacillin in various proportions with sodium cmc

SAMPLES	200MG CMC	100MG CMC	50MG CMC
WEEK 0	95.97%	98.34%	97.51%
WEEK 1	87.34%	88.41%	87.22%
% BREAKDOWN	8.99%	10.10%	10.55%
WEEK 2	83.27%	76.17%	82.16%
% BREAKDOWN	13.23%	22.54%	15.74%
WEEK 4	68.27%	64.82%	62.73%
% BREAKDOWN	28.86%	34.09%	35.67%
WEEK 8	41.63%	30.00%	21.12%
% BREAKDOWN	56.62%	69.49%	78.34%
WEEK 12	2.83%	6.64%	3.69%
% BREAKDOWN	97.05%	93.25	96.22%

3.6 DETERMINATION OF REACTION ORDER

First order

The derived equation is given as:

$\ln c = \ln c_0 - kt$ and comparing with $y = mx + c$

$y = \ln c$, $x = t$, $m = -k$ and $c = \ln c_0$

c= concentration after a certain period of time, t, c_0 = initial concentration and k = rate constant

Hence a plot of In c against time is a straight line with intercept, $\ln c_0$ and gradient, -k.

Zero order

The zero order equation is given as:

$c = c_0 - kt$ and comparing with $y = mx + c$

c= concentration after a certain period of time, t, c_0 = initial concentration and k = rate constant

Thus a plot of c against time, t is a straight line with intercept, c_0 and gradient,-k

3.6.1 VALUES TO BE PLOTTED FOR ZERO AND FIRST ORDERS FOR SAMPLES USED

3.6.1.1 250mg flucloxacillin and dried starch combinations

Table 15 Values plotted for zero and first orders for dried starch/flucloxacillin combinations

	200mg dried starch		100mg dried starch		50mg dried starch	
Time /week	conc	In conc.	conc.	In conc	conc	In conc
0	91.64	4.518	91.51	4.516	91.81	4.520
1	80.44	4.388	84.99	4.443	85.36	4.447
2	80.41	4.387	79.58	4.377	79.81	4.380
4	73.19	4.293	72.32	4.281	70.73	4.259
8	51.05	3.933	42.44	3.748	38.25	3.644
12	32.35	3.477	22.63	3.119	16.48	2.802

3.6.1.2 250mg flucloxacillin and undried starch combinations

Table 16 Values plotted for zero and first orders for undried starch/flucloxacillin combinations

	200mg undried starch		100mg undried starch		50mg undried starch	
Time /week	**conc**	**In conc**	**conc**	**In conc.**	**conc.**	**In conc**
0	97.20	4.577	99.44	4.600	100.29	4.608
1	89.22	4.491	90.05	4.500	89.22	4.491
2	84.75	4.440	86.01	4.454	87.82	4.575
4	73.80	4.301	75.40	4.323	72.20	4.279
8	55.60	4.018	50.68	3.926	44.03	3.785
12	36.78	3.605	26.57	3.280	18.45	2.915

3.6.1.3 250mg flucloxacillin and sodium CMC combinations

Table 17 Values plotted for zero and first orders for sodium cmc/flucloxacillin combinations

	200mg sodium CMC		100mg sodium CMC		50mg sodium CMC	
Time /week	**conc**	**In conc**	**conc**	**In conc**	**conc**	**In conc**
0	95.97	4.564	98.34	4.588	97.51	4.580
1	87.34	4.470	88.41	4.482	87.22	4.468
2	83.27	4.422	76.17	4.333	82.16	4.409
4	68.27	4.223	64.82	4.172	62.73	4.139
8	41.63	3.729	30.00	3.401	21.12	3.050
12	2.83	1.040	6.64	1.893	3.69	1.306

3.6.1.4 Samples and pure powder

Table 18 Values plotted for zero and first orders for capsule brands and pure flucloxacillin powder

	A1		B		C	
Time /week	**conc**	**In conc**	**conc**	**In conc**	**conc**	**In conc**
0	115.0	4.745	87.15	4.468	92.25	4.525
1	105.66	4.660	77.86	4.355	88.31	4.481
3	99.63	4.601	64.58	4.168	73.19	4.293
4	95.24	4.556	60.76	4.107	61.25	4.115
8	84.75	4.440	15.71	2.754	7.73	2.045
12	47.68	3.865	6.48	1.869	0.00	

	D		E		PURE POWDER	
Time /week	**conc**	**In conc**	**conc**	**In conc**	**conc**	**In conc**
0	86.59	4.461	55.23	4.012	91.14	4.512
1	78.97	4.369	44.53	3.796	82.04	4.407
3	78.60	4.364	37.64	3.628	76.88	4.342
4	71.46	4.269	31.73	3.457	74.17	4.306
8	19.64	2.978	9.82	2.284	20.87	3.038
12	0.73	-0.315	3.30	1.194	5.13	1.635

3.6.2 SAMPLE GRAPHS FOR SAMPLE 250MG FLUCLOXACILLIN AND 100MG DRIED STARCH MIXTURE

3.6.2.1 Zero order

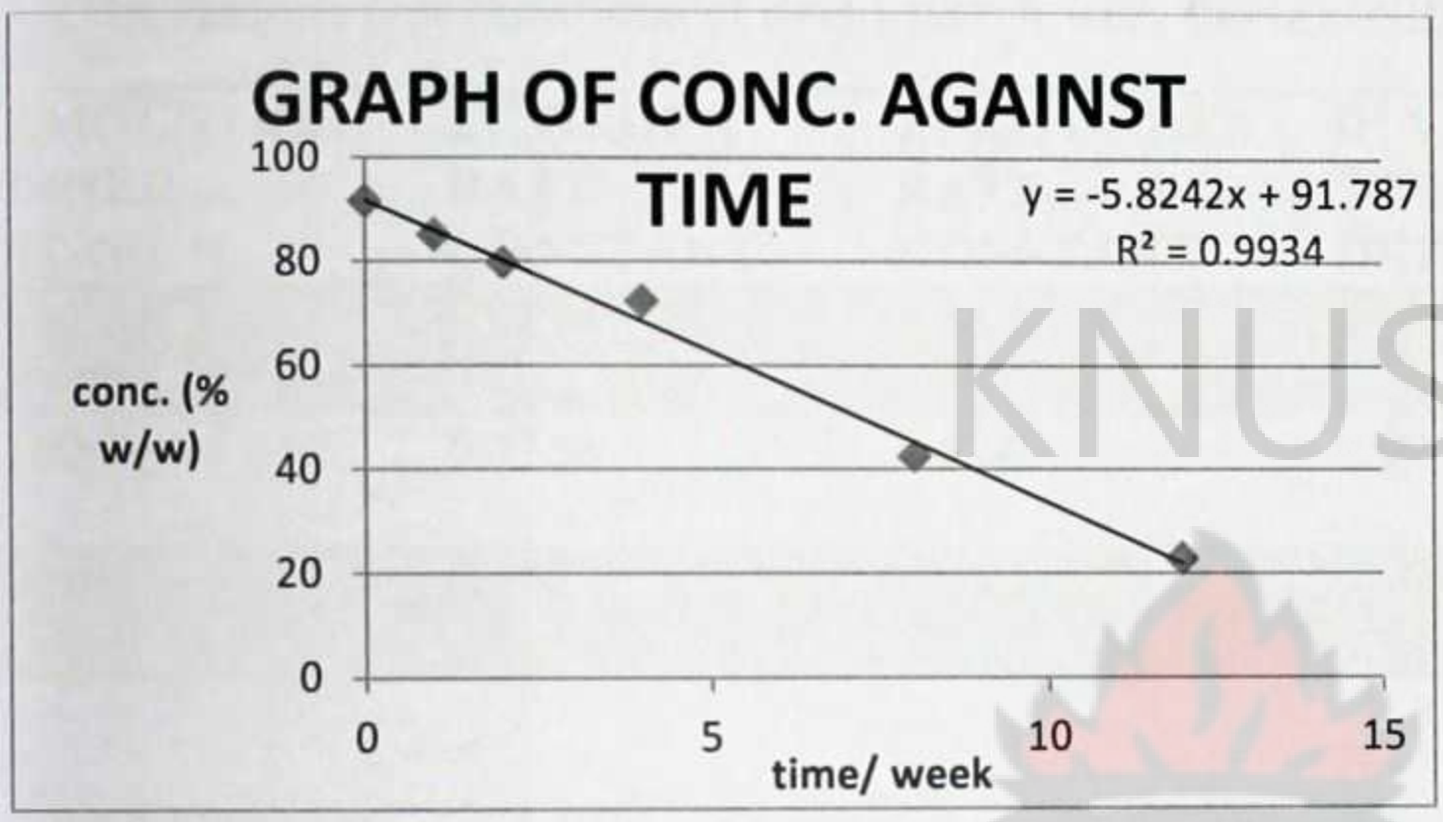


Figure 6 Zero order graph

3.6.2.2 First order

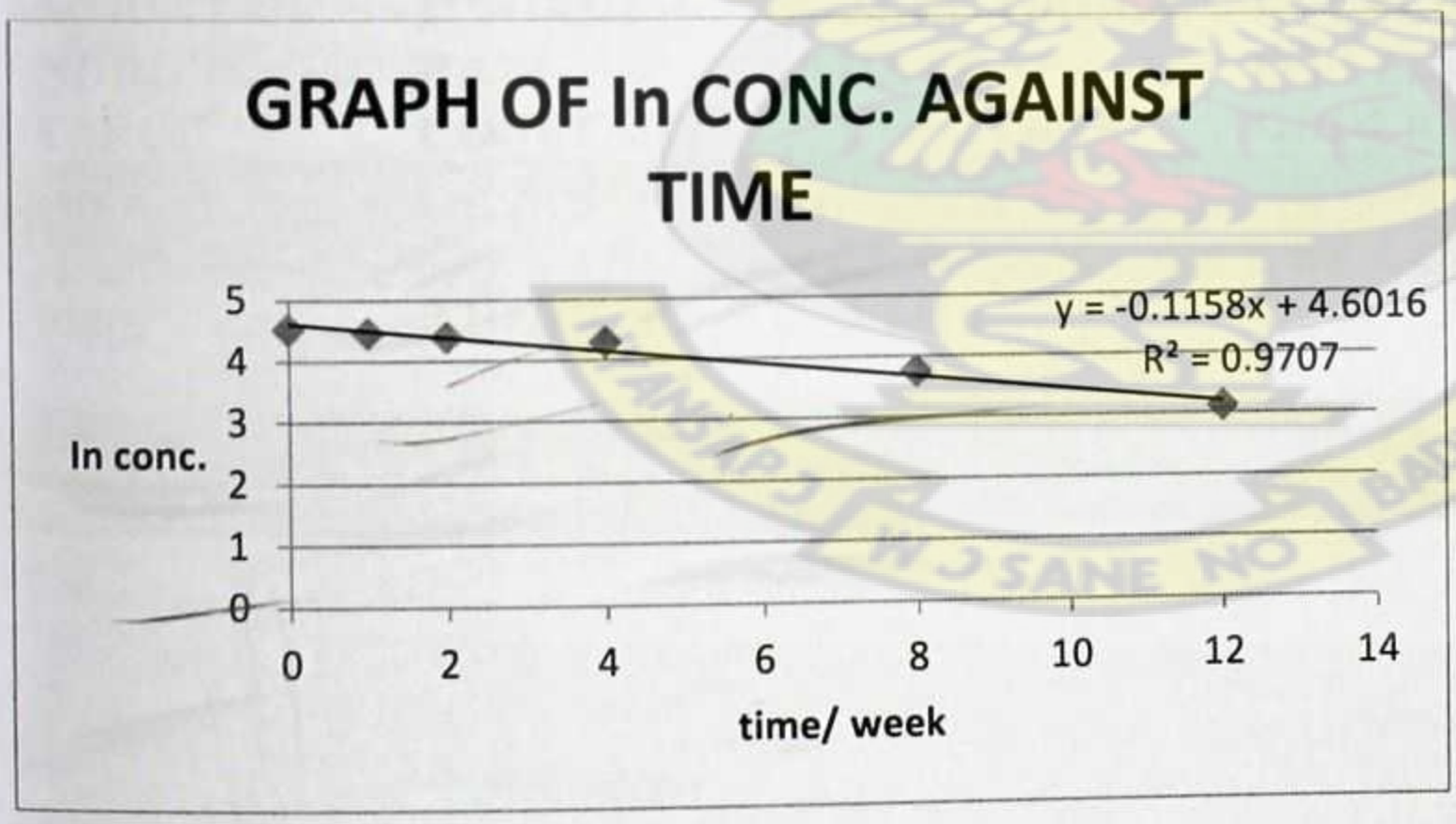


Figure 7 First order graph

3.6.3 COMPARISONS FOR RATE CONSTANTS (K) AND COEFFICIENT OF CORRELATION (R^2) VALUES FOR FIRST AND ZERO ORDERS FOR THE SAMPLES USED.

3.6.3.1 250mg flucloxacillin and dried starch combination

Table 19 Comparison of rate constants of the reaction orders and correlation coefficients for the various combinations of dried starch with flucloxacillin

AMOUNT OF DRIED STARCH	1ST ORDER RATE CONSTANT	ZERO ORDER RATE CONSTANT	R^2 VALUES FOR ZERO ORDER	R^2 VALUES FOR 1ST ORDER
200mg	0.0833	4.7464	0.9875	0.9735
100mg	0.1158	5.8242	0.9934	0.9707
50mg	0.1415	6.4123	0.9942	0.9600

3.6.3.2 250mg flucloxacillin and undried starch combination

Table 20 Comparison of rate constants of the reaction orders and correlation coefficients for the various combinations of undried starch with flucloxacillin

AMOUNT OF UNDRIED STARCH	1ST ORDER RATE CONSTANT	ZERO ORDER RATE CONSTANT	R^2 VALUES FOR ZERO ORDER	R^2 VALUES FOR 1ST ORDER
200mg	0.0787	4.9116	0.9966	0.9908
100mg	0.1061	5.9416	0.998	0.969
50mg	0.1388	6.7604	0.9965	0.9538

3.6.3.3 250mg flucloxacillin and sodium cmc combination

Table 21 Comparing rate constants the reaction orders and correlation coefficients for the various combinations of sodium cmc with flucloxacillin

3.6.3.4 Samples and pure powder

Table 22 Comparison of rate constants of the reaction orders and correlation coefficients

AMOUNT OF SODIUM CMC	1ST ORDER RATE CONSTANT	ZERO ORDER RATE CONSTANT	R^2 VALUES FOR ZERO ORDER	R^2 VALUES FOR 1ST ORDER
200mg	0.2651	7.5622	0.9924	0.8211
100mg	0.2154	7.6279	0.9919	0.9400
50mg	0.2686	8.1789	0.9819	0.9452

For capsule brands and pure flucloxacillin powder

SAMPLES	1ST ORDER RATE CONSTANT	ZERO ORDER RATE CONSTANT	R^2 VALUES FOR ZERO ORDER	R^2 VALUES FOR 1ST ORDER
A1	0.0657	5.0465	0.9404	0.8879
B	0.2286	7.1655	0.9597	0.9617
C	0.3116	8.6260	0.9472	0.8620
D	0.3807	7.7561	0.9433	0.8454
E	0.2383	4.3352	0.9575	0.9759
PURE POWDER	0.2439	7.7083	0.9494	0.9258

3.7 HPLC METHOD VALIDATION

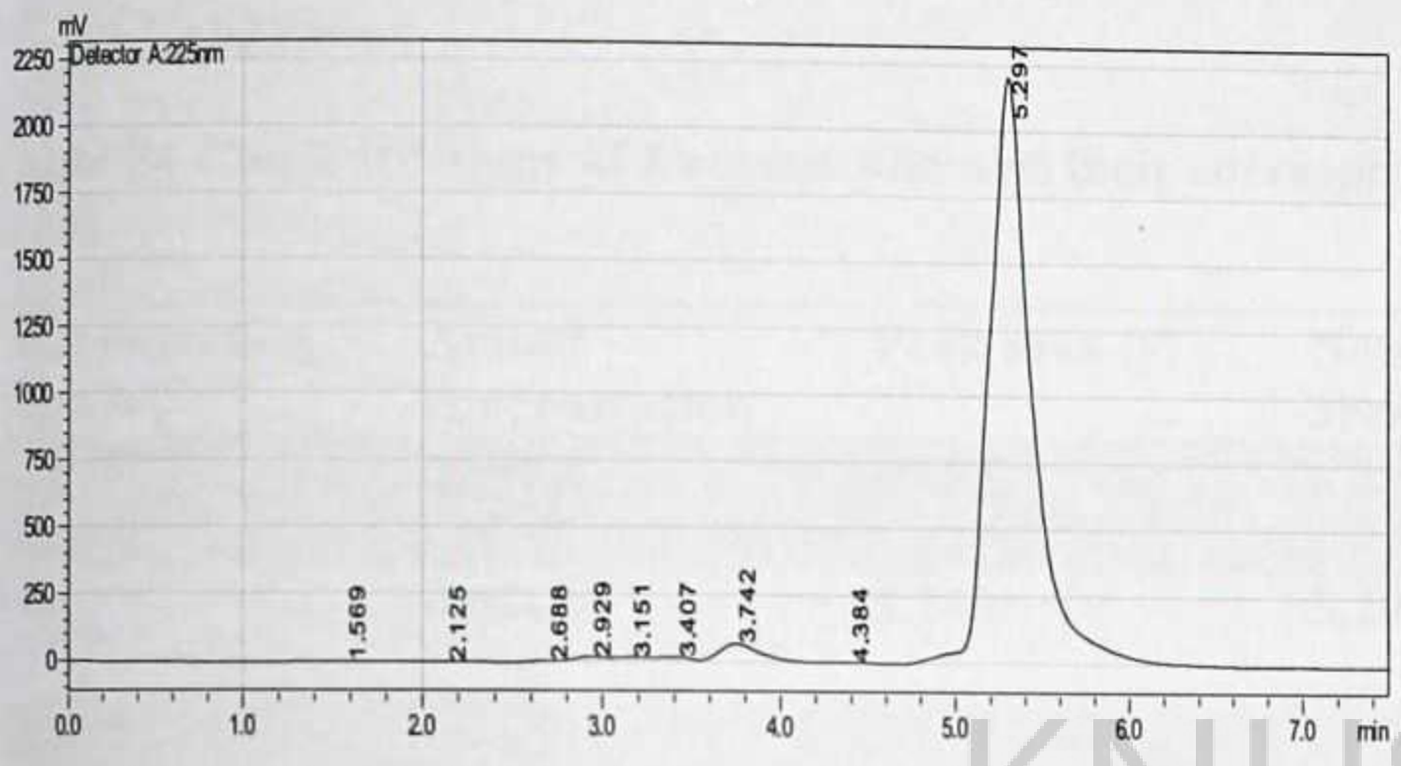


Figure 8 Chromatogram of flucloxacillin

Table 23 Chromatographic parameters used in method development

Chromatographic parameter	value
Flow rate	1.00ml/min
Retention time	5.36±0.07
Detection wavelength	225nm
Mobile phase	60% Methanol: 40% KH_2PO_4 pH =5
Temperature	ambient
Injection volume	100µl

3.7.1 LINEARITY

3.7.1.1 Calibration

Table 24 Concentrations of flucloxacillin and their corresponding peak and nominal peak areas

Concentration (%w/v)	Actual concentration	Peak area (y)	Nominal peak area (y_i)	Residual peak area ($y-y_i$)
0.10	0.0960	6.250	6.231	0.019
0.08	0.0768	5.240	5.208	0.032
0.05	0.0480	3.610	3.673	-0.063
0.03	0.0288	2.580	2.650	-0.070
0.01	0.0096	1.710	1.620	0.090

Percentage purity of flucloxacillin used is 96%

Sample calculation

Actual concentration = concentration × 0.96

= 0.10% w/v × 0.96

= 0.096% w/v ≈ 0.10% w/v

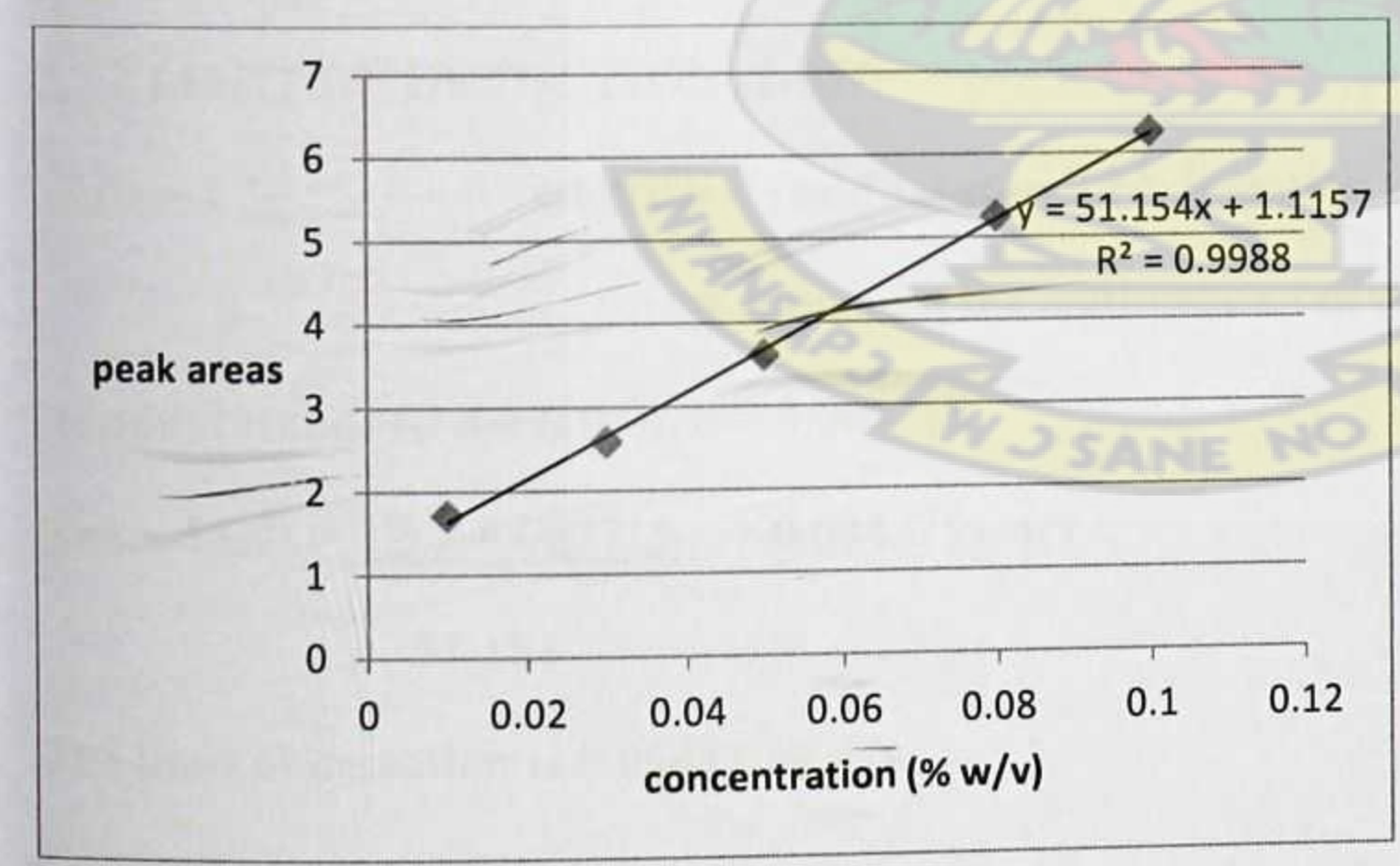


Figure 9 Calibration curve for pure flucloxacillin

Table 25 Data from calibration curve

slope	**51.154**
Correlation coefficient	**0.9988**
Y- intercept	**1.1157**
Range	**0.01%w/v – 0.10%w/v**

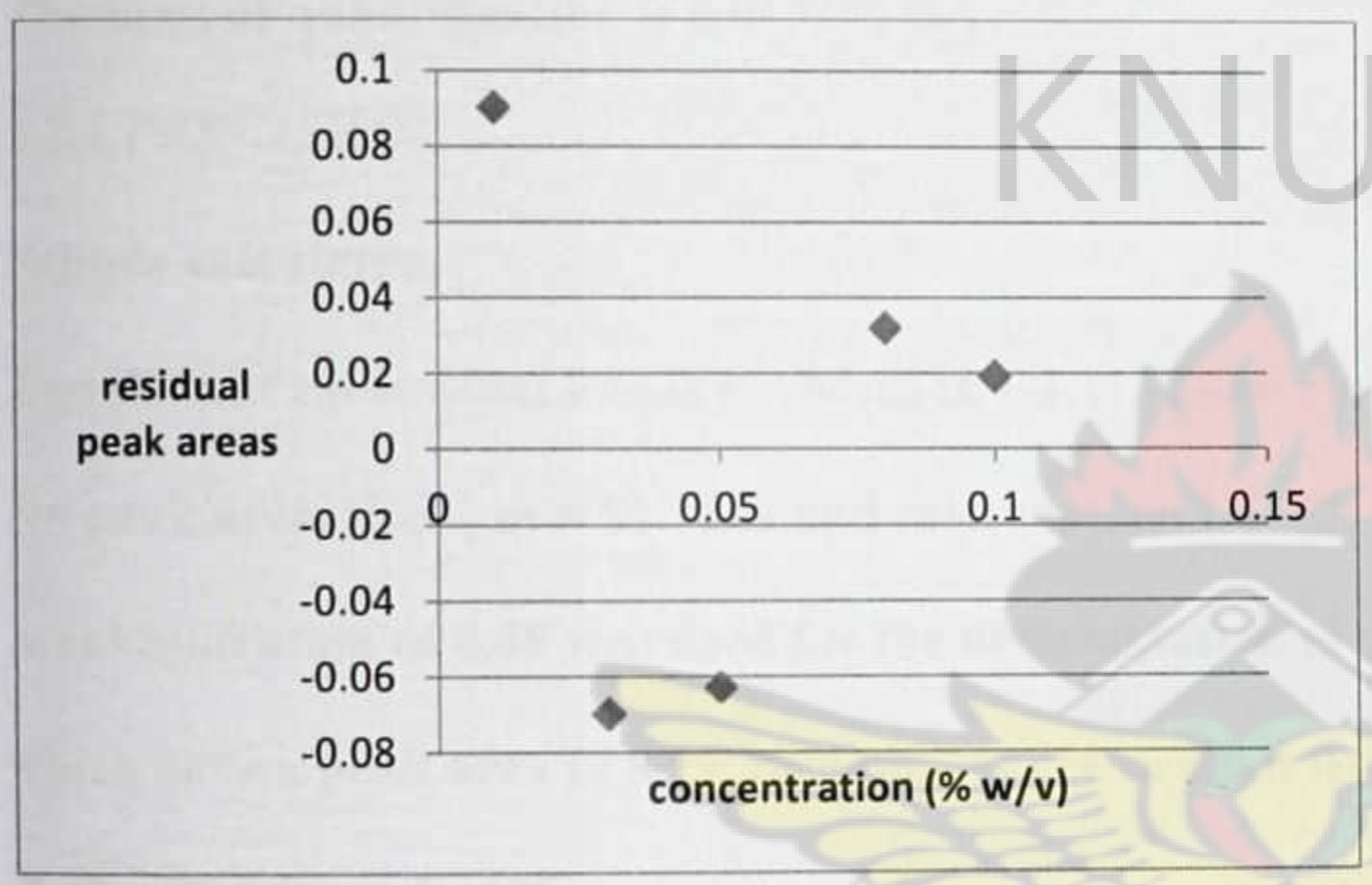


Figure 10 Residual plot

3.7.2 LIMIT OF DETECTION (LOD)

LOD = $\frac{3.3\sigma}{S}$ where σ = residual standard deviation

S = slope of the calibration curve

Residual standard deviation, σ = 0.067715

Hence LOD = $\frac{3.3 \times 0.067715}{51.154}$ = 0.00437% w/v

The limit of detection is 0.00437 % w/v.

3.7.3 LIMIT OF QUANTIFICATION (LOQ)

$$LOQ = \frac{10\sigma}{S}$$

$$\text{Hence LOQ} = \frac{10 \times 0.067715}{51.154} = 0.0132\% \text{ w/v}$$

The limit of quantification is 0.0132% w/v.

3.7.4 PRECISION

Sample calculation

Equation of calibration line is y = 51.154x + 1.1157 and comparing to y =mx + c

y= peak area, slope, m = 51.154x and intercept, c= 1.1157.

A concentration of 0.08 was used for the determination of precision. For determination 1 which gave a peak area of 5.17, its actual concentration from the curve is calculated as:

$$5.17 = 51.154x + 1.1157$$

$$x = \frac{5.17 - 1.1157}{51.154} = 0.0793\% \text{ w/v}$$

$$\text{Hence \% content} = \frac{\text{actual concentration from curve} \times 100\%}{\text{Nominal concentration}}$$

$$= \frac{0.0793\% \text{ w/v} \times 100\%}{0.080 \% \text{ w/v}}$$

$$= 99.125\% \approx 99.13\%$$

3.7.4.1 Intra-day Precision

Determination	% content	
1	99.13	
2	100.21	
3	98.90	
4	100.03	
5	99.62	Average = 99.82%
6	101.01	Relative standard deviation RSD = 0.77

3.7.4.2 Inter-day Precision

Determination	% content	
1	99.50	
2	98.68	
3	101.40	
4	99.02	
5	100.40	
6	100.21	
7	99.60	
8	98.50	
9	99.30	
10	100.70	
11	98.67	
12	100.30	
13	100.25	
14	99.10	
15	98.43	
16	100.10	
17	101.20	Average = 99.73
18	99.70	RSD = 0.91

3.7.5 ROBUSTNESS

Concentration of 0.08% w/v was used for the robustness.

3.7.5.1 Wavelength variation

Table 26 Percentage content for a concentration of 0.08% w/v upon wavelength variation

Wavelength /nm	Percentage content (%w/v)
223	100.65± 1.00
225	99.66± 0.99
227	99.54 ±1.66

3.7.5.2 Flow rate variation

Table 27 Percentage content for a concentration of 0.08%w/v upon flow rate variation

Flow rate (ml/min)	Percentage content (%w/v)
0.80ml/min	166± 0.71
1.00ml/min	99.66± 0.99
1.20ml/ min	98.93 ± 0.35

***A flow rate of 0.80ml/min very broad peaks and consequently large peak areas.**

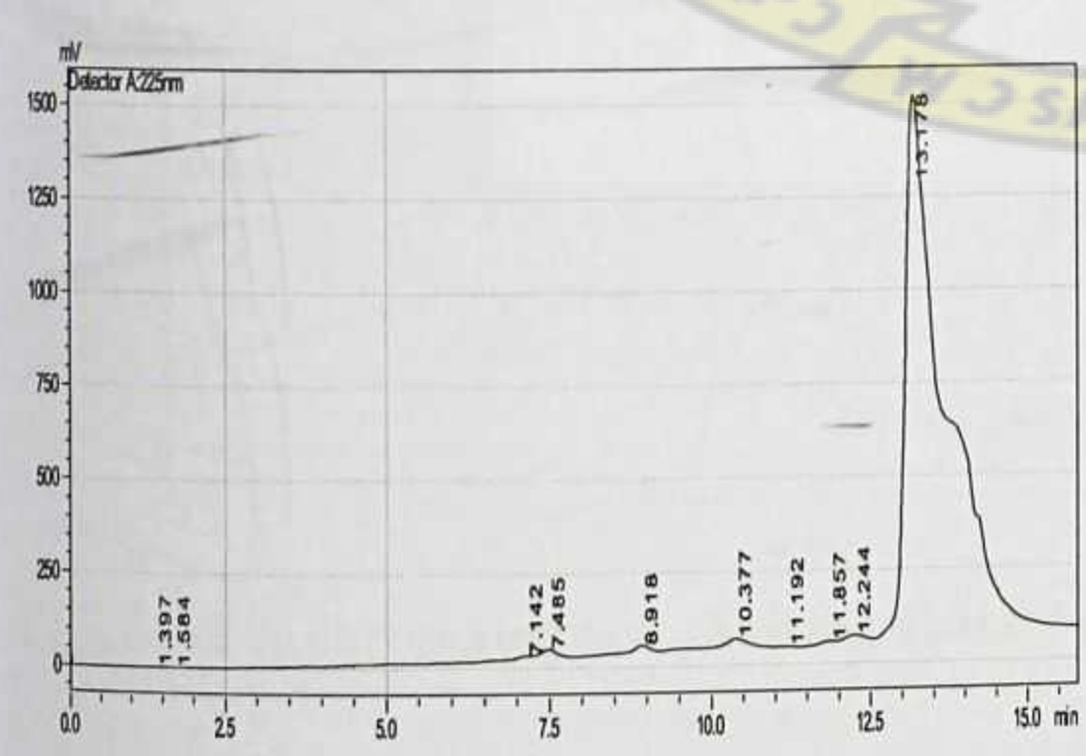


Figure 11 A broad peak at a flow rate of 0.80ml/min.

3.7.5.3 T- test calculation for robustness

For a detection wavelength of 223nm

$t = [(x-\mu)\sqrt{n}] / s$ (Vogel, 1989)

Where s, standard deviation = 1.00, n, number of samples = 3, x, the mean = 100.65, μ, the true mean = 99.66 and t is the confidence limit.

Therefore $t = [(100.65 - 99.66)\sqrt{3}]/ 1$

$= 1.7147 \approx 1.7145$

At 99% confidence level the confidence limit allowed is 6.965 for 2 degrees of freedom. The t- value calculated at 223nm is less than the critical value for the confidence limit hence there is no significant difference between the values at 225nm and 223nm.

3.7.6 SELECTIVITY

Table 28 Different retention times obtained for amoxicillin and flucloxacillin in a solution.

Analyte	Retention time/ min
Amoxicillin	8.93 ± 0.19
Flucloxacillin	5.44 ± 0.13

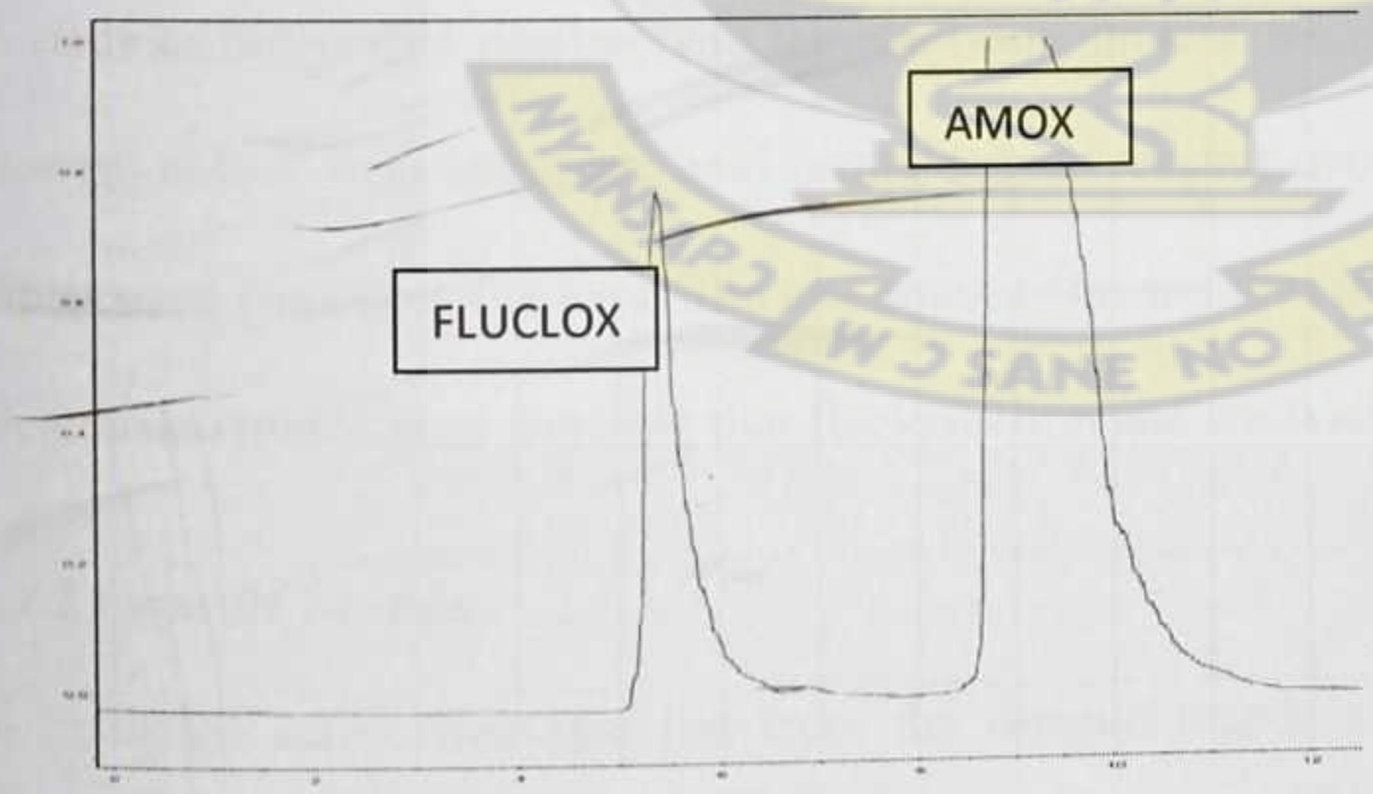


Figure 12 A chromatogram showing distinct peaks for amoxicillin and flucloxacillin

CHAPTER FOUR- DISCUSSION, CONCLUSION AND RECOMMENDATION

4.1 DISCUSSION

4.1.1IDENTIFICATION OF SAMPLES

It is always important that one ought to be sure of the substances being dealt with in a chemical laboratory. Sometimes these substances are used as reference standards to which other substances are compared for quantitative and qualitative analysis. One sure way of doing this is by subjecting the samples to identification tests to confirm their identities so that any conclusions drawn from results with such samples will be valid and authentic.

Additionally, some compounds could bear superficial resemblance to the sample being worked with and it is only tests of identity that can discriminate against these unwanted substances. Some tests for identification like HPLC are also able to detect adulteration with chemically related substances that can affect laboratory results significantly.

4.1.1.1 Pure flucloxacillin and pure amoxicillin

The samples responded positively to the identification test described in the **BP 2009** by giving a yellowish colour with sulphuric acid-formaldehyde reagent upon heating in a water- bath. The samples used were thus flucloxacillin and amoxicillin and the HPLC chromatograms of each also indicated one major peak showing that flucloxacillin and amoxicillin were largely present.

4.1.1.2 Capsule brands

The extracted active flucloxacillin from the capsule brands also tested positive by giving a yellow colour with sulphuric acid-formaldehyde reagent after warming in a water-bath for a minute. The HPLC chromatograms also showed major peaks indicating the presence of flucloxacillin in the capsule brands.

4.1.1.3 Excipients

The excipients employed tested positive for their identification tests; starch gave a blue-black colour with iodine solution and sodium carboxyl-methylcellulose gave a precipitate with copper sulphate solution.

4.1.2 IDENTIFICATION OF EXCIPIENTS IN CAPSULE BRANDS

4.1.2.1 Test for starch

Samples A1, B and E contained starch as an excipient but C and D had no starch in them. The amount of starch in A1 was more than in the other two since it gave intense blue- black coloration whereas the other two only gave blue- black specks meaning a small amount of starch was present.

4.1.2.2 Test for magnesium stearate

Magnesium stearate is used as a lubricant in tablet and capsule formulations. It has the ability to react with moisture to give magnesium hydroxide which is alkaline and can promote breakdown of flucloxacillin. None of the capsules contained magnesium stearate.

4.1.2.3 Test for sodium cmc

All the capsules did not contain sodium cmc.

4.1.3 PHARMACOPOEIAL TESTS

4.1.3.1 Disintegration test

Capsule disintegration constitutes a measure of quality assurance and to some extent dictates the bioavailability and hence the pharmacokinetic profile of a drug. A drug that takes an unduly long time to disintegrate would mean that serum and blood levels of the drug may not be attained in time and this could affect therapeutic outcomes.

The **BP 2009** states that the disintegration time for hard gelatin capsules should not exceed 30 minutes. All the capsule brands with the exception of sample D that disintegrated after 53 minutes passed the test. The failure of sample D may have been due to cross-linking of the gelatin capsules to reduce their solubility in the disintegration medium. The poor solubility may have led to the long disintegration time. Additionally, colouring agents used in imparting colour to the shells like titanium dioxide are water insoluble as such capsules impregnated with a lot of this colouring agent or similar ones like the iron oxides would take long to disintegrate as the disintegration medium employed was aqueous and may not have permeated the shells readily. **(Bhatt and Agrawal, 2007)**

4.1.3.2 Uniformity of content

Uniformity of content is one of many in- process controls that are performed. The test is designed to ensure that every capsule within a batch contains the amount of drug substance intended with very little variation among the capsules within the batch. Hence the test controls and assesses the variability of dosage forms like capsules by ensuring that each contains equal amounts of active ingredients and also ensures that powders filled into capsules have the same consistency. It is when they have the same consistency that they will flow evenly into the capsule shells with little or no variations in their weights in the shells.

The **BP 2009** states that for capsules with an average weight of more than 300mg, not more than two should have deviations greater than 7.5% and none should deviate by more than 15%.The average weight of capsule brands A1, B, C and E were more than 300mg. Capsule brand D had an average weight of less than 300mg. For capsules whose average weights are below 300mg the **BP 2009** states that not more than two should have deviations greater than 10% and none should deviate by more than 20%. Based on these observations only brand B passed the test.

4.1.4 MOISTURE CONTENT DETERMINATION

Starch is hygroscopic and is used as a disintegrant in capsule and tablet formulation. In formulating moisture sensitive drugs, it is very important not to use starch of a high moisture content to prevent premature degradation of the drug. Also in capsule formulations, the capsule shell can take up water from starch that has a high moisture content, swell and distort. The starch used had a moisture content of 11% which is less than the 15% stated in the **BP 2009** implying that the starch sample passed the test.

4.1.5 DETERMINATION OF IODINE ABSORBING IMPURITIES

The limit for iodine absorbing impurities in flucloxacillin is 5% according to the **BP 2003**. All the samples passed the test except sample E which failed with a value of 14.61%.

One major problem associated with the use of penicillin antibiotics is the occurrence of allergic reactions in sensitive individuals. These reactions are caused by degradation products of the penicillins and are quantifiable because they can react with iodine. A penicillin product like flucloxacillin is likely to cause such a reaction in a sensitive individual if the levels of these impurities are high. With the exception of sample E all the samples passed the test.

Iodine absorbing impurity can also be used as an index of penicillin breakdown. A very high level is therefore indicative of an appreciable breakdown. It can be said that a high amount of the flucloxacillin in sample E had broken down since it had a low active content of 55.23%. Using such a product may cause an allergic reaction in susceptible persons and will also constitute under-dosage that could lead to bacterial resistance development.

Those samples that passed the test may have been well stored and kept away from acidic and alkaline substances and moisture that could induce breakdown. Sample E may have been exposed to any of these notably moisture in humid environments due to poor packaging and storage of the raw material and/ or the finished product.

4.1.6 ASSAY OF FLUCLOXACILLIN IN CAPSULE AND PURE FLUCLOXACILLIN

The percentage content of flucloxacillin in capsules should be 92.5% to 101.0% and 95% to 101% for the pure powder as stated in the **BP 2007**.All the 250mg capsule brands used and the pure powder did not pass the test as their contents were outside their respective ranges.

Sample A1 had a high average weight value of 0.3769g, passed the test for iodine absorbing impurities and had a percentage content of 115%.These imply that generally a larger amount than stated of flucloxacillin and a good amount of starch (an intense blue coloration was seen for test for starch) were put into the capsules in that batch hence the values for the content and average weight respectively. Sample B also had a high average weight value of 0.3823g, passed narrowly for iodine absorbing impurities and had a percentage content of 87.15%. It means that less flucloxacillin than 250mg per capsule was present and good amounts of one or more excipients not tested for may have been added to give the high average weight as it contained no magnesium stearate or sodium cmc and had a small amount of starch. Sample C gave a content of 92.25% meaning that the amounts in the capsules were close to the stated amount. Sample D had a relatively low average weight of 0.2596g and a content of 86.59%. It can be inferred that generally the capsules of brand D originally had lower than the 250mg flucloxacillin per capsule stated with very little breakdown as the iodine absorbing impurity value was 1.88%. Sample E in spite of its high average weight of 0.3785g had a very low content of 55.23%. Clearly the

amounts of flucloxacillin in the capsules of E or most of them within the batch were very low. The high breakdown value of 14.61% when added to the content assuming a 0% breakdown will give nothing close to 92.5% and this implies that very low amounts of flucloxacillin were encapsulated with a small amount of starch. The high average weight is thus explained by the presence of high amounts of some unknown substance or excipient other than those tested for. With the exception of sample A1 it can be said that the low amounts of flucloxacillin in the samples may be due to the following:

- Poor storage of the drug and exposure to conditions like moisture from the environment, formulation, capsule shells etc and alkaline or acidic substances that readily break it down.
- Using lower than the stated amounts in production by the manufacturer.

The pure powder failed the content test but passed for the iodine absorbing impurity content. The additive result for both content and iodine absorbing impurity gives a value of about 95% which lies in the range for the pure powder. If follows therefore that the pure failed due to breakdown with poor storage being the most likely explanation.

4.1.7 STABILITY STUDIES

4.1.7.1 Preparation of flucloxacillin/ excipient mixtures

The various proportions of 200mg, 100mg and 50mg of excipient and 250mg flucloxacillin mixtures were chosen using the average weight of the capsules as a guide. The capsules did not appear to have been very fully stuffed with their contents hence such combinations were deemed feasible should they be encapsulated with some other excipients that are often used in small amounts.

4.1.7.2 Determination of the order of the reaction

The samples were assayed over a period of three months or twelve weeks and their percentage contents converted to concentration terms needed to plot graphs for zero and first order reactions. The focal point of the experiment was to find out how well the excipients used could stabilize the drug in the presence of moisture. Moisture is always in the air and more so in high amounts because of the high humidity of our atmosphere. Consideration was thus given to a possible first order reaction and more appropriately a pseudo-first order because the amount of moisture is in far excess of the drug as it is always present in air and the amounts of it will not change even after reaction with the drug and the reaction taken to be dependent only on the drug amounts present.

A zero order was also seen as a possibility because it was also thought that irrespective of the amounts of water and drug that may be present, there should be a reaction between them once the two substances are present. Also most solid phase reactions are known to follow zero order kinetics.

A second order kinetics was ruled out because two different concentration terms; concentration of the drug and of water were needed and it was not possible to measure the concentration of moisture the samples were exposed to.

After obtaining zero and first order graphs for the samples it was seen that most of the samples followed zero order kinetics. The co-efficients of correlation obtained for these samples were higher than those obtained for first order. The coefficient of correlation shows the strength of correlation between y (concentration terms) and x (time) values and is loosely related to linearity. These showed there were stronger degrees of correlation between the concentration

terms and the time for zero order. The linear graphs for these samples also looked better than the first order graphs.

For these samples the reaction did not depend on the concentration of flucloxacillin or moisture and in the case of the capsule brands on any other chemical substance that may have been present.

Samples B and E however followed first order kinetics with their co-efficients of correlation higher than for their zero order graphs. This implies that their concentration terms for first order showed a stronger and better correlation with time than those for zero order.

4.1.7.3 Comparing reaction rate constants and predicting the most stable formulation

The concentration of flucloxacillin in the capsules fell with time upon exposure to moisture in air in a room. A confirmation that flucloxacillin in capsules readily breaks down with time when it comes into contact with moisture.

A careful look at the k (rate constant) values revealed that the rate constants for the various amounts of dried starch was smallest followed by undried starch and then sodium cmc for the same amounts of each excipient. It is worth noting that the value for the rate constant for pure flucloxacillin (7.7083%w/w /week) was higher than all these except that for 50mg sodium cmc which gave a rate constant of 8.1789%w/w $week^{-1}$. It was observed that the rate constant for any amount of dried starch or undried starch was smaller than any of the rate constants obtained for any proportion of sodium cmc with flucloxacillin. All the rate constants obtained for the proportions of dried and undried starch were lower than that for only pure flucloxacillin.

The reaction rate constant, k, is a very useful measure and indicator of how rapidly a reaction occurs with time. A high k value therefore denotes a fast reaction rate which translates into a

very rapid breakdown and an unstable product. The opposite is true for a small rate constant. It was observed that in general dried starch formed the most stable product with flucloxacillin and sodium cmc gave the least stable product.

Starch and sodium cmc are hygroscopic substances and it was thought that their inclusion would make flucloxacillin stable as they will take up any moisture present in the formulation thereby protecting the drug from moisture induced hydrolysis. This protection is thought to be possible because both substances have hydroxyl groups projecting from their backbones and can link up with water molecules via hydrogen bonding.

A direct relation between water absorption and rate of breakdown was thus established. Over the period of study the exposed amounts of dried starch, undried starch and sodium cmc respectively absorbed 0.84%, 2.82% and 12.50% of moisture. Dried starch formed the most stable product because it absorbed the least moisture making it the least hygroscopic and sodium cmc formed the least stable product because it took up the most moisture and proved to be the most hygroscopic. Undried starch formed a product less stable than that of dried starch but more stable than that of sodium cmc. The undried starch since it was not heated to remove water molecules, the adhering water molecules readily associated with incoming ones to cause a higher absorption than dried starch that was dried to constant weight prior to use.

Of note is the trend of reducing rate constants with increase in the amounts of the excipients used. The more excipient used the higher the protection offered to flucloxacillin and the more stable the product. This is seen in the rate constant obtained for 50mg sodium cmc/ 250mg flucloxacillin combination which was even less stable than pure flucloxacillin by comparing their rate constants. Before the third month elapsed, this product formed a hard caked substance

whereas those for the 200mg and 100mg sodium cmc were quite powdery. It follows that the 50mg proportion was inadequate and partly dissolved and yielded a caked substance that retained a lot of moisture in its core and caused the fastest breakdown even faster than pure flucloxacillin alone.

It can be recalled that only samples B and E followed a first order reaction while the other sample brands followed a zero order reaction. For effective comparison and for the sake of feasibility the zero order rate constants were used. Brand E was the most stable product and brand C was the least stable. E contained only a small amount of starch which was evident as blue- black specks with iodine. It also gave the lowest percentage content of 55.23% meaning that the amount of starch and the amount of flucloxacillin may be nearly proportional. With the effect of increasing amount of starch on a fixed amount of flucloxacillin already established, the stability of E could be attributed to the starch present. Sample A1 was the second most stable and tested to contain a lot of starch which manifested as intense blue- black coloration. A1 had a high percentage content of 115%. This means that the ratio of starch to flucloxacillin could be lower than that for E hence the higher stability for E. E and B strictly speaking followed first order kinetics and may contain other chemical substances that increased stability. B was less stable than A1 but more stable than D and D also showed a higher stability than C. Both B and D had nearly the same active contents which were fairly high (i.e. B- 87.15% and D-86.59%) but the small amount of starch present in B may have been responsible for it being more stable than D which contained no starch but less stable than A1 because of a possible lower starch-flucloxacillin ratio than A1. C being the least stable is attributable to one or a combination of the following reasons: the absence of starch, formulation with moist or hydrated excipients and the presence of chemical substances that promoted breakdown. C and D were seen to be less stable

than pure flucloxacillin alone while A1, E and B were more stable than the pure flucloxacillin because of the above reasons.

4.1.8 HPLC METHOD DEVELOPMENT AND VALIDATION

4.1.8.1 Method development

The mobile phase system of methanol and potassium dihydrogen orthophosphate (KH_2PO_4) adjusted to pH 5 was used. Flucloxacillin has been assayed in literature with several mobile phase systems involving combinations of solvents like acetonitrile which is expensive and various buffers which are not readily available. The choice of methanol and KH_2PO_4 was made because they are cheap and readily obtainable. A detection wavelength of 225nm was chosen from literature and as it also gave sharp and nice peaks of the drug. The trial was begun with a 50:50 mixture of the two solvents and this gave a retention time of about 13 minutes at a flow rate of 1.00ml/min. Since the retention time was quite long the amount of methanol was stepped up to 60% with the buffer constituting 40% of the mobile phase and this reduced the retention time to around 5.35 minutes. These conditions were considered optimum for an HPLC method.

4.1.8.2 Method validation

The calibration plot for linearity gave a straight line with a coefficient of correlation, r^2, of 0.9988. A high r^2 does not imply linearity as it only implies that there is a strong correlation between the y and x values. An r^2 of 0.9988 hence tells that the concentration terms and the peak areas had a strong correlation. It is difficult to tell linearity from a scatter diagram in spite of a high r^2 value since they are only loosely related. Hence to confirm linearity a residual plot was done. For a residual plot an equal distribution between negative and positive residual values should confirmed linearity. But for a five point calibration curve there can only be three positives

and two negatives which was attained or vice versa. The response to flucloxacillin was thus linear with concentration over the range 0.01% to 0.10% i.e. different concentrations gave proportional peak areas.

The limits of detection (LOD) and quantification (LOQ) calculated from the curve were 0.00437% w/v and 0.0132% w/v respectively. It means that for the chromatographic conditions developed, the least unquantifiable analyte concentration that will give a response or a signal is 0.00437% w/v below which signals will not be seen. Also with the method and its conditions, the smallest quantifiable analyte concentration that produces a signal is 0.0132% w/v below which signals will be produced by analyte concentrations that cannot be quantitated. These values seem to be quite high but for a method designed to assay flucloxacillin in formulations where there are sufficiently high concentrations of it such a method with the developed conditions is satisfactory.

The method was precise since the intra- day and inter- day determinations gave relative standard deviations of less than 2%. A low precision of less than two implies determinations of a particular concentration of the analyte under the chromatographic conditions will yield results that are reproducible or very close.

Selectivity was confirmed as the method gave two distinct peaks for flucloxacillin and amoxicillin at different retention times of 5.44± 0.13 minutes and 8.93 ± 0.19 minutes respectively without any interference from each other. This means that flucloxacillin can comfortably be assayed in the presence of amoxicillin.

Once the method was linear, specific and precise, accuracy was inferred. This means that if a reference sample of known purity with a concentration within the range were assayed, the

concentration calculated would be very close to the concentration of the reference material made if not the same.

The method proved to be robust with an increase in flow rate from 1.00ml/min to 1.20ml/min as there was no significant difference in their calculated values for percentage content. This was shown by a t- test as the calculated t- values were below the critical value of 6.965 at 99% confidence interval. At a flow rate of 0.80ml/min a broad peak was obtained with a large peak area which gave a large percentage content of 166.24± 0.71 which was significantly different from the expected 99.66± 0.99 as determined by the t-test. It is therefore not suitable to carry out determinations at this flow rate using the chromatographic conditions. Varying the detection wavelength also showed how robust the method was as it did not produce any significant changes in the percentage content as the calculated t- values were below the critical value at 99% confidence interval.

4.2 CONCLUSION

4.2.1 IDENTIFICATION TESTS

All the samples – pure flucloxacillin, pure amoxicillin, capsule brands and the excipients used passed the identification test.

4.2.2 IDENTIFICATION OF EXCIPIENTS IN CAPSULE BRANDS

All the brands did not contain magnesium stearate and sodium carboxyl-methylcellulose.

Capsule brands A1, B and E contained starch. Brands C and D did not contain starch.

4.2.3 PHARMACOPOEIAL TESTS

4.2.3.1 Disintegration test

All the brands passed except brand D.

4.2.3.2 Uniformity of content

Only brand B passed. All the other brands failed.

4.2.4 MOISTURE CONTENT DETERMINATION

The starch had a moisture content of 11.10% w/w.

4.2.5 DETERMINATION OF IODINE ABSORBING IMPURITIES

All the capsule brands and the pure powder passed except capsule brand E

4.2.6 ASSAY OF FLUCLOXACILLIN

All the capsule brands and the pure flucloxacillin failed.

4.2.7 STABILITY STUDIES

4.2.7.1 Reaction order determination

Capsule brands B and E followed first order kinetics. The other capsule brands and the various flucloxacillin-excipient combinations followed a zero order reaction.

4.2.7.2 Stability of the formulations

Dried starch formed the most stable product and sodium cmc gave the least stable product.

Weight for weight dried starch was a better stabilizer of flucloxacillin than undried starch.

4.2.8 HPLC METHOD VALIDATION

The proposed HPLC method is suitable for the assay of flucloxacillin in formulations.

4.3 RECOMMENDATIONS

- Stability studies should be carried out with other excipients and if possible a combination of two or three excipients in differing amounts.
- A hygrometer should be used to investigate the effect of humidity changes on flucloxacillin stability.

APPENDIX
MILLIEQUIVALENT CALCULATIONS

Preparation of 250ml of 0.01M $Na_2S_2O_3$.$5H_2O$

248.1800g in 1000ml ≡ 1M $Na_2S_2O_3.5H_2O$

2.4818g in 1000ml ≡ 0.01M

0.6205g in 250ml ≡ 0.01M

Preparation of primary standard, KIO_3 solution for 0.01M $Na_2S_2O_3$.$5H_2O$

214.0000g in 1000ml ≡ 6M $Na_2S_2O_3$

35.6667g in 1000ml ≡ 1M

0.3566g in 1000ml ≡ 0.01M

0.1783g in 500ml ≡ 0.01M

Preparation of 0.01M iodine, I_2 solution

2(126.9000g) in 1000ml ≡ 1M I_2

2.5380g in 1000ml ≡ 0.01M

0.5076g in 200ml ≡ 0.01M

Preparation of 2M H_2SO_4

98.0700g in 1000ml ≡ 1M

196.1400g in 1000ml ≡ 2M

19.6140g in 100ml ≡ 2M

But assay of H_2SO_4 is 98%

98% = 19.6140g

$$100\% = \frac{100\% \times 19.6140g}{98\%}$$

= 20.0000g

Specific gravity of H_2SO_4 = 1.835g/ml

1.835g = 1ml

$$20.0000g = \frac{20.0000g \times 1ml}{1.835g}$$

= 10.91ml

Preparation of 1M NaOH

40.0000g in 1000ml ≡ 1M NaOH

4.0000g in 100ml ≡ 1M NaOH

% purity of NaOH = 99%

99% = 4.0000g

$$100\% = \frac{100\% \times 4.0000g}{99\%}$$

= 4.0404g

Preparation of 1M HCl

36.4500g in 1000ml ≡ 1M HCl

3.6450g in 100ml ≡ 1M HCl

Assay = 36%

36% = 3.6450g

$$100\% = \frac{100\% \times 3.6450g}{36\%}$$

= 10.1250g

Specific gravity = 1.18g/ml

1.18g = 1ml

$$10.1250g = \frac{10.1250g \times 1ml}{1.18g}$$

= 8.58 ml

Preparation of 2.40% w/v Glacial acetic acid

This implies 2.40g made to 100ml

Specific gravity = 1.048g/ml- 1.0511g/ml

Average value = 1.0495g/ml

Assay = 99%

99% = 2.4000g

$$100\% = \frac{100\% \times 2.4000g}{99\%}$$

$$= 2.4242g$$

1.0495g = 1ml

$$2.4242g = \frac{2.4242g \times 1ml}{1.0495g}$$

$$= 2.3099ml \approx 2.31ml$$

$$\text{But for } 250ml = \frac{250ml \times 2.31ml}{100ml}$$

= 5.78ml of glacial acetic acid

Preparation of 0.02M $Na_2S_2O_3.5H_20$

248.1800g in 1000ml ≡ 1M $Na_2S_2O_3.5H_20$

4.9636g in 1000ml ≡ 0.02M

2.4818g in 500ml ≡ 0.02M

Preparation of primary standard, KIO_3 solution for 0.02M $Na_2S_2O_3.5H_20$

214.0000g in 1000ml ≡ 6M $Na_2S_2O_3$

35.6667g in 1000ml ≡ 1M

0.7133g in 1000ml ≡ 0.01M

0.3567g in 500ml ≡ 0.01M

UNIFORMITY OF CONTENT TABLES

Capsule brand A1

Caps. no	wt of cap	wt of shell	wt of content	deviation	% deviation
1	0.4756	0.0758	0.3998	-0.0213	-5.63
2	0.426	0.0789	0.3471	0.0314	8.30
3	0.4492	0.0778	0.3714	0.0071	1.88
4	0.379	0.0757	0.3033	0.0752	19.87
5	0.4687	0.0761	0.3926	-0.0141	-3.73
6	0.4521	0.075	0.3771	0.0014	0.37
7	0.4366	0.0761	0.3605	0.018	4.76
8	0.4687	0.0752	0.3935	-0.015	-3.96
9	0.4734	0.0814	0.392	-0.0135	-3.57
10	0.4773	0.084	0.3933	-0.0148	-3.91
11	0.4617	0.0759	0.3858	-0.0073	-1.93

Caps. no	Wt of cap	Wt of shell	Wt of content	Deviation	% deviation
12	0.4517	0.074	0.3777	0.0008	0.21
13	0.4844	0.0758	0.4086	-0.0301	-7.95
14	0.4669	0.0773	0.3896	-0.0111	-2.93
15	0.4767	0.0791	0.3976	-0.0191	-5.05
16	0.4363	0.0743	0.362	0.0165	4.36
17	0.4477	0.0773	0.3704	0.0081	2.14
18	0.4495	0.0758	0.3737	0.0048	1.27
19	0.4576	0.0792	0.3784	0.0001	0.03
20	0.4681	0.0775	0.3906	-0.0121	-3.20

Weight of 20 caps = 9.1041g weight of 20 shells = 1.5340g

Capsule brand B

Caps no.	wt of cap	wt of shell	wt of content	deviation	% deviation
1	0.4741	0.0778	0.3963	-0.014	-3.66
2	0.4646	0.0769	0.3877	-0.0054	-1.41
3	0.4599	0.0789	0.381	0.0013	0.34
4	0.4626	0.0782	0.3844	-0.0021	-0.55
5	0.4552	0.0764	0.3788	0.0035	0.92
6	0.452	0.0779	0.3741	0.0082	2.14
7	0.4532	0.0769	0.3763	0.006	1.57
8	0.4728	0.077	0.3958	-0.0135	-3.53
9	0.4608	0.0775	0.3833	-0.001	-0.26
10	0.4513	0.0784	0.3729	0.0094	2.46
11	0.4423	0.0752	0.3671	0.0152	3.98
12	0.4657	0.0827	0.383	-0.0007	-0.18
13	0.451	0.0826	0.3684	0.0139	3.64
14	0.4621	0.0782	0.3839	-0.0016	-0.42
15	0.4601	0.0768	0.3833	-0.001	-0.26
16	0.4606	0.0788	0.3818	0.0005	0.13
17	0.4678	0.0791	0.3887	-0.0064	-1.67
18	0.4695	0.0801	0.3894	-0.0071	-1.86
19	0.4637	0.0776	0.3861	-0.0038	-0.99
20	0.4561	0.0772	0.3789	0.0034	0.89

Weight of 20 caps = 9.1940g weight of 20 shells = 1.5486g

Capsule brand C

cap no	wt of cap	wt of shell	wt of content	deviation	% deviation
1	0.3832	0.0777	0.3055	0.0495	13.94
2	0.4513	0.0761	0.3752	-0.0202	-5.69
3	0.4499	0.0762	0.3737	-0.0187	-5.27
4	0.4483	0.0765	0.3718	-0.0168	-4.73
5	0.4782	0.0839	0.3943	-0.0393	-11.07
6	0.4354	0.073	0.3624	-0.0074	-2.08
7	0.4111	0.076	0.3351	0.0199	5.61
8	0.4626	0.0777	0.3849	-0.0299	-8.42
9	0.4803	0.0767	0.4036	-0.0486	-13.69
10	0.4178	0.0785	0.3393	0.0157	4.42
11	0.3765	0.0731	0.3034	0.0516	14.54
12	0.405	0.0759	0.3291	0.0259	7.30
13	0.4215	0.0746	0.3469	0.0081	2.28
14	0.4173	0.0751	0.3422	0.0128	3.61
15	0.4491	0.0748	0.3743	-0.0193	-5.44
16	0.4586	0.0779	0.3807	-0.0257	-7.24
17	0.4764	0.0796	0.3968	-0.0418	-11.77
18	0.4111	0.0805	0.3306	0.0244	6.87
19	0.4461	0.0774	0.3687	-0.0137	-3.86
20	0.3605	0.0819	0.2786	0.0764	21.52

Weight of 20 caps = 8.6397g weight of 20 shells = 1.5388g

Capsule brand D

cap no.	wt of cap	wt of shell	wt of content	deviation	% deviation
1	0.3847	0.0796	0.3051	-0.0455	-17.53
2	0.3299	0.078	0.2519	0.0077	2.97
3	0.32	0.0771	0.2429	0.0167	6.43
4	0.2919	0.0763	0.2156	0.044	16.95
5	0.3527	0.0806	0.2721	-0.0125	-4.82
6	0.3624	0.0764	0.286	-0.0264	-10.17
7	0.3292	0.0776	0.2516	0.008	3.08
8	0.3351	0.08	0.2551	0.0045	1.73
9	0.3193	0.0812	0.2381	0.0215	8.28
10	0.3697	0.0815	0.2882	-0.0286	-11.02
11	0.3001	0.0747	0.2254	0.0342	13.17
12	0.3112	0.0803	0.2309	0.0287	11.06
13	0.3268	0.0769	0.2499	0.0097	3.74

cap no.	wt of cap	wt of shell	wt of content	deviation	% deviation
14	0.339	0.0769	0.2621	-0.0025	-0.96
15	0.3715	0.079	0.2925	-0.0329	-12.67
16	0.3632	0.0811	0.2821	-0.0225	-8.67
17	0.359	0.0783	0.2807	-0.0211	-8.13
18	0.3401	0.081	0.2591	0.0005	0.19
19	0.3622	0.0769	0.2853	-0.0257	-9.90
20	0.3306	0.0755	0.2551	0.0045	1.73

Weight of 20 caps = 6.7566g weight of 20 shells = 1.5651g

Capsule brand E

cap no.	wt of cap	wt of shell	wt of content	deviation	% deviation
1	0.4756	0.0758	0.3998	-0.0213	-5.63
2	0.426	0.0789	0.3471	0.0314	8.30
3	0.4492	0.0778	0.3714	0.0071	1.88
4	0.379	0.0757	0.3033	0.0752	19.87
5	0.4687	0.0761	0.3926	-0.0141	-3.73
6	0.4521	0.075	0.3771	0.0014	0.37
7	0.4366	0.0761	0.3605	0.018	4.76
8	0.4687	0.0752	0.3935	-0.015	-3.96
9	0.4734	0.0814	0.392	-0.0135	-3.57
10	0.4773	0.084	0.3933	-0.0148	-3.91
11	0.4617	0.0759	0.3858	-0.0073	-1.93
12	0.4517	0.074	0.3777	0.0008	0.21
13	0.4844	0.0758	0.4086	-0.0301	-7.95
14	0.4669	0.0773	0.3896	-0.0111	-2.93
15	0.4767	0.0791	0.3976	-0.0191	-5.05
16	0.4363	0.0743	0.362	0.0165	4.36
17	0.4477	0.0773	0.3704	0.0081	2.14
18	0.4495	0.0758	0.3737	0.0048	1.27
19	0.4576	0.0792	0.3784	0.0001	0.03
20	0.4681	0.0775	0.3906	-0.0121	-3.20

Weight of 20 capsules = 9.1041g weight of 20 shells = 1.5340g

PREPARATION 250MG FLUCLOXACILLIN/ EXCIPIENT MIXTURES

Preparation of dried starch and flucloxacillin mixture

Preparation	250mg flucloxacillin + 200mg dried starch	250mg flucloxacillin + 100mg dried starch	250mg flucloxacillin + 50mg dried starch
Weight of flucloxacillin/g	2.0146	2.0048	2.0003
Weight of dried starch /g	1.6117	0.8020	0.4005
Total weight by addition/g	3.6263	2.8068	2.4008
weight of mixture + dish/g	54.5826	54.4254	53.1547
weight of dish/g	50.9930	51.6839	50.8812
Weight of mixture obtained from balance/g	3.5896	2.7415	2.2735

Weight of empty dish = 43.3928g

Weight of dish and dried starch only = 46.2450g

Preparation of undried starch and flucloxacillin mixture

Preparation	250mg flucloxacillin + 200mg undried starch	250mg flucloxacillin + 100mg undried starch	250mg flucloxacillin + 50mg undried starch
Weight of flucloxacillin/g	2.0029	2.0017	2.0034
Weight of undried starch /g	1.6026	0.8007	0.4006
Total weight by addition/g	3.6055	2.8024	2.4040
weight of mixture + dish/g	39.9755	46.2066	43.2089
weight of dish/g	36.4863	43.4470	40.8313
Weight of mixture obtained from balance/g	3.4892	2.7596	2.3776

Weight of empty dish = 80.3893g

Weight of dish and undried starch only = 84.7382g

Preparation of sodium cmc and flucloxacillin mixture

Preparation	250mg flucloxacillin + 200mg sodium cmc	250mg flucloxacillin + 100mg sodium cmc	250mg flucloxacillin + 50mg sodium cmc
Weight of flucloxacillin/g	2.0077	2.0034	2.0014
Weight of sodium cmc /g	1.6062	0.8013	0.4003
Total weight by addition/g	3.6139	2.8047	2.4017
weight of mixture + dish/g	54.6748	50.0223	53.5342
weight of dish/g	51.1061	47.2652	51.1883
Weight of mixture obtained from balance/g	3.5687	2.7571	2.3459

Weight of empty dish = 49.8950g

Weight of dish and sodium cmc only = 53.2063g

Moisture content determination in starch used

Weight of empty Petri dish = 36.2800g

Weight of dish + starch = 54.3000g

Weight of dish + dried starch after heating to constant weight = 52.3000g

Weight lost = 54.3000g – 52.3000g

= **2.0000g**

Weight of starch used = 54.3000g -36.2800g

= **18.0200g**

= 2.0000g × 100% = **11.10%w/w**
18.0200g

Sample calculations for moisture content for excipients

***Sodium CMC*:**

Weight of moisture gained

= 53.6202g- 53.2063g

= **0.4139g**

Weight of Sodium CMC used

= weight of Petri dish + Sodium CMC – weight of empty dish

= 53.2063g – 49.8950g

= **3.3113g**

% moisture content = 0.4139g × 100%

3.3113g

= **12.50% w/w**

Capsule brands used

Brand code	Manufacturer
A1	Ernest Chemist
B	M and G Pharmaceutical Ltd.
C	Kinapharma Ltd.
D	Letap
E	GR industries Ltd

IODINE ABSORBING IMPURITIES RESULTS

SAMPLE A1

Standardization of 0.01M $Na_2S_2O_3.5H_2O$

Volume of pipette used is 20.00ml

Titration	1	2	3
Final	19.30	38.60	19.80
Initial	0.00	19.30	0.30
Volume used	19.30	19.30	19.50

Average titre = 19.37ml

Blank

Titration	1	2	3
Final	20.10	19.90	19.70
Initial	0.30	0.00	0.00
Volume used	19.80	19.90	19.70

Average titre = 19.80ml

Assay

Titration	1	2	3
Final	16.30	32.60	48.90
Initial	0.10	16.30	32.60
Volume used	16.20	16.30	16.30

Average titre = 16.27ml

Standardization of 0.01M $Na_2S_2O_3.5H_2O$

Volume of pipette used is 25ml

Titration	1	2	3
Final	24.60	25.20	25.00
Initial	0.00	0.00	0.00
Volume used	24.60	25.20	25.00

Average titre = 25.10ml

Blank determination

Titration	1	2	3
Final	19.20	38.80	19.40
Initial	0.00	19.20	0.00
Volume used	19.20	19.60	19.40

Average titre: 19.40ml

SAMPLE B

Titration	1	2
Final	14.80	29.80
Initial	0.00	14.80
Volume used	14.80	15.00

Average titre = 14.90ml

SAMPLE C

Titration	1	2
Final	16.90	33.90
Initial	0.10	16.90
Volume used	16.80	17.00

Average titre = 16.90ml

SAMPLE D

Titration	1	2
Final	17.40	35.00
Initial	0.00	17.40
Volume used	17.40	17.60

Average titre = 17.50ml

SAMPLE E

Titration	1	2
Final	5.20	10.60
Initial	0.00	5.20
Volume used	5.20	5.40

Average titre = 5.30ml

- **Sample calculation for iodine absorbing impurities**

For pure powder

Standardization of 0.01M $Na_2S_2O_3.5H_2O$

Volume of pipette used is 25ml

Titration	1	2	3
Final	24.60	25.20	25.00
Initial	0.00	0.00	0.00
Volume used	24.60	25.20	25.00

Average titre = 25.10ml

Weight of I_2 to be used to prepare 1000ml of 0.01M = 2.5380g

Weight taken = 2.5379g

Factor of KIO_3 = $\frac{\text{actual weight}}{\text{Nominal weight}}$

$= \frac{0.1782g}{0.1783g}$

PURE SAMPLE

Titration	1	2
Final	35.00	16.60
Initial	19.40	1.00
Volume used	15.60	15.60

Average titre = 15.60 ml

= 0.9994

Factor of $Na_2S_2O_3.5H_2O$

$= \frac{\text{factor of } KIO_3 \times \text{volume of } KIO_3}{\text{Volume of } Na_2S_2O_3.5H_2O}$

$= \frac{0.9994 \times 25.00ml}{25.10ml}$

= 0.9954

Blank determination

Titration	1	2	3
Final	19.20	38.80	19.40
Initial	0.00	19.20	0.00
Volume used	19.20	19.60	19.40

Average titre = 19.30ml

Weight of flucloxacillin powder taken = 0.1250g

Assay

Titration	1	2
Final	35.00	16.60
Initial	19.40	1.00
Volume used	15.60	15.60

Average titre = 15.60 ml

Volume of thiosulphate equivalent to volume of iodine absorbed by impurities

= Blank – assay

= (19.30- 15.60) ml

= 3.70 ml

Actual volume = 3.70ml ×factor of $Na_2S_2O_3.5H_2O$

= 3.70ml × 0.9954

= 3.68 ml

From milliequivalent:

ASSAY OF FLUCLOXAXILLIN IN BRANDS FOR 12 WEEKS

WEEK 0

STANDARDIZATION OF 0.02M $Na_2S_2O_3.5H_2O$

Weight of iodate to be used = 0.3567g

Weight of iodate used = 0.3567g

0.524mg of iodine absorbing impurities in 1ml ≡ 0.01M $Na_2S_2O_3.5H_2O$

Hence 3.68 ml of 0.01M $Na_2S_2O_3.5H_2O$ reacted with = $\frac{3.68\ ml \times 0.524\ mg}{1ml}$

= 1.92832mg ≈ 1.9283mg

25ml of solution = 125.0000mg of flucloxacillin

10ml = $\frac{10ml \times 125.0000mg}{25ml}$

= 50.0000mg of flucloxacillin was present in the 10ml

Hence % of iodine absorbing impurities = $\frac{1.9283mg \times 100\%}{50.0000mg}$

= 3.86 % w/w

The percentage of iodine absorbing impurities present in the pure sample is **3.86 % w/w**

Volume of pipette used is 25.00 ml

Titration	1	2	3
Final	26.90	40.70	27.10
Initial	0.00	13.40	0.00
Volume used	26.90	27.30	27.10

Average titre = 27.00 ml

SAMPLE B

ASSAY

Titration	1	2
Final	15.80	31.70
Initial	0.00	15.80
Volume used	15.80	15.90

Average titre = 15.85ml

BLANK

Titration	1	2
Final	23.70	23.50
Initial	0.20	0.00
Volume	23.50	23.50

Average titre = 23.50ml

SAMPLE C

ASSAY

Titration	1	2	3
Final	15.50	32.60	15.70
Initial	0.00	15.50	0.00
Volume used	15.50	17.10	15.70

Average titre = 15.60ml

BLANK

Titration	1	2
Final	23.60	47.40
Initial	0.00	23.60
Volume	23.60	23.80

Average titre = 23.70ml

SAMPLE D

ASSAY

Titration	1	2
Final	16.20	32.50
Initial	0.00	16.20
Volume used	16.20	16.30

Average titre = 16.25ml

BLANK

Titration	1	2
Final	23.80	47.70
Initial	0.00	23.80
Volume	23.80	23.90

Average titre = 23.85ml

SAMPLE E

ASSAY

Titration	1	2	3
Final	17.50	35.70	17.60
Initial	0.00	17.50	0.00
Volume used	17.50	18.20	17.60

Average titre = 17.55ml

BLANK

Titration	1	2
Final	23.80	47.70
Initial	0.00	23.80
Volume	23.80	23.90

Average titre = 22.40ml

PURE FLUCLOXACILLIN POWDER

ASSAY

Titration	1	2
Final	15.70	31.60
Initial	0.00	15.70
Volume used	15.70	15.90

Average titre = 15.80ml

BLANK

Titration	1	2
Final	23.80	47.60
Initial	0.00	23.80
Volume	23.80	23.90

Average titre = 23.80ml

SAMPLE A1

STANDARDIZATION OF 0.02M $Na_2S_2O_3.5H_2O$

Volume of pipette used is 20.00 ml

Titration	1	2
Final	20.30	40.60
Initial	0.00	20.30
Volume used	20.30	20.30

Average titre = 20.30 ml

WEEK 1

Weight of KIO_3 to be taken = 0.7183g
weight taken = 0.1782g

Standardization of 0.02M $Na_2S_2O_3.5H_2O$

Volume of pipette used is 20.00ml

Titration	1	2	3	4
Final	23.10	44.80	26.00	48.20
Initial	0.00	23.10	4.00	26.00
Volume used	23.10	21.70	22.00	22.20

Average titre = 22.10ml

SAMPLE A1

Assay

Titration	1	2	3
Final	15.00	30.00	14.80
Initial	0.00	15.00	0.00
Volume used	15.00	15.00	14.80

Average titre: 14.93ml

ASSAY

Titration	1	2	3
Final	14.50	14.40	17.60
Initial	0.00	0.00	3.10
Volume used	14.50	14.40	14.50

Average titre = 14.67ml

BLANK

Titration	1	2	3
Final	24.20	24.30	24.10
Initial	0.00	0.00	0.00
Volume	24.20	24.30	24.10

Average titre = 24.20ml

Blank

Titration	1	2	3
Final	24.40	48.40	24.50
Initial	0.00	24.40	0.00
Volume used	24.40	24.40	24.50

Average titre: 24.43ml

Sample B

Assay

Titration	1	2	3
Final	16.60	33.20	49.70
Initial	0.00	16.60	33.20
Volume used	16.60	16.60	16.50

Average titre = 16.57

Blank

Titration	1	2	3
Final	23.70	47.20	23.50
Initial	0.00	23.70	0.00
Volume	23.70	23.50	23.50

Average titre = 23.57ml

SAMPLE C

Assay

Titration	1	2	3
Final	39.90	15.90	31.90
Initial	24.00	0.00	15.90
Volume used	15.90	15.90	16.00

Average titre = 15.93ml

Blank

Titration	1	2	3
Final	23.90	47.80	24.00
Initial	0.00	24.00	0.10
Volume	23.90	23.80	23.90

Average titre = 23.87ml

SAMPLE D

Assay

Titration	1	2	3
Final	16.70	33.70	16.90
Initial	0.00	16.70	0.00
Volume used	16.70	17.00	16.90

Average titre = 16.80ml

Blank

Titration	1	2	3
Final	24.00	47.90	23.80
Initial	0.00	24.00	0.00
Volume	24.00	23.90	23.80

Average titre = 23.90ml

SAMPLE E

Assay

Titration	1	2	3
Final	39.60	17.70	35.40
Initial	21.70	0.00	17.70
Volume used	17.90	17.70	17.70

Average titre = 16.57ml

Blank

Titration	1	2	3
Final	21.70	43.60	21.70
Initial	0.00	21.70	0.00
Volume	21.70	21.90	21.70

Average titre = 21.77ml

PURE SAMPLE

Assay

Titration	1	2	3	4	5
Final	39.00	15.90	32.20	16.00	32.10
Initial	23.40	0.00	15.90	0.00	16.00
Volume used	15.60	15.90	16.30	16.00	16.10

Average titre = 16.00ml

Blank

Titration	1	2	3
Final	23.30	46.70	23.40
Initial	0.00	23.30	0.00
Volume	23.30	23.40	23.40

Average titre = 23.37ml

WEEK 4

Standardization of 0.02M $Na_2S_2O_3$
Weight of iodate to be used = 0.1783g
weight of iodate used = 0.1782g

Volume of pipette used is 20.00ml

Titration	1	2	3
final	20.90	41.80	39.40
initial	0.20	20.90	18.80
Volume used	20.70	20.90	20.60

Average titre = 20.65ml

SAMPLE A1

Assay

Titration	1	2	3
Final	15.00	14.90	29.80
Initial	0.00	0.00	14.90
Volume used	15.00	14.90	14.90

Average titre: 14.93ml

Blank

Titration	1	2	3
Final	22.90	45.90	22.90
Initial	0.00	22.90	0.00
Volume used	22.90	23.00	22.90

Average titre: 22.93ml

SAMPLE B

Assay

Titration	1	2	3
Final	33.60	44.90	17.40
Initial	16.40	27.80	0.00
Volume	17.20	17.10	17.40

Average titre = 17.15ml

Blank

Titration	1	2	3
Final	22.30	44.80	22.20
Initial	0.00	22.30	0.00
Volume used	22.30	22.50	22.20

Average titre = 22.25ml

SAMPLE C

Assay

Titration	1	2	3
Final	18.00	34.90	45.90
Initial	1.20	18.00	29.50
Volume used	16.80	16.90	16.40

Average titre = 16.85ml

Blank

Titration	1	2	3
Final	22.00	44.10	33.90
Initial	0.00	22.00	12.00
Volume used	22.00	22.10	21.90

Average titre = 22.00ml

SAMPLE D

Assay

Titration	1	2	3
Final	23.80	40.90	34.80
Initial	6.80	23.80	17.90
Volume used	17.00	17.10	16.90

Average titre = 17.00ml

Blank

Titration	1	2	3
Final	25.50	22.90	46.00
Initial	0.30	0.00	22.90
Volume used	25.20	22.90	23.10

Average titre = 23.00ml

SAMPLE E

Assay

Titration	1	2	3
Final	17.50	17.60	17.50
Initial	0.00	0.00	0.00
Volume used	17.50	17.60	17.50

Average titre: 17.53ml

Blank

Titration	1	2	3
Final	20.20	40.40	20.20
Initial	0.00	20.20	0.00
Volume used	20.20	20.20	20.20

Average titre: 20.20ml

PURE POWDER

Assay

Titration	1	2	3
Final	16.70	33.90	22.50
Initial	0.80	16.70	6.60
Volume used	15.90	17.20	15.90

Average titre = 15.90ml

Blank

Titration	1	2	3
Final	23.70	46.00	29.40
Initial	1.20	23.70	7.00
Volume used	22.50	23.30	22.40

Average titre = 22.40ml

WEEK 8

Standardization of 0.02M $Na_2S_2O_3$

Weight of iodate to be used = 0.1783g
weight of iodate used = 0.1782g

Volume of pipette used = 20.00ml

Titration	1	2	3
Final	20.10	40.10	28.90
Initial	0.10	20.10	8.80
Volume used	20.00	20.00	20.10

Average titre = 20.03ml

SAMPLE A1

Assay

Titration	1	2	3
Final	16.60	32.50	48.30
Initial	0.90	16.60	32.50
Volume used	15.70	15.90	15.80

Average titre = 15.80ml

Blank

Titration	1	2	3
Final	22.80	45.50	23.30
Initial	0.10	22.80	0.20
Volume used	22.70	22.70	23.10

Average titre = 22.70ml

SAMPLE B

Assay

Titration	1	2	3
Final	19.80	39.70	25.40
Initial	0.00	19.80	5.50
Volume used	19.80	19.90	19.90

Average titre = 19.87ml

Blank

Titration	1	2	3
Final	21.50	42.70	24.00
Initial	0.40	21.50	2.90
Volume used	20.90	21.20	21.10

Average titre = 21.15ml

SAMPLE C

Assay

Titration	1	2	3
Final	42.50	21.50	41.30
Initial	22.80	1.70	21.50
Volume used	19.70	19.80	19.80

Average titre = 19.77ml

Blank

Titration	1	2	3
Final	21.80	42.20	22.80
Initial	1.40	21.80	2.40
Volume used	20.40	20.40	20.40

Average titre = 20.40ml

SAMPLE D

Assay

Titration	1	2	3
Final	27.80	47.30	42.20
Initial	8.30	27.80	22.70
Volume	19.50	19.50	19.50

Average titre = 19.50ml

Blank

Titration	1	2	3
Final	21.90	42.90	25.20
Initial	0.70	21.90	4.10
Volume used	21.20	21.00	21.10

Average titre = 21.10ml

SAMPLE E

Assay

Titration	1	2	3
Final	19.80	39.40	21.00
Initial	0.10	19.80	1.30
Volume used	19.70	19.60	19.70

Average titre = 19.67ml

Blank

Titration	1	2	3
Final	20.50	41.10	24.50
Initial	0.10	20.50	4.10
Volume used	20.40	20.60	20.40

Average titre = 20.47ml

PURE POWDER

Assay

Titration	1	2	3
Final	20.20	39.80	31.80
Initial	0.50	20.20	12.30
Volume used	19.70	19.60	19.50

Average titre = 19.60ml

Blank

Titration	1	2	3
Final	21.20	42.50	23.00
Initial	0.00	21.20	1.60
Volume used	21.20	21.30	22.40

Average titre = 21.30ml

WEEK 12

Standardization of 0.02M thiosulphate

Weight of iodate = 0.1783g weight of iodate used = 0.1781g

Volume of pipette used is 20.00ml

Titration	1	2	3
Final	21.30	41.40	33.60
Initial	1.20	21.30	13.50
Volume used	20.10	20.10	20.10

Average titre: 20.10ml

SAMPLE A 1

Assay

Titration	1	2	3
Final	18.20	36.00	30.20
Initial	0.40	18.20	12.40
Volume used	17.80	17.80	17.80

Average titre: 17.80ml

Blank

Titration	1	2	3
Final	23.00	44.60	21.80
Initial	1.30	23.00	0.00
Volume used	21.70	21.60	21.80

Average titre: 21.70ml

SAMPLE B

Assay

Titration	1	2	3
Final	43.30	21.10	41.40
Initial	22.90	0.70	21.10
Volume used	20.40	20.40	20.30

Average titre: 20.37ml

Blank

Titration	1	2	3
Final	23.00	44.60	21.80
Initial	1.30	23.00	0.00
Volume used	21.70	21.60	21.80

Average titre: 21.70ml

SAMPLE C

Assay

Titration	1	2	3
Final	20.60	41.10	48.20
Initial	0.10	20.60	27.80
Volume used	20.50	20.50	20.40

Average titre: 20.47ml

Blank

Titration	1	2	3
Final	22.20	42.70	34.00
Initial	1.80	22.20	13.50
Volume used	20.40	20.50	20.50

Average titre: 20.47ml

SAMPLE D

Assay

Titration	1	2	3
Final	45.50	24.50	45.80
Initial	24.00	3.20	24.50
Volume used	21.50	21.30	21.30

Average titre: 21.37ml

Blank

Titration	1	2	3
Final	22.50	44.00	24.00
Initial	1.10	22.50	2.60
Volume used	21.40	21.50	21.40

Average titre: 21.43ml

SAMPLE E

Titration	1	2	3
Final	20.50	41.00	23.50
Initial	0.20	20.50	3.00
Volume used	20.30	20.50	20.50

Average titre: 20.43ml

Activation

Titration	1	2	3
Final	21.70	42.40	20.90
Initial	0.90	21.70	0.30
Volume used	20.80	20.70	20.60

Average titre: 20.70ml

PURE POWDER

Assay

Titration	1	2	3
Final	20.80	41.20	23.90
Initial	0.00	20.80	3.20
Volume used	20.80	20.40	20.70

Average titre: 20.75ml

Activation

Titration	1	2	3
Final	21.60	42.70	21.60
Initial	0.30	21.60	0.50
Volume used	21.30	21.10	21.10

Average titre: 21.17ml

ASSAY OF 25OMG FLUCLOXACILLIN AND DRIED STARCH MIXTURE

WEEK O

250MG FLUCLOXACILLIN AND 200MG STARCH

Assay

Titration	1	2	3
Final	15.50	31.00	46.60
Initial	0.00	15.50	31.00
Volume used	15.50	15.50	15.60

Average titre = 15.53ml

Blank

Titration	1	2	3
Final	23.10	46.20	42.40
Initial	0.00	23.10	19.70
Volume	23.10	23.10	22.70

Average titre = 23.10ml

250MG FLUCLOXACILLIN AND 100MG STARCH

Assay

Titration	1	2	3
Final	38.60	23.40	39.00
Initial	23.10	7.80	23.40
Volume used	15.50	15.60	15.60

Average titre = 15.57ml

Assay

Titration	1	2	3
Final	23.20	40.20	23.10
Initial	0.00	17.10	0.00
Volume	23.20	23.10	23.10

Average titre = 23.13ml

250MG FLUCLOXACILLIN AND 50MG STARCH

Assay

Titration	1	2	3
Final	15.30	30.50	45.60
Initial	0.00	15.30	30.50
Volume used	15.30	15.20	15.10

Average titre = 16.03ml

Without Assay (blank)

Titration	1	2	3
Final	23.30	46.30	23.10
Initial	0.10	23.30	0.00
Volume	23.20	23.00	23.10

Average titre = 23.10ml

WEEK 1

Prep of 0.02M $Na_2S_2O_3.5H_20$ and standardization

Weight of KIO_3 to be used = 0.1783g
weight used = 0.1782g

Volume of pipette used is 20.00ml

Titration	1	2	3
Final	22.80	44.10	27.40
Initial	1.00	22.80	5.80
Volume used	21.80	21.30	21.60

Average titre = 21.70ml

250mg flucloxacillin + 200mg dried starch

Assay

Titration	1	2	3
Final	16.00	31.90	47.90
Initial	0.10	16.00	31.90
Volume used	15.90	15.90	16.00

Average titre = 15.93ml

250mg flucloxacillin + 100mg dried starch

Assay

Titration	1	2	3
Final	15.70	31.30	46.80
Initial	0.00	15.70	31.30
Volume used	15.70	15.60	15.50

Average titre = 15.60ml

Blank

Titration	1	2	3
Final	22.80	46.00	23.00
Initial	0.00	22.80	0.00
Volume used	22.80	23.20	23.00

Average titre = 23.10ml

250mg flucloxacillin + 50mg starch

Assay

Titration	1	2	3
Final	18.70	34.20	49.70
Initial	3.30	18.70	34.20
Volume used	15.40	15.50	15.50

Average = 15.47ml

Blank

Titration	1	2	3
Final	22.90	46.00	23.00
Initial	0.00	22.90	0.00
Volume used	22.90	23.10	23.00

Average titre: 23.00ml

WEEK 2

Standardization of thiosulphate

Weight of iodate to used = 0.1783g
weight used = 0.1782g

Volume of pipette used = 20.00ml

Titration	1	2	3
Final	22.30	43.00	41.50
Initial	1.90	22.30	20.90
Volume used	20.40	20.70	20.60

Average titre = 20.65ml

250mg flucloxacillin + 200mg dried starch

Assay

Titration	1	2	3
Final	19.40	35.50	20.30
Initial	3.50	19.60	4.40
Volume used	15.90	15.90	15.90

Average titre = 15.90ml

Blank

Titration	1	2	3
Final	25.50	48.20	22.90
Initial	2.90	25.50	2.00
Volume used	22.60	22.70	22.90

Average titre = 22.65ml

250mg flucloxacillin + 100mg dried starch

Assay

Titration	1	2	3
Final	17.20	33.10	49.00
Initial	1.20	17.20	33.10
Volume used	16.00	15.90	15.90

Average titre = 15.93ml

Blank

Titration	1	2	3
Final	22.60	45.20	30.40
Initial	0.00	22.60	7.80
Volume used	22.60	22.60	22.60

Average titre = 22.60ml

250mg flucloxacillin + 50mg dried starch

Assay

Titration	1	2	3
Final	15.80	31.50	47.40
Initial	0.00	15.80	31.50
Volume used	15.80	15.70	15.90

Average titre = 15.80ml

Blank

Titration	1	2	3
Final	22.50	45.40	23.50
Initial	0.00	22.50	1.00
Volume used	22.50	22.90	22.50

Average titre = 22.50ml

WEEK 4

Standardization of thiosulphate

Weight of iodate to used = 0.1783g
weight used = 0.1782g

Volume of pipette used = 20.00ml

Titration	1	2	3
Final	22.30	43.00	41.50
Initial	1.90	22.30	20.90
Volume used	20.40	20.70	20.60

Average titre = 20.65ml

250mg flucloxacillin + 200mg dried starch

Assay

Titration	1	2	3
Final	19.40	35.50	20.30
Initial	3.50	19.60	4.40
Volume used	15.90	15.90	15.90

Average titre = 15.90ml

Blank

Titration	1	2	3
Final	25.50	48.20	22.90
Initial	2.90	25.50	2.00
Volume used	22.60	22.70	22.90

Average titre = 22.65ml

250mg flucloxacillin + 100mg dried starch

Assay

Titration	1	2	3
Final	17.20	33.10	49.00
Initial	1.20	17.20	33.10
Volume used	16.00	15.90	15.90

Average titre = 15.93ml

Blank

Titration	1	2	3
Final	22.60	45.20	30.40
Initial	0.00	22.60	7.80
Volume used	22.60	22.60	22.60

Average titre = 22.60ml

250mg flucloxacillin + 50mg dried starch

Assay

Titration	1	2	3
Final	15.80	31.50	47.40
Initial	0.00	15.80	31.50
Volume used	15.80	15.70	15.90

Average titre = 15.80ml

Blank

Titration	1	2	3
Final	22.50	45.40	23.50
Initial	0.00	22.50	1.00
Volume used	22.50	22.90	22.50

Average titre = 22.50ml

WEEK 8

Weight of iodate to be used = 0.1783g

weight of iodate used = 0.1782g

Standardization of thiosulphate

Volume of pipette used is 20.00ml

Titration	1	2	3
Final	19.80	39.80	19.90
Initial	0.00	19.80	0.00
Volume used	19.80	20.00	19.90

Average titre: 19.90ml

250mg flucloxacillin + 200mg dried starch

Assay

Titration	1	2	3
Final	18.60	36.80	22.60
Initial	0.50	18.60	4.40
Volume used	18.10	18.20	18.10

Average titre: 18.17ml

Blank

Titration	1	2	3
Final	23.30	45.60	22.40
Initial	1.00	23.30	0.10
Volume used	22.30	22.30	22.30

Average titre: 22.30ml

250mg flucloxacillin + 100mg dried starch

Assay

Titration	1	2	3
Final	18.60	37.20	25.90
Initial	0.00	18.60	7.30
Volume used	18.60	18.60	18.60

Average titre: 18.60ml

Blank

Titration	1	2	3
Final	44.50	24.60	46.70
Initial	22.50	2.60	24.60
Volume used	22.00	22.00	22.10

Average titre: 22.03ml

250mg flucloxacillin + 50mg dried starch

Assay

Titration	1	2	3
Final	21.10	39.90	46.00
Initial	2.40	21.10	27.30
Volume used	18.70	18.80	18.70

Average titre: 18.73ml

Blank

Titration	1	2	3
Final	22.40	44.30	25.80
Initial	0.60	22.40	4.00
Volume used	21.80	21.90	21.80

Average titre: 21.83ml

WEEK 12

Standardization of 0.02M thiosulphate

Weight of iodate to be used: 0.1783g
weight used: 0.1781g

Volume of pipette used is 20.00ml

Titration	1	2	3
Final	30.40	41.00	20.00
initial	10.40	20.90	0.00
Volume used	20.00	20.10	20.00

Average titre: 20.05ml

250mg Flucloxacillin and 200mg dried starch

Assay

Titration	1	2	3
Final	20.40	39.80	36.40
initial	1.00	20.40	17.20
Volume used	19.40	19.40	19.20

Average titre: 19.33ml

Blank

Titration	1	2	3
Final	25.60	47.60	26.20
initial	3.60	25.60	4.30
Volume used	22.00	22.00	21.90

Average titre: 21.97ml

250mg Flucloxacillin and 100mg dried starch

Assay

Titration	1	2	3
Final	22.90	42.70	21.90
initial	3.20	22.90	2.10
Volume used	19.70	19.80	19.80

Average titre: 19.77ml

Blank

Titration	1	2	3
Final	21.90	43.50	22.30
initial	0.30	21.90	0.70
Volume used	21.60	21.60	21.60

Average titre: 21.60ml

250mg flucloxacillin and 50mg dried starch

Assay

Titration	1	2	3
Final	22.40	42.80	43.40
initial	2.10	22.40	23.10
Volume used	20.30	20.40	20.30

Average titre: 20.33ml

Blank

Titration	1	2	3
Final	22.80	44.20	25.90
initial	1.40	22.80	4.70
Volume used	21.40	21.40	22.20

Average titre: 21.67ml

ASSAY OF 250MG FLUCLOXACILLIN AND UNDRIED STARCH MIXTURE

WEEK 0

Standardization of 0.02M $Na_2S_2O_3.5H_2O$

Weight of KIO_3 used = 0.1783g weight taken = 0.1782g

Volume of pipette used is 20.00ml

Titration	1	2	3
Final	20.40	40.60	37.90
Initial	0.10	20.40	17.80
Volume used	20.30	20.20	20.10

Average titre = 20.20ml

250mg flucloxacillin + 200mg undried starch

Assay

Titration	1	2	3
Final	37.70	14.70	29.60
Initial	22.80	0.00	14.70
Volume used	14.90	14.70	14.90

Average titre: 14.83ml

Blank

Titration	1	2	3
Final	23.20	45.90	22.80
Initial	0.30	23.20	0.00
Volume used	22.90	22.70	22.80

Average titre = 22.80ml

250mg flucloxacillin + 100mg undried starch

Assay

Titration	1	2	3
Final	14.70	29.40	44.20
Initial	0.00	14.70	29.40
Vol. used	14.70	14.70	14.80

Average titre: 14.73ml

Blank

Titration	1	2	3
Final	18.70	41.70	22.80
Initial	0.00	18.70	0.00
Volume used	18.70	23.00	22.80

Average titre: 22.90ml

250mg flucloxacillin + 50mg undried starch

Assay

Titration	1	2	3
Final	14.70	43.90	29.20
Initial	0.00	29.40	14.70
Volume used	14.70	14.50	14.50

Average titre: 14.56ml

Blank

Titration	1	2	3
Final	23.60	46.40	22.70
Initial	0.70	23.60	0.00
Volume used	22.90	22.80	22.70

Average titre: 22.80ml

WEEK 1

Standardization of thiosulphate

Weight of iodate to be used = 0.1783g
weight used = 0.1782g

Volume of pipette used is 20.00ml

Titration	1	2	3
Final	20.80	41.80	20.80
Initial	0.10	20.80	0.00
Volume used	20.70	21.00	20.80

Average titre = 20.75ml

250mg flucloxacillin and 200mg undried starch combination

Assay

Titration	1	2	3
Final	17.40	33.20	49.00
Initial	1.40	17.40	33.20
Volume used	16.00	15.80	15.80

Average titre = 15.87ml

Blank

Titration	1	2	3
Final	23.70	47.20	23.30
Initial	0.00	23.70	0.00
Volume used	23.70	23.50	23.30

Average titre = 23.40ml

250mg flucloxacillin and 100mg undried starch combination

Assay

Titration	1	2	3
Final	15.80	31.60	47.30
Initial	0.00	15.80	31.60
Volume used	15.80	15.80	15.70

Average titre = 15.77ml

Blank

Titration	1	2	3
Final	23.40	46.80	23.30
Initial	0.00	23.40	0.00
Volume used	23.40	23.40	23.30

Average titre = 23.37ml

250mg flucloxacillin and 50mg undried starch

Assay

Titration	1	2	3
Final	15.70	31.40	47.30
Initial	0.00	15.70	31.40
Volume used	15.70	15.70	15.90

Average titre = 15.77ml

Blank

Titration	1	2	3
Final	23.40	46.90	24.20
Initial	0.10	23.30	0.90
Volume used	23.30	23.60	23.30

Average titre = 23.30ml

WEEK 2

Standardization of thiosulphate

Weight of iodate to be used = 0.1783g
weight used = 0.1783g

Volume of pipette used is 20.00ml

Titration	1	2	3
Final	21.70	42.10	46.20
Initial	1.70	21.70	25.70
Volume used	20.00	20.40	20.50

Average titre = 20.45ml

250mg flucloxacillin and 200mg undried starch

Assay

Titration	1	2	3
Final	17.10	33.30	17.30
Initial	0.30	17.10	1.20
Vol. used	16.80	16.20	16.10

Average titre = 16.15ml

Blank

Titration	1	2	3
Final	24.60	48.10	26.20
Initial	1.30	24.60	3.10
Vol. used	23.30	23.50	23.10

Average titre = 23.20ml

250mg flucloxacillin and 100mg undried starch

Assay

Titration	1	2	3
Final	16.90	33.00	49.00
Initial	0.50	16.90	33.00
Vol. used	16.40	16.10	16.00

Average titre = 16.05ml

Blank

Titration	1	2	3
Final	40.90	25.60	26.50
Initial	17.60	0.80	3.40
Vol. used	23.30	24.80	23.10

Average titre = 23.20ml

250mg flucloxacillin and 50mg undried starch

Assay

Titration	1	2	3
Final	20.70	36.70	43.40
Initial	4.70	20.70	27.40
Vol. used	16.00	16.00	16.00

Average titre = 16.00ml

Blank

Titration	1	2	3
Final	24.30	47.50	24.50
Initial	0.90	24.30	1.00
Volume used	23.40	23.20	23.50

Average titre = 23.30ml

WEEK 4

Weight of iodate to be used = 0.1783g
weight of iodate used = 0.1783g

Weight of thiosulphate used = 4.9636

Standardization of thiosulphate

Volume of pipette used is 20.00ml

Titration	1	2	3
Final	20.60	41.10	44.00
Initial	0.20	20.60	23.40
Volume	20.40	20.50	20.60

Average titre = 20.50ml

250mg flucloxacillin + 200mg undried starch

Assay

Titration	1	2	3
Final	17.90	35.00	47.50
Initial	0.60	17.90	30.50
Volume	17.30	17.10	17.00

Average titre = 17.05ml

Blank

Titration	1	2	3
Final	24.70	48.00	46.30
Initial	1.60	24.70	23.10
Volume	23.10	23.30	23.20

Average titre = 23.20ml

250mg flucloxacillin + 100mg undried starch.

Assay

Titration	1	2	3
Final	18.60	35.40	48.10
Initial	1.90	18.60	31.30
Volume	16.70	16.80	16.80

Average titre = 16.77ml

Blank

Titration	1	2	3
Final	23.20	46.30	46.30
Initial	0.20	23.20	23.50
Volume	23.00	23.10	22.80

Average titre = 23.05ml

250mg flucloxacillin + 50mg undried starch

Assay

Titration	1	2	3
Final	18.10	34.90	48.70
Initial	1.20	18.10	32.10
Volume	16.90	16.80	16.60

Average titre = 16.85ml

Blank

Titration	1	2	3
Final	24.00	46.90	23.40
Initial	1.10	24.00	0.60
Volume	22.90	22.90	22.80

Average titre = 22.87ml

WEEK 8

Weight of iodate to be used = 0.1783g
weight of iodate used = 0.1782g

Weight of thiosulphate used = 4.9637g

Standardization of thiosulphate

Volume of pipette used is 20.00ml

Titration	1	2	3
Final	19.80	39.80	19.90
Initial	0.00	19.80	0.00
Volume used	19.80	20.00	19.90

Average titre: 19.90ml

250mg flucloxacillin + 200mg undried starch

Assay

Titration	1	2	3
Final	17.90	35.50	26.80
Initial	0.20	17.90	9.30
Volume used	17.70	17.60	17.50

Average titre: 17.60ml

Blank

Titration	1	2	3
Final	22.00	44.10	24.30
Initial	0.00	22.00	2.10
Volume used	22.00	22.10	22.20

Average titre: 22.10ml

250mg flucloxacillin + 100mg undried starch

Assay

Titration	1	2	3
Final	18.00	35.80	22.20
Initial	0.10	18.00	4.30
Volume used	17.90	17.80	17.90

Average titre: 17.87ml

Blank

Titration	1	2	3
Final	23.60	45.60	25.60
Initial	1.60	23.60	3.70
Volume used	22.00	22.00	21.90

Average titre: 21.97ml

250mg flucloxacillin + 50mg undried starch

Assay

Titration	1	2	3
Final	18.80	36.70	39.10
Initial	0.90	18.80	21.30
Volume used	17.90	17.90	17.80

Average titre: 17.87ml

Blank

Titration	1	2	3
Final	21.70	43.10	21.50
Initial	0.30	21.70	0.00
Volume used	21.40	21.40	21.50

Average titre: 21.43ml

WEEK 12

Standardization of 0.02M thiosulphate

Weight of iodate to be used: 0.1783g
weight used: 0.1781g

Volume of pipette used is 20.00ml

Titration	1	2	3
Final	30.40	41.00	20.00
initial	10.40	20.90	0.00
Volume used	20.00	20.10	20.00

Average titre: 20.05ml

250mg Flucloxacillin and 200mg undried starch

Assay

Titration	1	2	3
Final	20.30	39.30	34.40
initial	1.30	20.30	15.30
Volume used	19.00	19.00	19.10

Average titre: 19.03ml

Blank

Titration	1	2	3
Final	21.90	44.00	25.90
initial	0.00	21.90	3.80
Volume used	21.90	22.10	22.10

Average titre: 22.03ml

250mg Flucloxacillin and 100mg undried starch

Assay

Titration	1	2	3
Final	20.50	40.10	21.70
initial	0.90	20.50	2.10
Volume used	19.60	19.60	19.60

Average titre: 19.60ml

Blank

Titration	1	2	3
Final	24.30	46.00	26.80
initial	2.50	24.30	5.00
Volume used	21.80	21.70	21.80

Average titre: 21.77ml

250mg flucloxacillin and 50mg undried starch

Assay

Titration	1	2	3
Final	20.70	40.80	29.60
initial	0.70	20.70	9.50
Volume used	20.00	20.10	20.10

Average titre: 20.07ml

Blank

Titration	1	2	3
Final	43.30	22.60	44.10
initial	21.60	1.10	22.60
Volume used	21.70	21.50	21.50

Average titre: 21.57ml

ASSAY OF 250MG FLUCLOXACILLIN AND SODIUM CMC MIXTURES

WEEK 0

Standardization of thiosulphate solution

Weight of iodate to be taken = 0.1783g
weight of iodine taken = 0.1782g

Volume of pipette used is 20.00ml

Titration	1	2	3
Final	20.80	41.80	20.80
Initial	0.10	20.80	0.00
Volume used	20.70	21.00	20.80

Average titre = 20.75ml

250mg flucloxacillin + 200mg sodium CMC

Assay

Titration	1	2	3
Final	15.80	31.40	47.10
Initial	0.00	15.80	31.40
Vol. used	15.80	15.60	15.70

Average titre = 15.70ml

Blank

Titration	1	2	3
Final	23.40	47.20	23.90
Initial	0.00	23.40	0.10
Vol. used	23.40	23.80	23.80

Average titre = 23.80ml

250mg flucloxacillin + 100mg sodium CMC

Assay

Titration	1	2	3
Final	15.10	30.20	45.40
Initial	0.00	15.10	30.20
Vol. used	15.10	15.10	15.20

Average titre = 15.13ml

Blank

Titration	1	2	3
Final	23.40	46.80	23.90
Initial	0.00	23.40	0.40
Vol. used	23.40	23.40	23.50

Average titre = 23.43ml

250mg flucloxacillin + 50mg sodium CMC

Assay

Titration	1	2	3
Final	16.30	31.80	47.30
Initial	0.90	16.30	31.80
Volume used	15.40	15.50	15.50

Average titre = 15.47ml

Blank

Titration	1	2	3
Final	23.60	47.30	23.80
Initial	0.00	23.60	0.00
Vol. used	23.60	23.70	23.30

Average titre = 23.65ml

WEEK 1

Standardization of thiosulphate

Weight of iodate to be used = 0.1783g
weight of iodate used = 0.1783g

Volume of pipette used is 20.00ml

Titration	1	2	3
Final	21.00	41.80	41.60
Initial	0.20	21.00	21.00
Volume used	20.80	20.80	20.60

Average titre = 20.73ml

250mg flucloxacillin + 200mg sodium CMC

Assay

Titration	1	2	3
Final	16.30	31.60	46.80
Initial	1.00	16.30	31.60
Volume used	15.30	15.30	15.20

Average titre = 15.27ml

Blank

Titration	1	2	3
Final	23.00	45.60	25.60
Initial	0.40	23.00	2.90
Volume used	22.60	22.60	22.70

Average titre = 22.63ml

250mg flucloxacillin + 100mg sodium CMC

Assay

Titration	1	2	3
Final	17.00	32.30	47.40
Initial	1.80	17.00	32.30
Vol. used	15.20	15.30	15.10

Average titre = 15.20ml

Blank

Titration	1	2	3
Final	25.30	48.00	40.10
Initial	2.70	25.30	17.70
Volume used	22.60	22.70	22.40

Average titre = 22.65ml

250mg flucloxacillin + 50mg sodium CMC

Assay

Titration	1	2	3
Final	15.10	30.20	45.20
Initial	0.40	15.10	30.20
Volume used	14.70	15.10	15.00

Average titre = 15.05ml

Blank

Titration	1	2	3
Final	22.40	45.40	27.50
Initial	0.00	22.40	5.10
Volume used	22.40	23.00	22.40

Average titre = 22.40

WEEK 2

Weight of iodate to be used = 0.1783g
weight of iodate used = 0.1783g

Weight of thiosulphate used = 4.9635

Standardization of thiosulphate

Volume of pipette used is 20.00ml

Titration	1	2	3
Final	20.60	41.30	45.60
Initial	0.00	20.60	4.00
Volume	20.60	20.70	20.70

Average titre = 20.67ml

250mg flucloxacillin + 200mg sodium CMC

Assay

Titration	1	2	3
Final	17.60	34.20	49.10
Initial	1.10	17.60	32.60
Volume	16.50	16.60	16.50

Average titre = 16.53ml

Blank

Titration	1	2	3
Final	23.50	47.10	44.10
Initial	0.00	23.50	20.60
Volume	23.50	23.60	23.50

Average titre = 23.53ml

250mg flucloxacillin + 100mg sodium CMC

Assay

Titration	1	2	3
Final	40.60	21.50	38.30
Initial	23.70	4.30	21.50
Volume	16.90	17.20	16.80

Average titre = 16.85ml

Blank

Titration	1	2	3
Final	23.30	46.60	23.70
Initial	0.10	23.30	0.10
Volume	23.20	23.30	23.60

Average titre = 23.25ml

250mg flucloxacillin + 50mg sodium CMC

Assay

Titration	1	2	3
Final	17.40	34.10	48.00
Initial	1.00	17.40	31.80
Volume	16.40	16.70	16.20

Average titre = 16.30ml

Blank

Titration	1	2	3
Final	23.80	46.90	26.00
Initial	0.50	23.80	2.80
Volume	23.30	23.10	23.20

Average titre = 23.20ml

WEEK 4

Standardization of 0.02M thiosulphate

Weight of iodate to be used = 0.1783g
weight of iodate used = 0.1782g

Volume of pipette used = 20.00ml

Titration	1	2	3
Final	20.10	40.10	31.20
Initial	0.20	20.10	11.20
Volume used	19.90	20.00	20.00

Average titre = 19.97 ml

250 mg flucloxacillin and 100mg Na CMC

Assay

Titration	1	2	3
Final	26.60	43.50	37.40
Initial	9.50	26.60	20.30
Volume used	17.10	16.90	17.10

Average titre = 17.03ml

Blank

Titration	1	2	3
Final	23.10	45.50	46.70
Initial	0.80	23.10	24.50
Volume used	22.30	22.40	22.20

Average titre = 22.30ml

250mg Flucloxacillin and 200mg Na CMC

Assay

Titration	1	2	3
Final	17.80	34.60	47.90
Initial	0.90	17.80	31.30
Volume used	16.90	16.80	16.60

Average titre = 16.85ml

Blank

Titration	1	2	3
Final	22.80	45.20	27.30
Initial	0.40	22.80	4.90
Volume used	22.40	22.40	22.40

Average titre = 22.40

250mg flucloxacillin and 50mg Na CMC

Assay

Titration	1	2	3
Final	16.70	33.30	41.40
Initial	0.00	16.70	24.70
Volume used	16.70	16.60	16.70

Average titre = 16.67ml

Activation

Titration	1	2	3
Final	22.30	44.00	24.60
Initial	0.50	22.30	2.80
Volume used	21.80	21.70	21.80

Average titre = 21.77ml

WEEK 8

Standardization of thiosulphate solution

Weight of iodate to be taken = 0.1783g
weight of iodate taken = 0.1781g

Volume of pipette used is 20.00ml

Titration	1	2	3
Final	20.10	40.10	22.80
Initial	0.00	20.10	2.70
Volume used	20.10	20.00	20.10

Average titre: 20.07ml

250mg Flucloxacillin and 200mg Na CMC

Assay

Titration	1	2	3
Final	19.40	38.00	19.70
Initial	0.80	19.40	1.10
Volume used	18.60	18.60	18.60

Average titre: 18.60ml

Blank

Titration	1	2	3
Final	23.40	45.40	25.30
Initial	1.40	23.40	3.30
Volume used	22.00	22.00	22.00

Average titre: 22.00ml

250mg Flucloxacillin and 100mg NaCMC

Assay

Titration	1	2	3
Final	19.60	38.60	24.00
Initial	0.30	19.60	4.80
Volume used	19.30	19.00	19.20

Average titre: 19.25ml

Blank

Titration	1	2	3
Final	21.80	43.40	22.00
Initial	0.00	21.80	0.30
Volume used	21.80	21.60	21.70

Average titre: 21.70ml

250mg Flucloxacillin and 50mg Na CMC

Assay

Titration	1	2	3
Final	20.80	40.50	48.40
Initial	1.00	20.80	28.80
Volume used	19.80	19.70	19.60

Average titre: 19.70ml

Blank

Titration	1	2	3
Final	21.90	43.50	21.70
Initial	0.10	21.90	0.10
Vol used	21.80	21.60	21.60

Average titre: 21.67ml

WEEK 12

Standardization of 0.02M thiosulphate

Weight of iodate to be used: 0.1783g
weight used: 0.1782g

Volume of pipette used is 20.00ml

Titration	1	2	3
Final	19.90	39.90	21.90
initial	0.00	19.90	1.80
Volume used	19.90	20.00	20.10

Average titre: 20.00ml

250mg Flucloxacillin and 200mg sodium CMC

Assay

Titration	1	2	3
Final	23.30	43.60	23.60
initial	3.00	23.30	3.40
Volume used	20.30	20.30	23.20

Average titre: 21.27ml

Blank

Titration	1	2	3
Final	23.20	44.70	31.50
initial	2.10	23.20	10.00
Volume used	21.10	21.50	21.50

Average titre: 21.50ml

250mg Flucloxacillin and 100mg Na CMC

Assay

Titration	1	2	3
Final	22.90	43.80	23.20
initial	1.90	22.90	2.30
Vol. used	21.00	20.90	20.90

Average titre: 20.93ml

Blank

Titration	1	2	3
Final	22.20	43.70	26.70
initial	0.70	22.20	5.30
Volume used	21.50	21.50	21.40

Average titre: 21.47ml

250mg flucloxacillin and 50mg sodium CMC

Assay

Titration	1	2	3
Final	25.40	46.40	27.20
initial	4.40	25.40	6.00
Volume used	21.00	21.00	21.20

Average titre: 21.07ml

Blank

Titration	1	2	3
Final	21.70	43.00	23.30
initial	0.30	21.70	1.90
Volume used	21.40	21.30	21.40

Average titre: 21.37ml

Sample calculations for assay of flucloxacillin

Sample A1

Week 0

Weight of powder in 20 caps

= 9.0487g – 1.5103g

= 7.5384g

Average weight of powder in a cap

$$= \frac{7.5384}{20}$$

= 0.3769g

0.2500g of flucloxacillin = 0.3769

0.1000g of flucloxacillin = $\frac{0.100g \times 0.3769g}{0.2500g}$

= 0.1508g

Weight used for the assay was **0.1508g**

STANDARDIZATION OF 0.02M $Na_2S_2O_3.5H_2O$

Volume of pipette used is 20.00 ml

Titration	1	2
Final	20.30	40.60
Initial	0.00	20.30
Volume used	20.30	20.30

Average titre = 20.30 ml

ASSAY

Titration	1	2	3
Final	14.50	14.40	17.60
Initial	0.00	0.00	3.10
Volume used	14.50	14.40	14.50

Average titre = 14.67ml

BLANK

Titration	1	2	3
Final	24.20	24.30	24.10
Initial	0.00	0.00	0.00
Volume	24.20	24.30	24.10

Average titre = 24.20ml

Factor of KIO_3 = actual weight / nominal weight

$$= \frac{0.1785}{0.1784}$$

$$= 1.0006$$

Factor of $Na_2S_2O_3.5H_2O$

$$= \frac{\text{Factor of } KIO_3 \times \text{volume of } KIO_3}{\text{Volume of } Na_2S_2O_3.5H_2O}$$

$$= \frac{1.0006 \times 20.00ml}{20.30ml} = 0.9858$$

Hence factor of $Na_2S_2O_3.5H_2O$ is **0.9858**

Volume of thiosulphate consumed in assay = blank – assay

=24.20ml – 14.67ml

= **9.53ml**

Actual volume of thiosulphate = 9.53ml × factor of thiosulphate

= 9.53ml × 0.9858

= **9.39ml**

The volume of thiosulphate equivalent to the volume of iodine that reacted with flucloxacillin is 9.39ml.

Weight of flucloxacillin present in 0.1508g used to prepare 100ml was 0.1000g

Hence the 10ml aliquots used would contain 0.0100g of flucloxacillin.

From milliequivalent the amount of active drug present was:

$$= \frac{9.39ml \times 0.00123g \text{ of flucloxacillin}}{1ml}$$

$$= 0.0115497g \approx 0.0115g$$

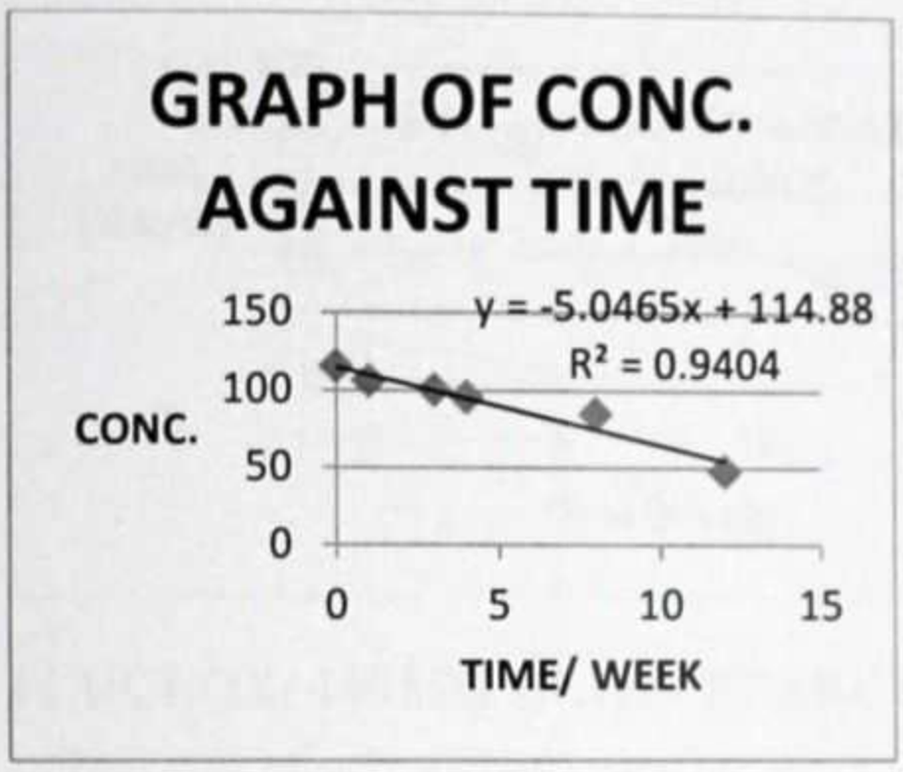
Percentage content = $\frac{0.0115g}{0.0100g} \times 100\%$

= 115.0 % w/w

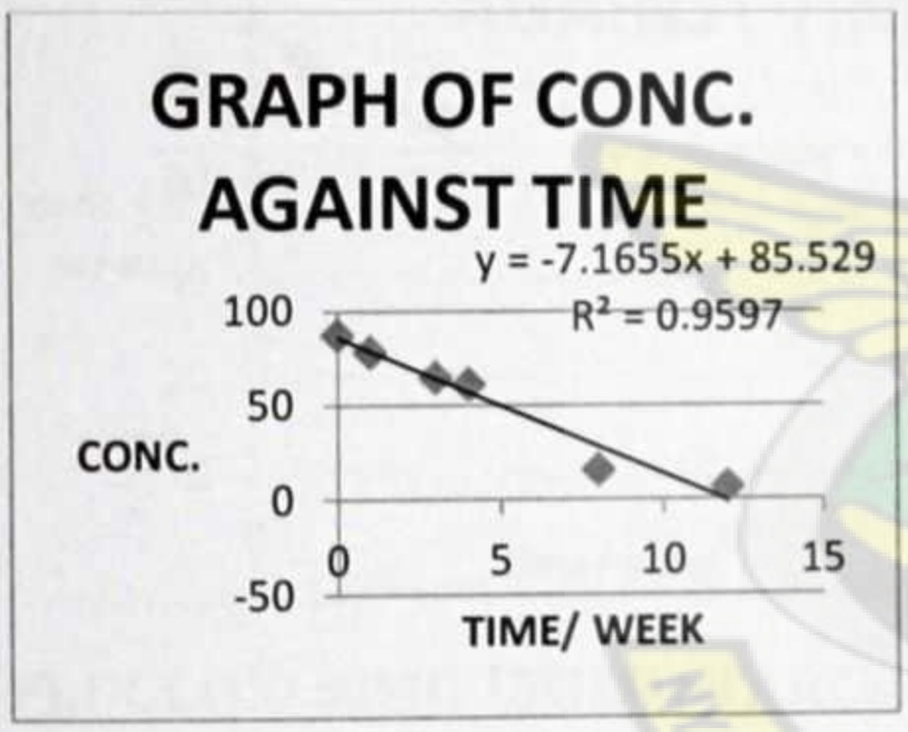
ORDER GRAPHS

ZERO ORDER GRAPH

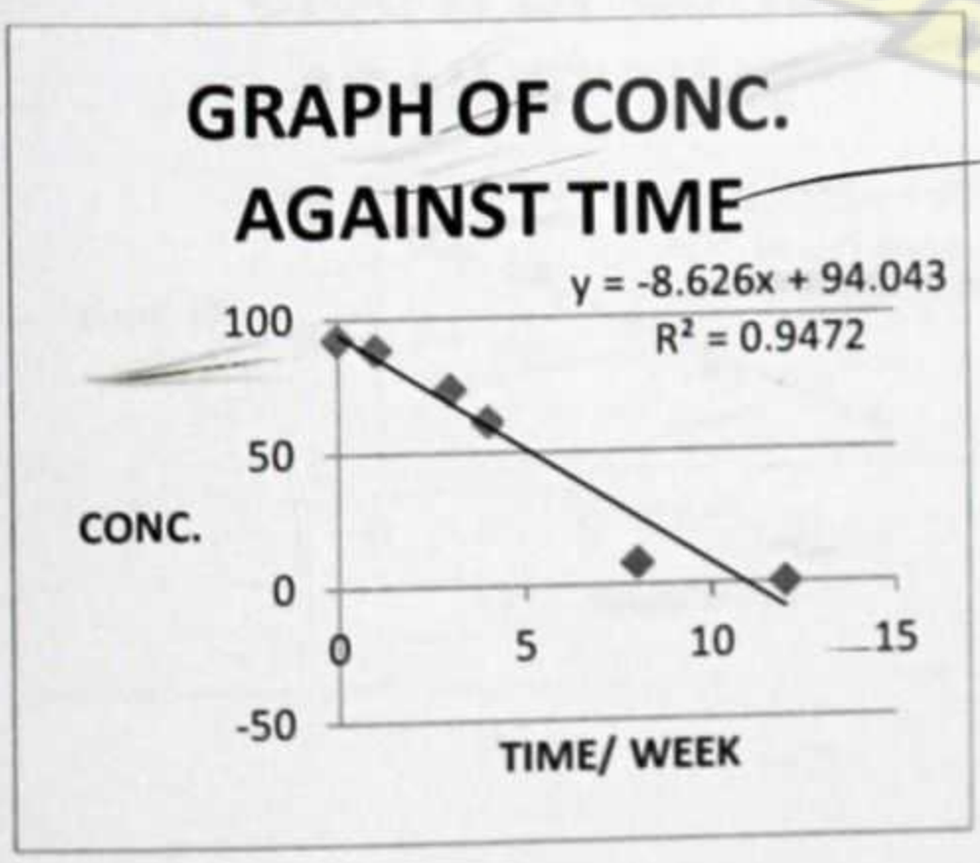
SAMPLE A1



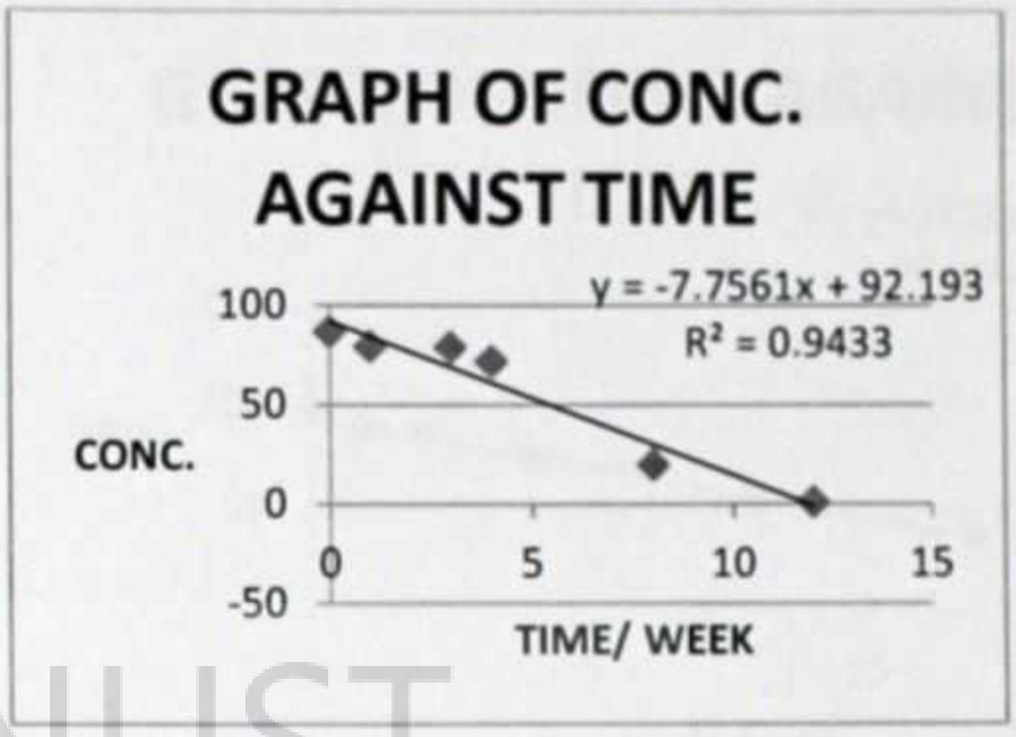
SAMPLE B



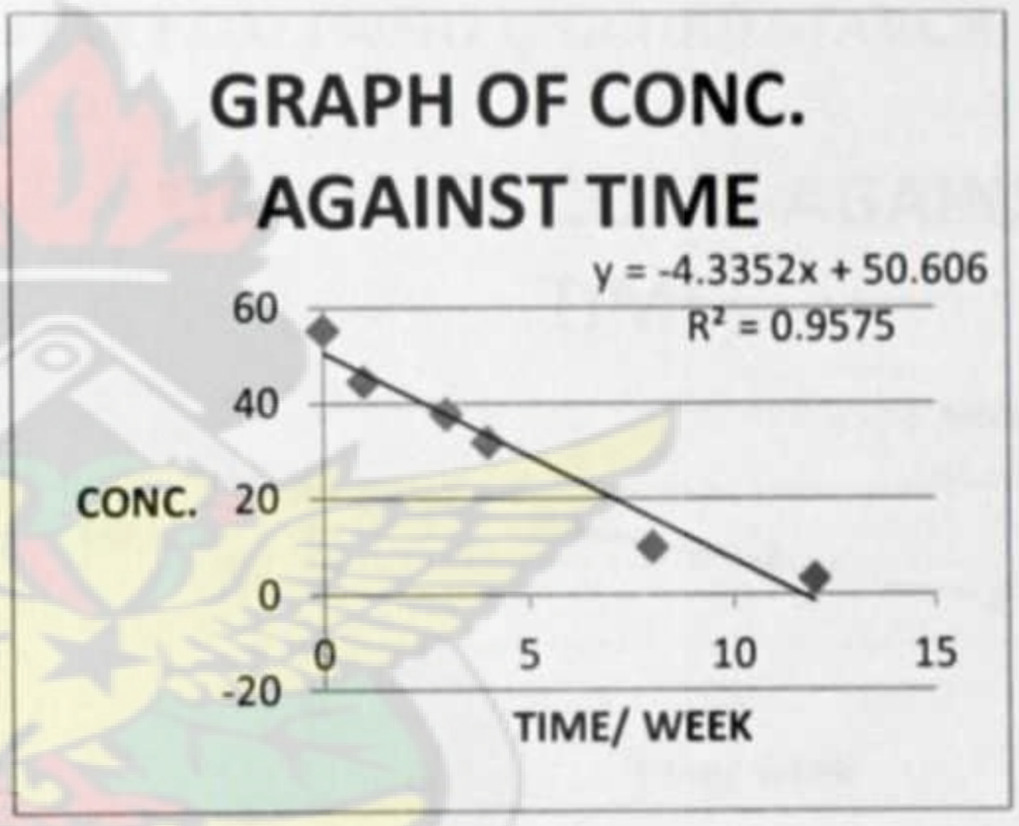
SAMPLE C



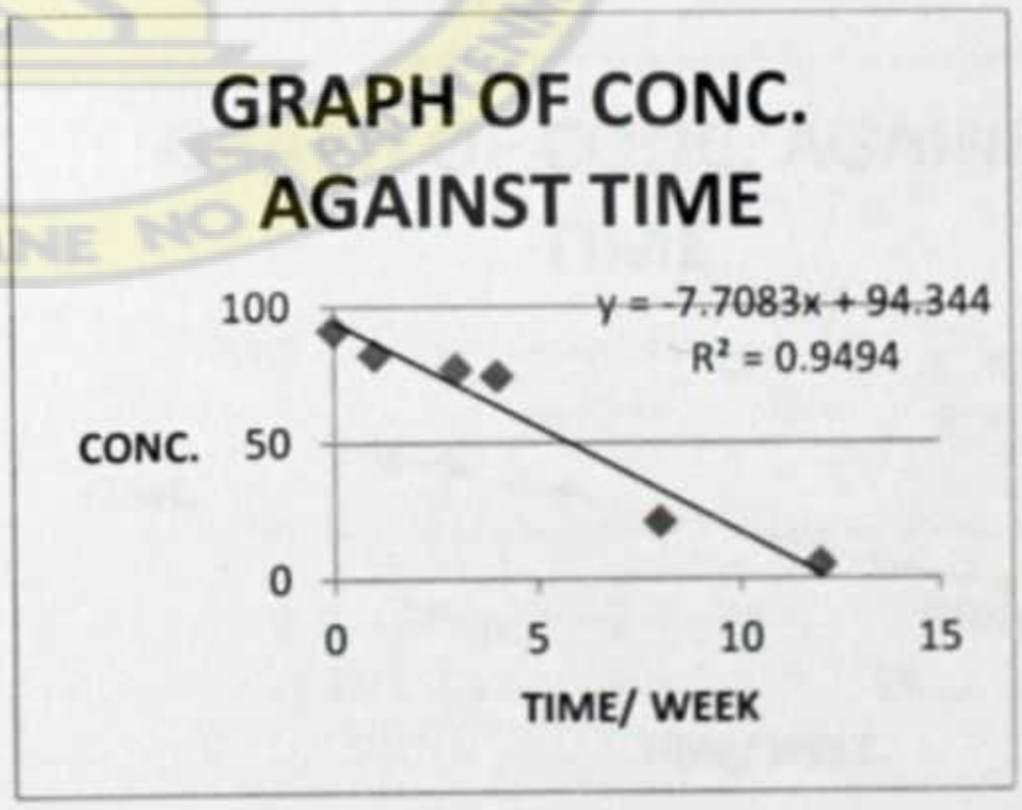
SAMPLE D



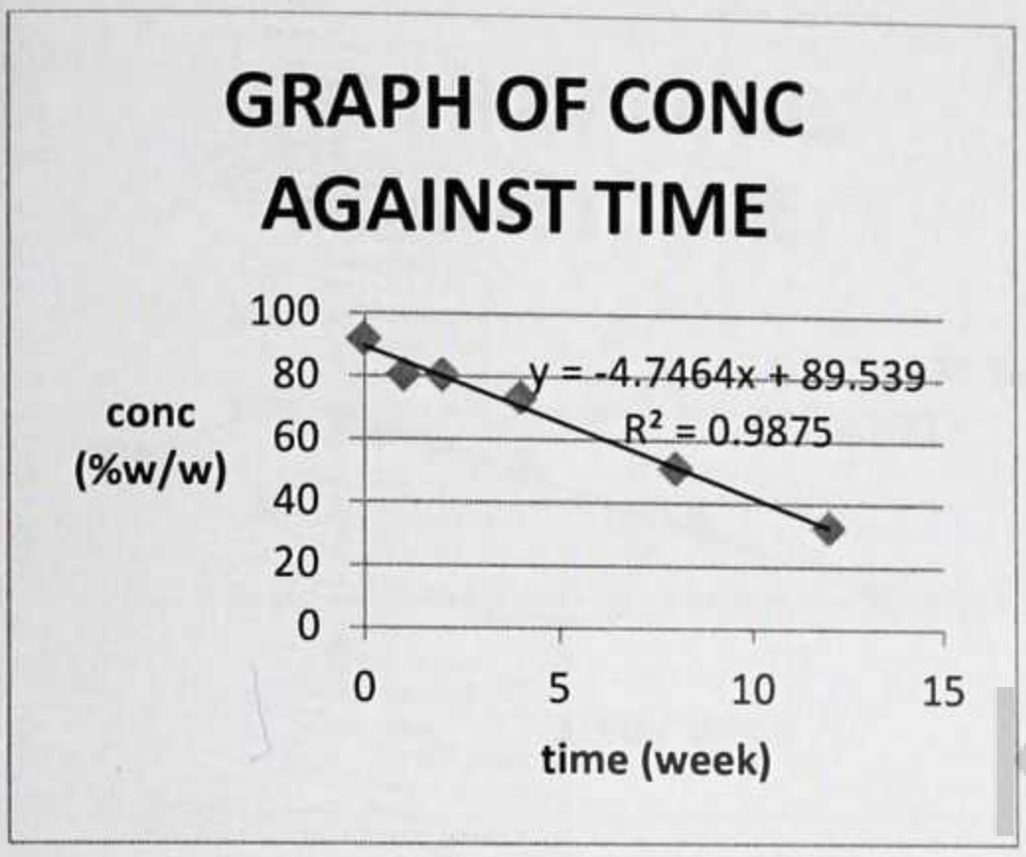
SAMPLE E



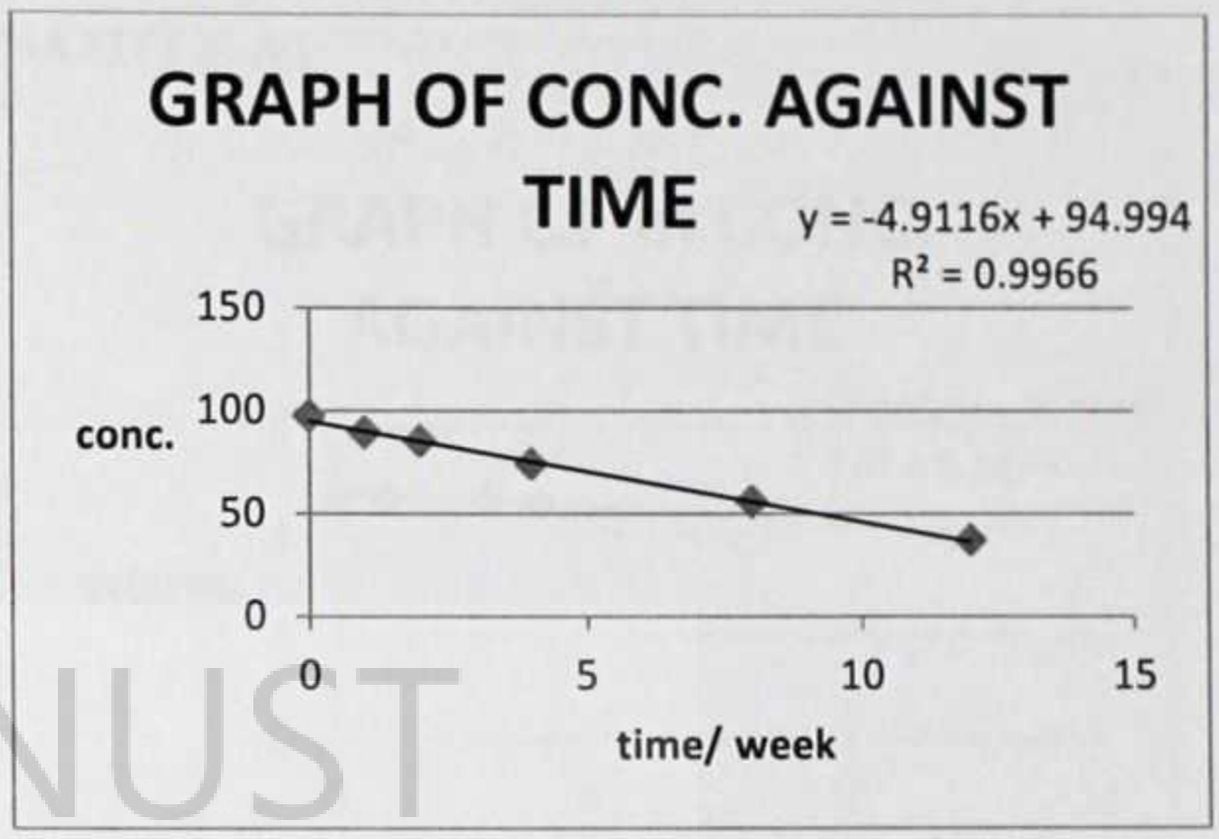
PURE POWDER



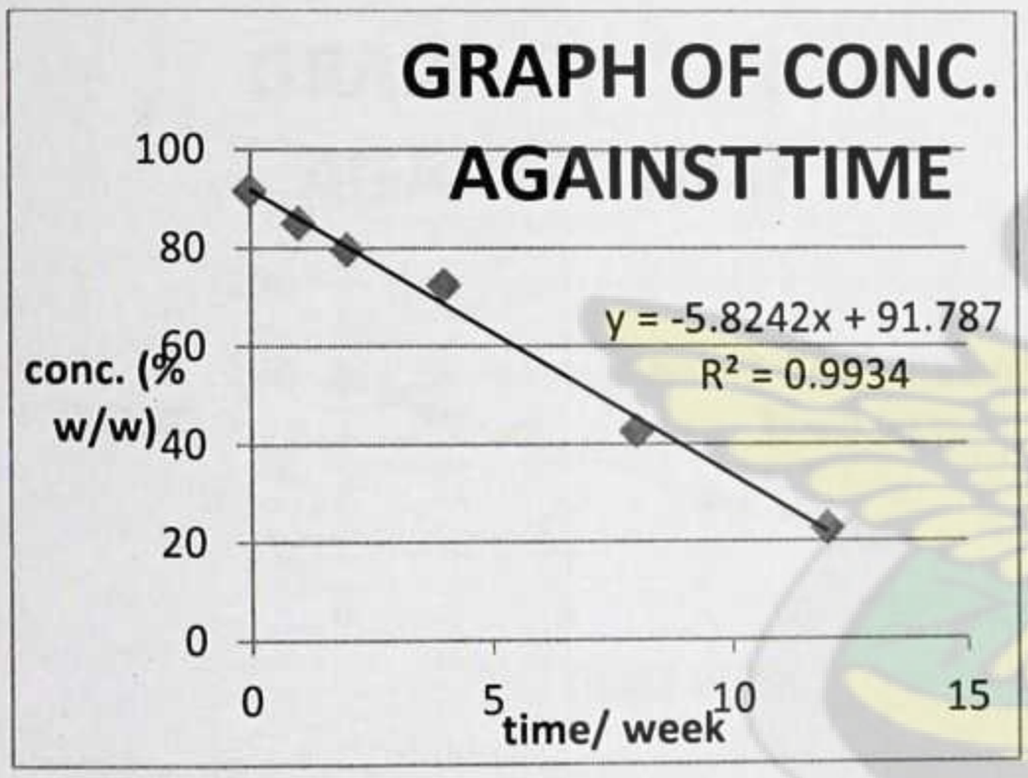
FLUCLOX/ 200MG DRIED STARCH



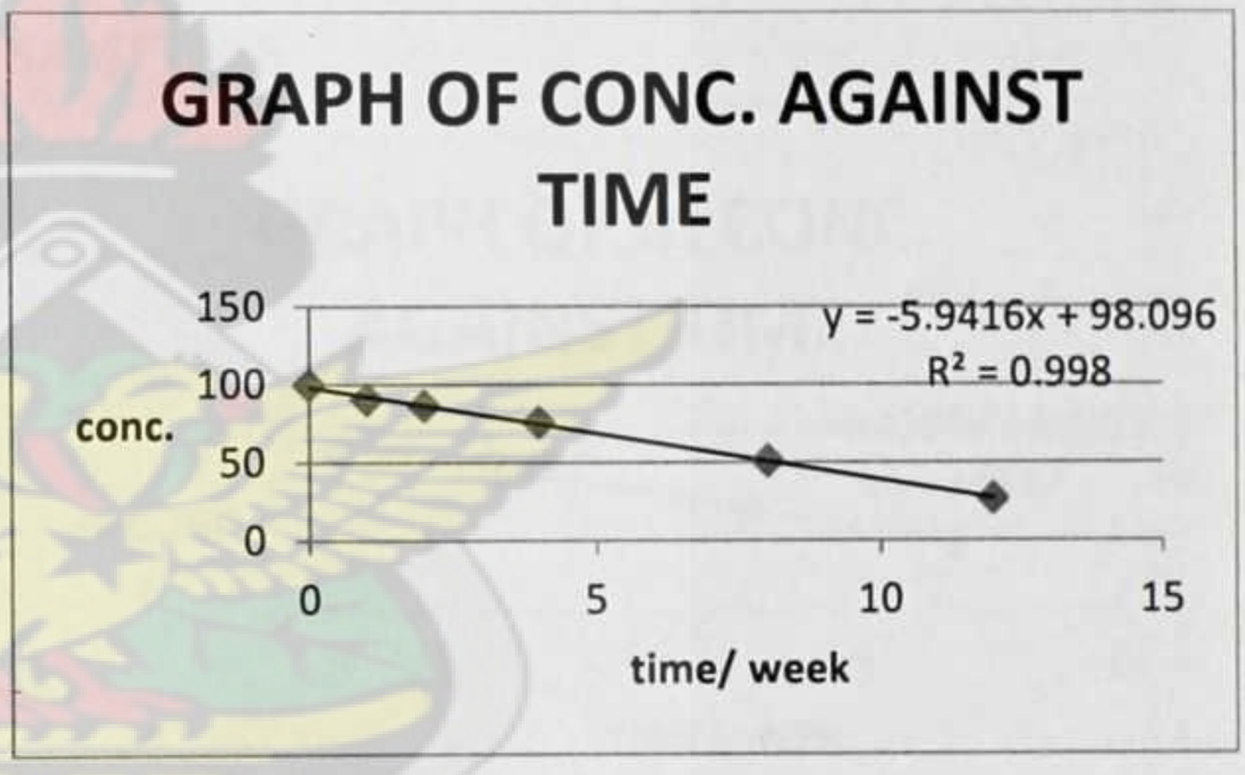
FLUCLOX/ 200MG UNDRIED STARCH



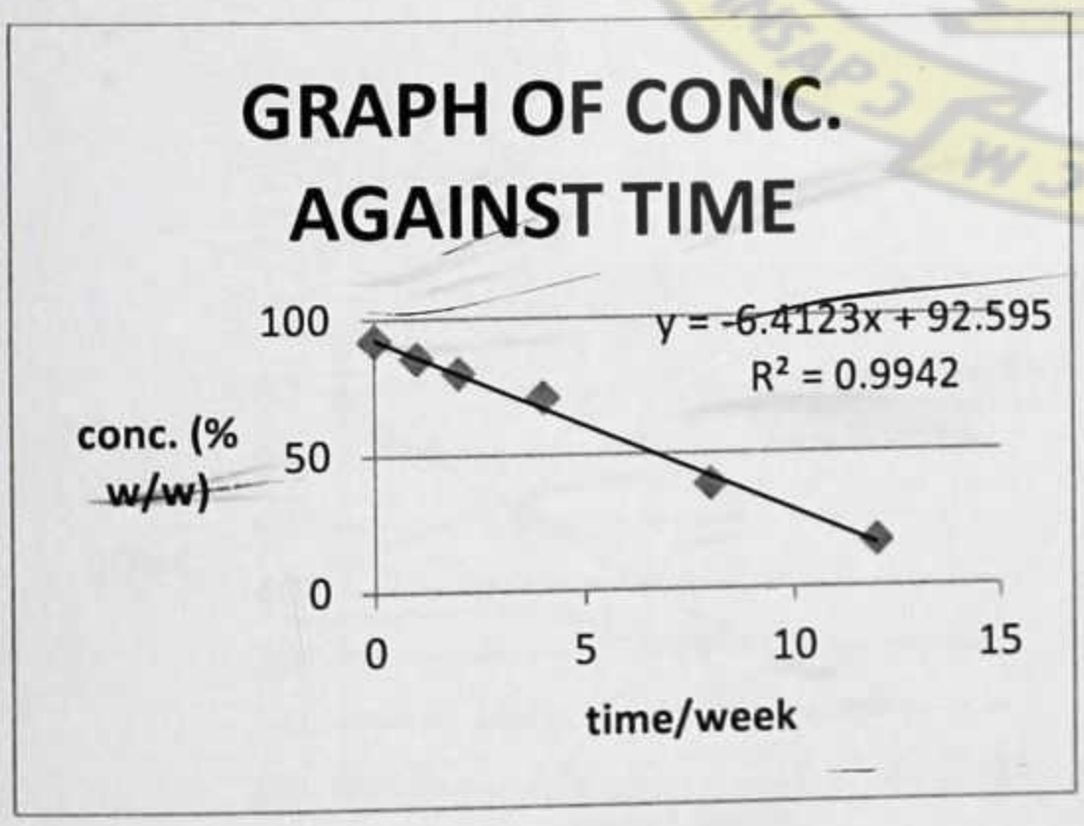
FLUCLOX/ 100MG DRIED STARCH



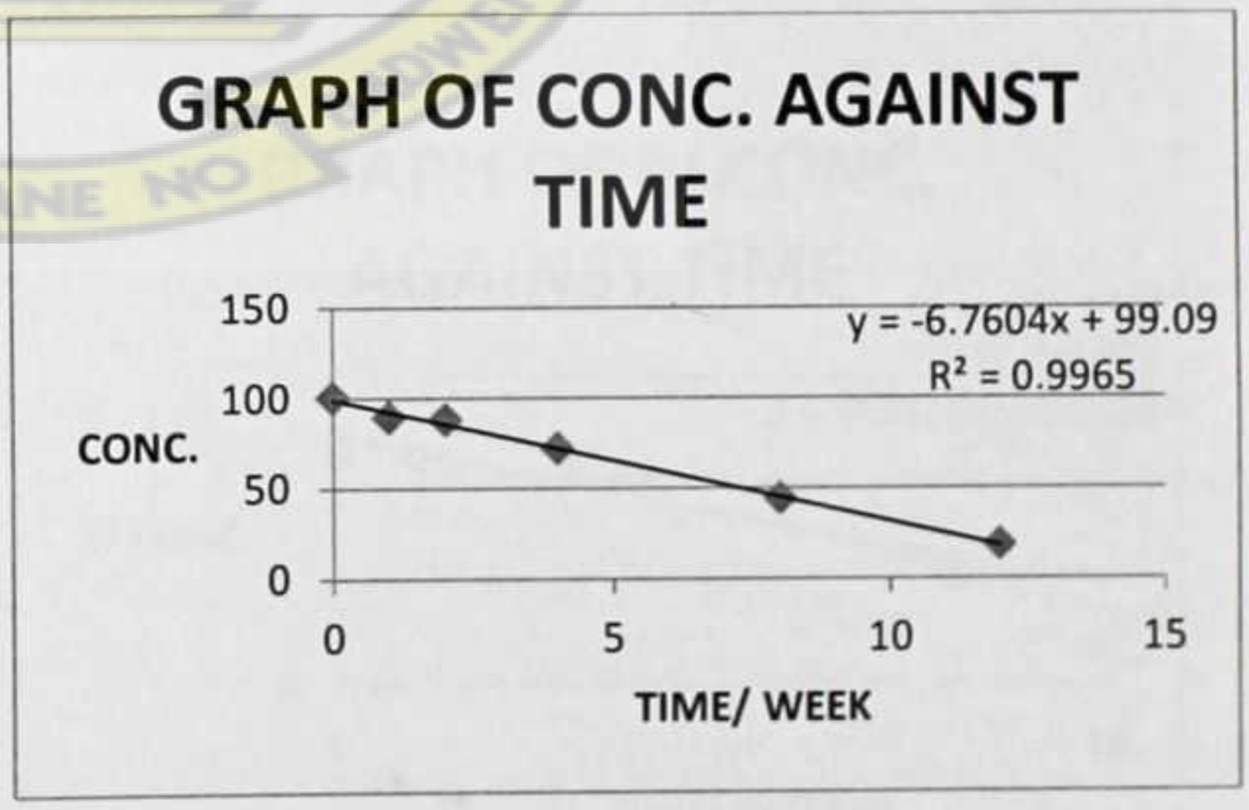
FLUCLOX/ 100MG UNDRIED STARCH



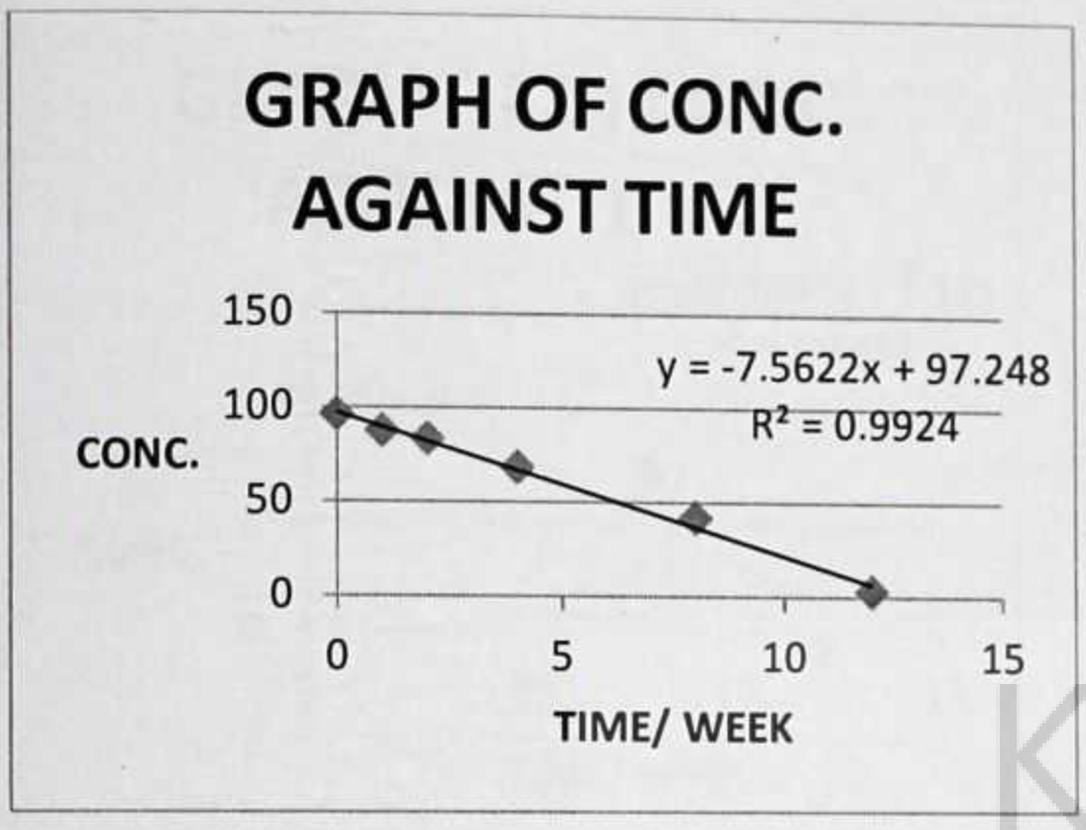
FLUCLOX/ 50MG DRIED STARCH



FLUCLOX/ 50MG UNDRIED STARCH

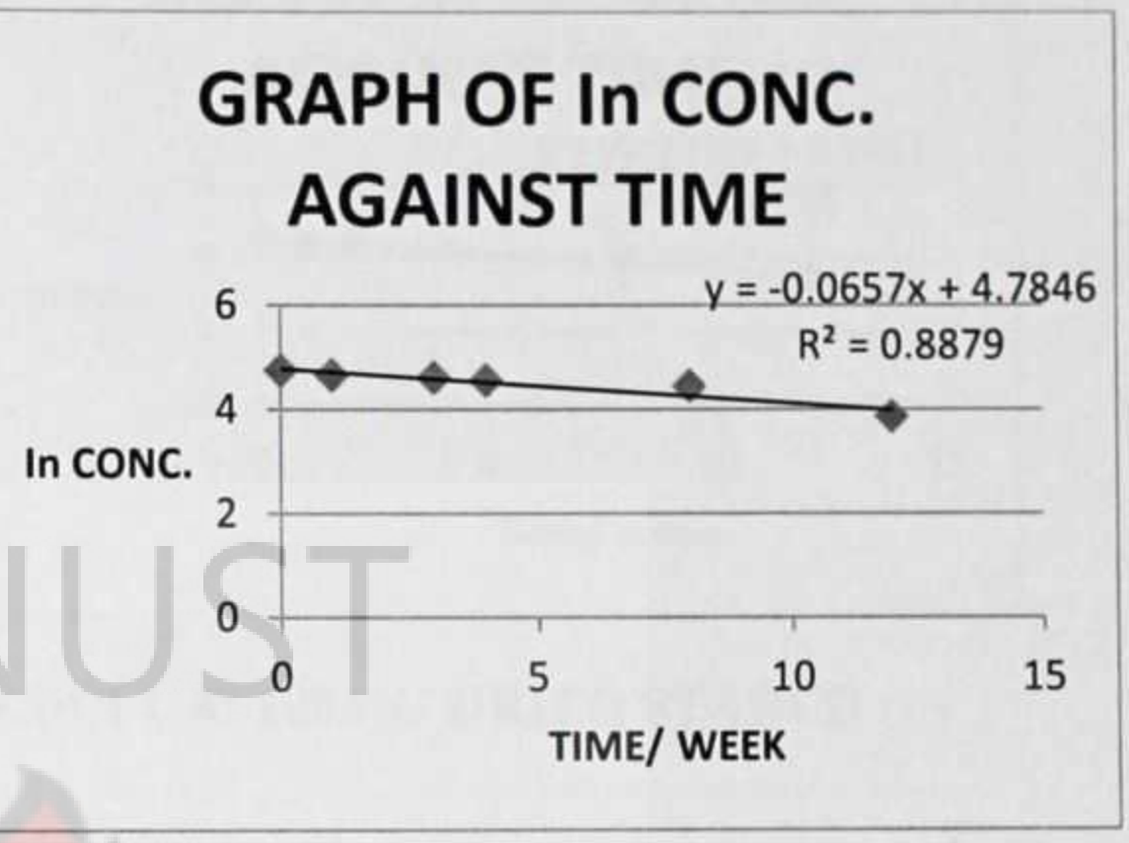


FLUCLOX/ 200MG SODIUM CMC

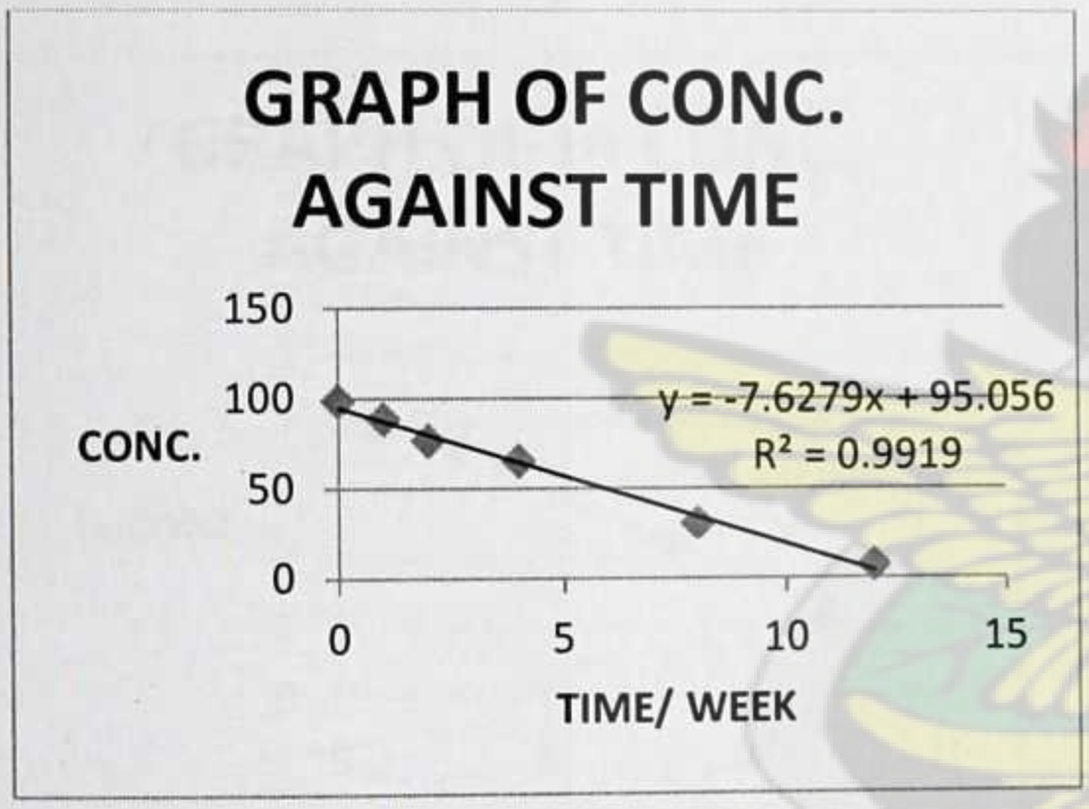


FIRST ORDER GRAPHS

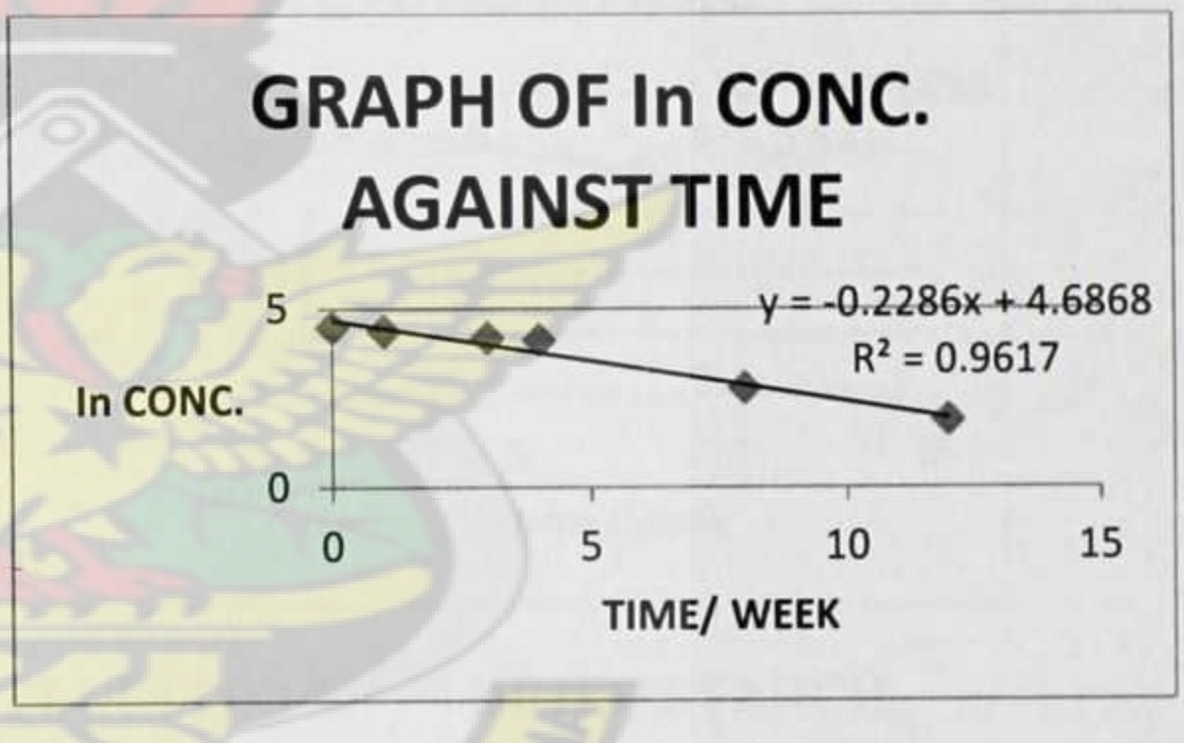
SAMPLE A1



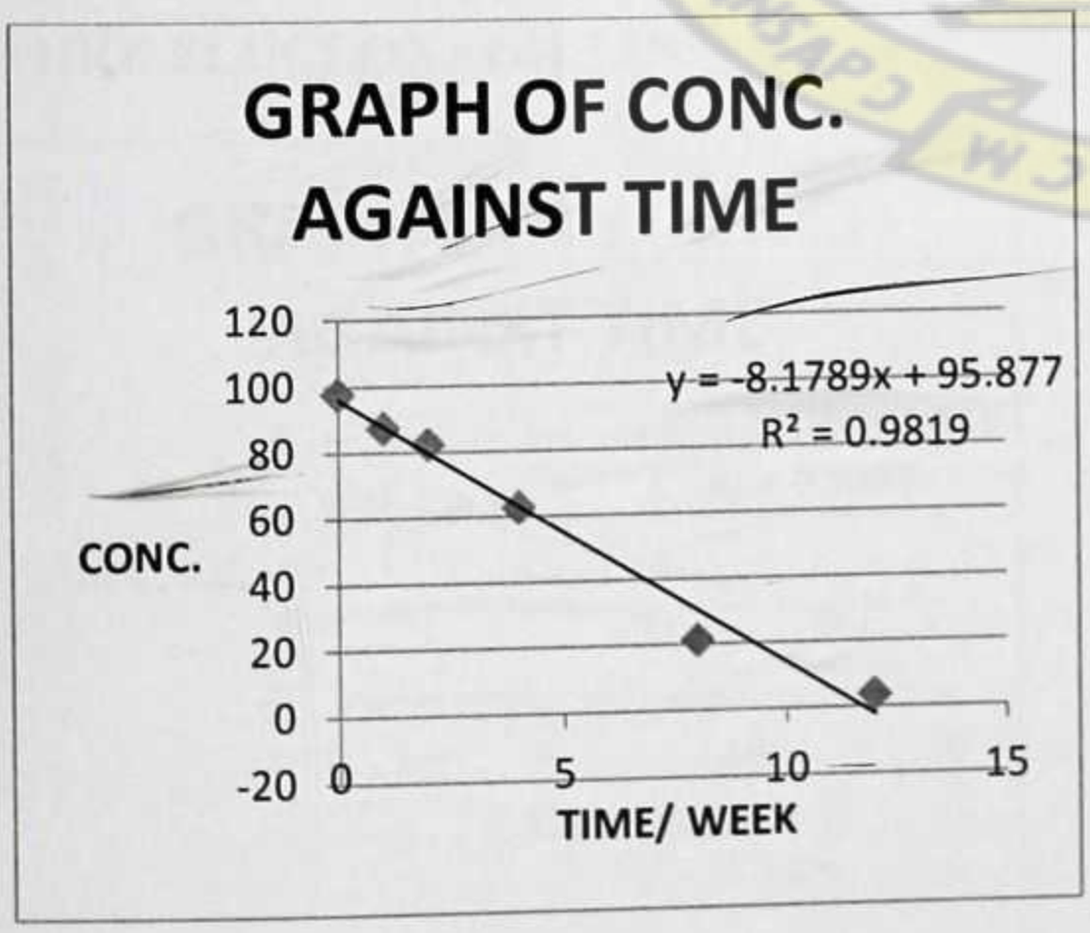
FLUCLOX 100MG SODIUM CMC



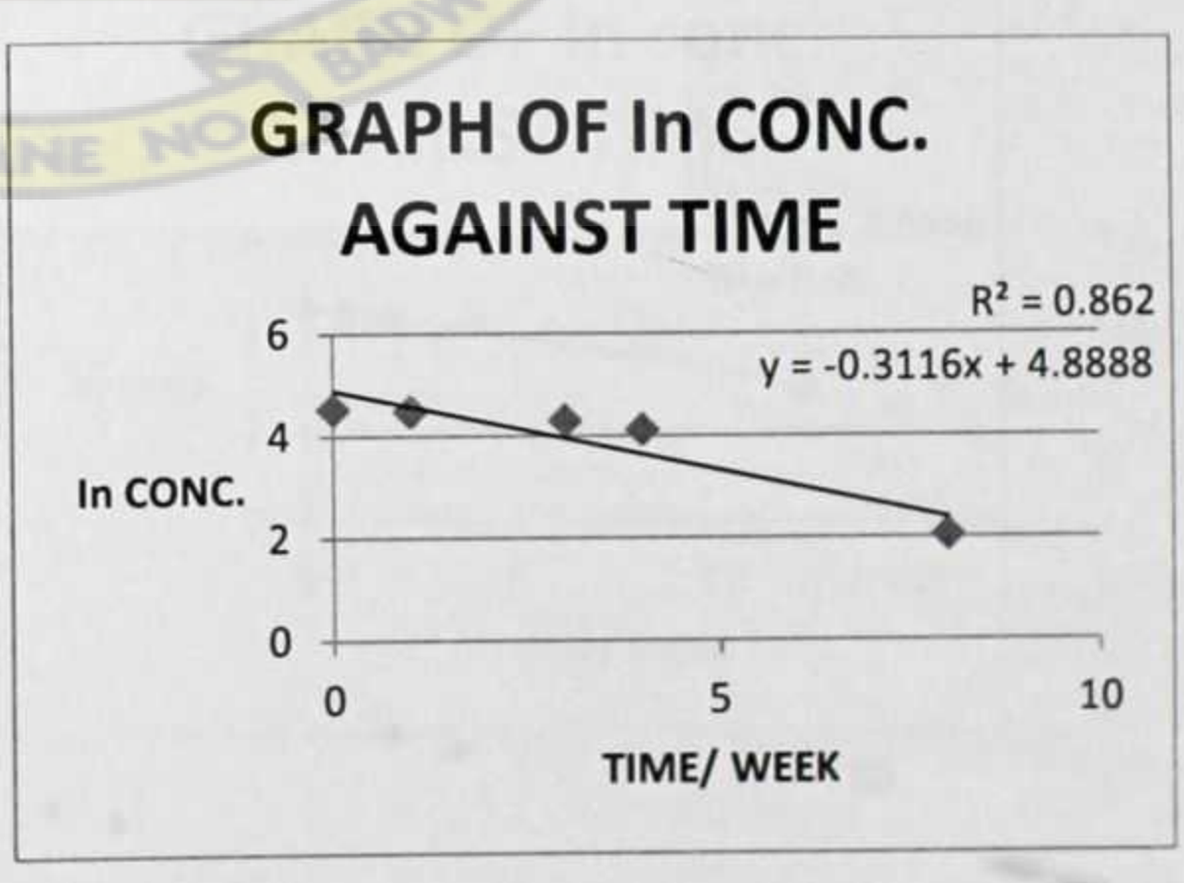
SAMPLE B



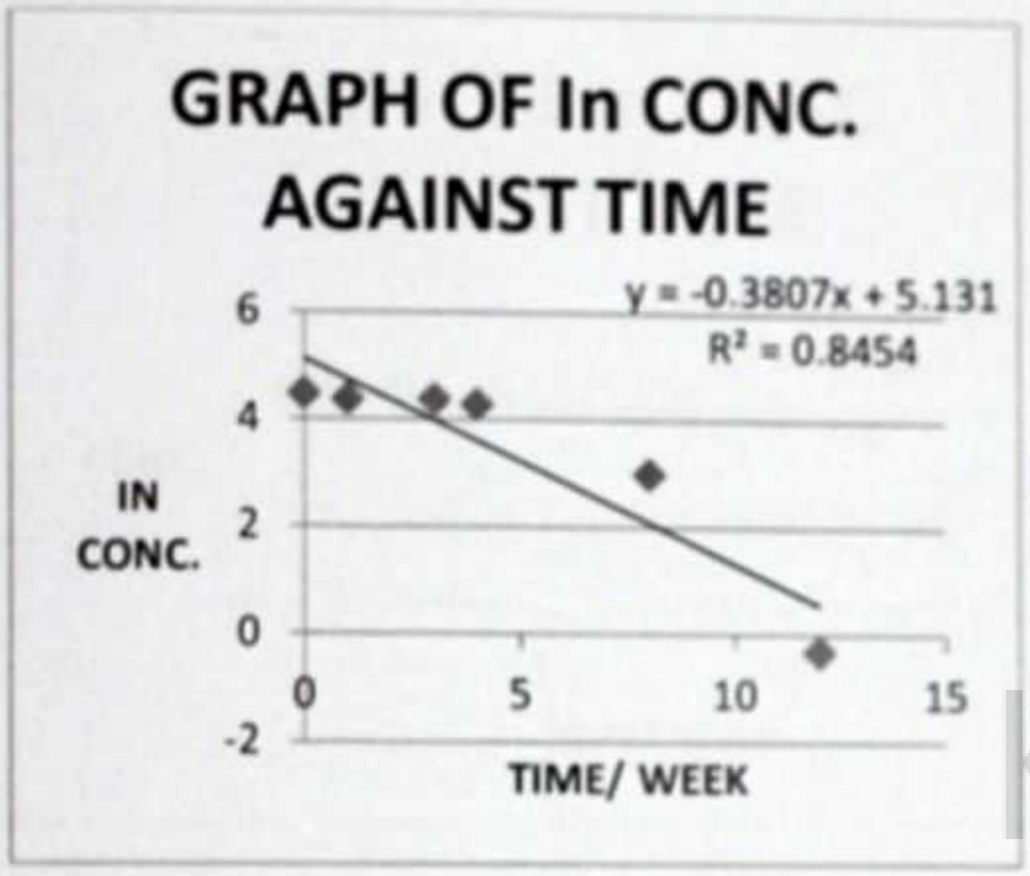
FLUCLOX 50MG SODIUM CMC



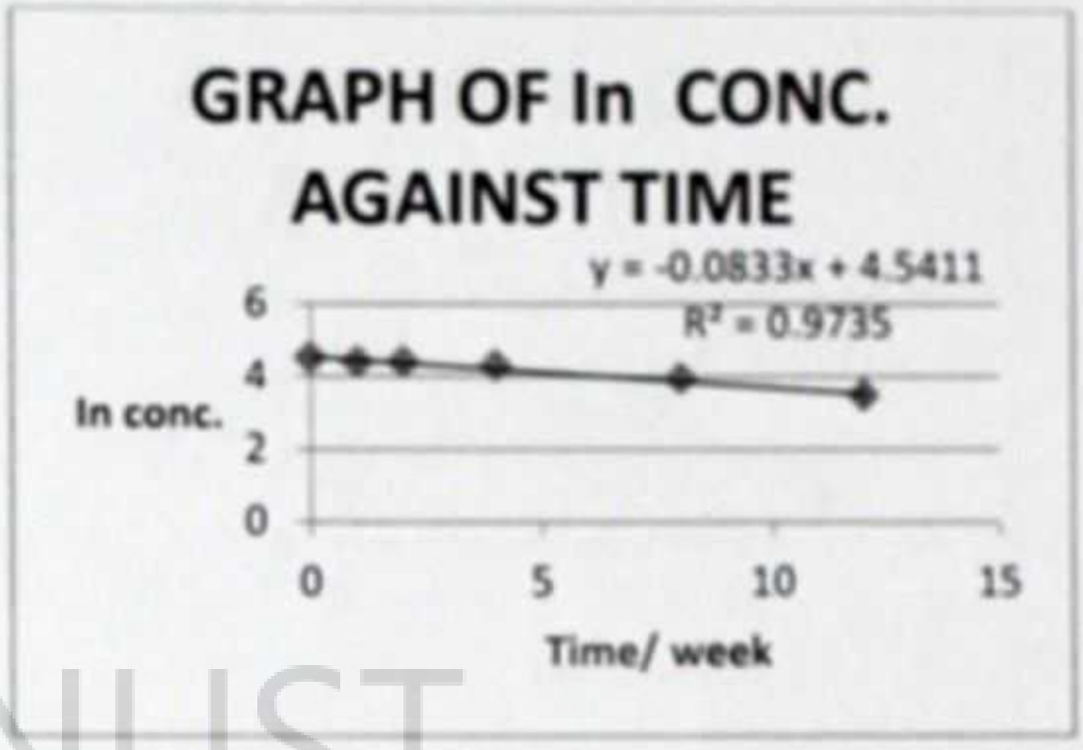
SAMPLE C



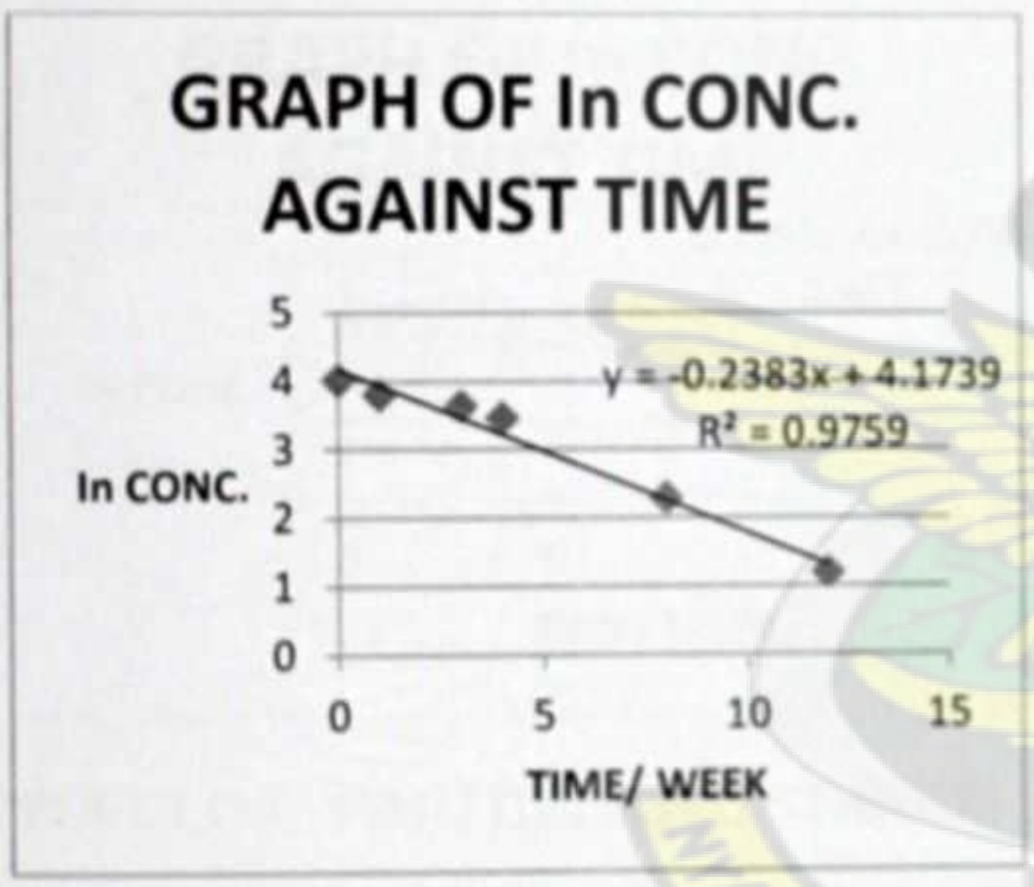
SAMPLE D



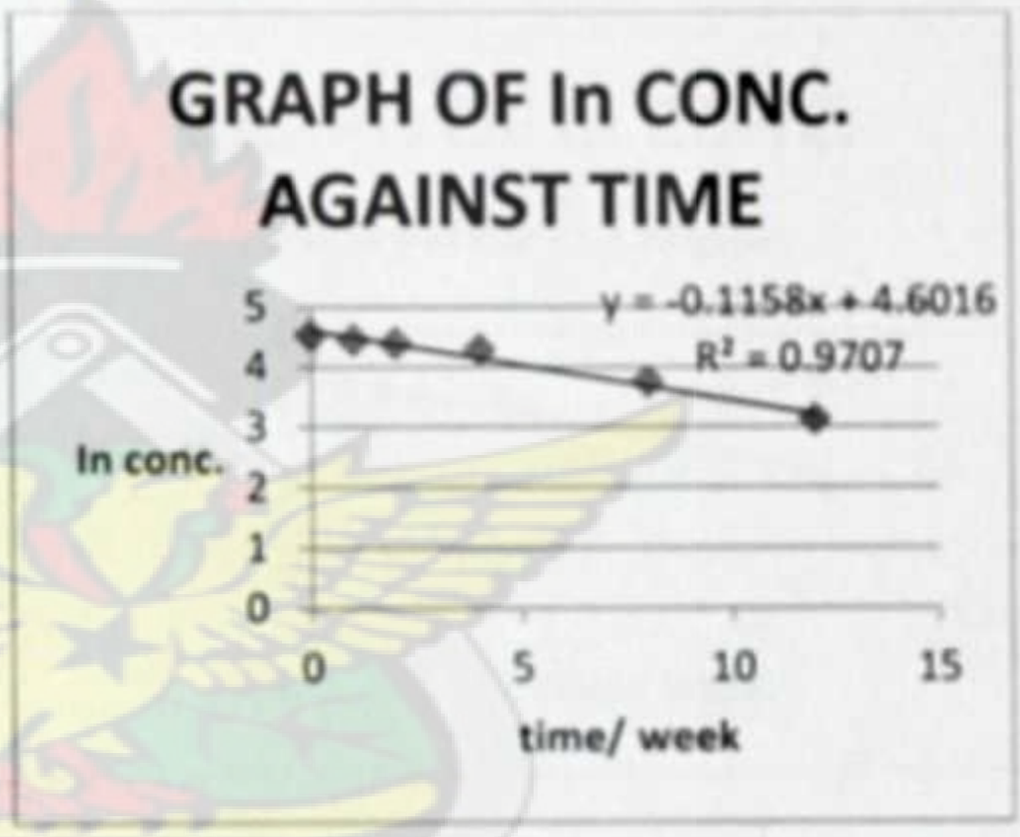
FLUCLOX/ 200MG DRIED STARCH



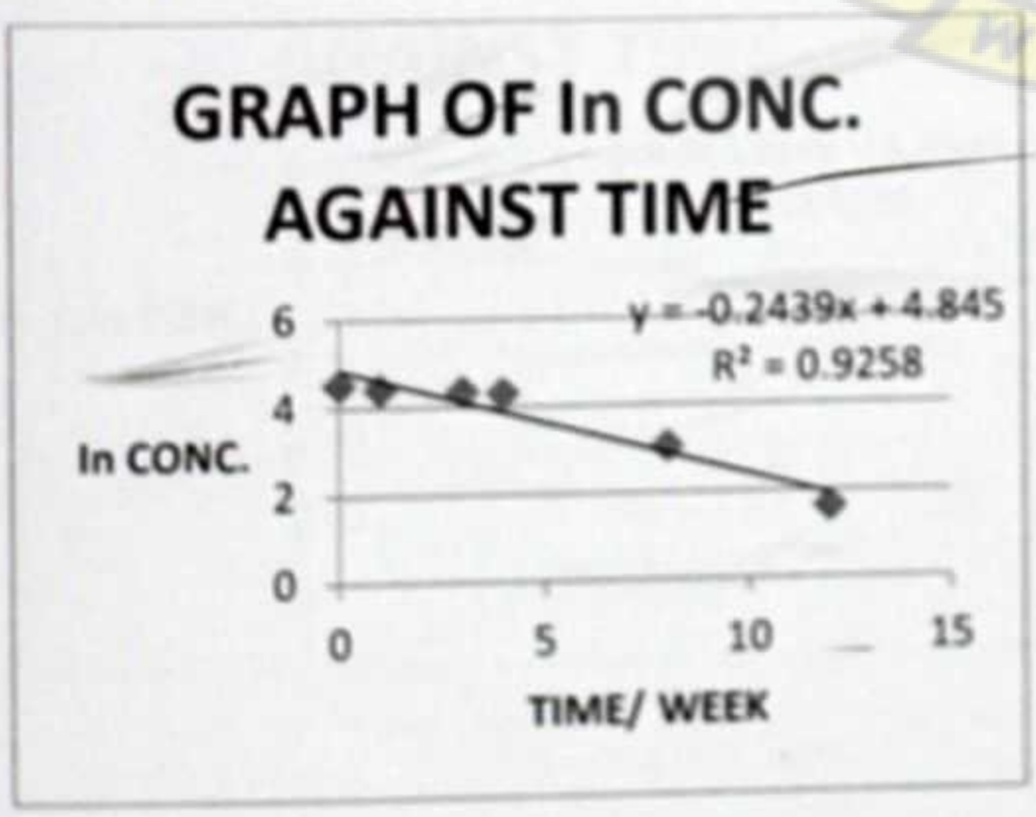
SAMPLE E



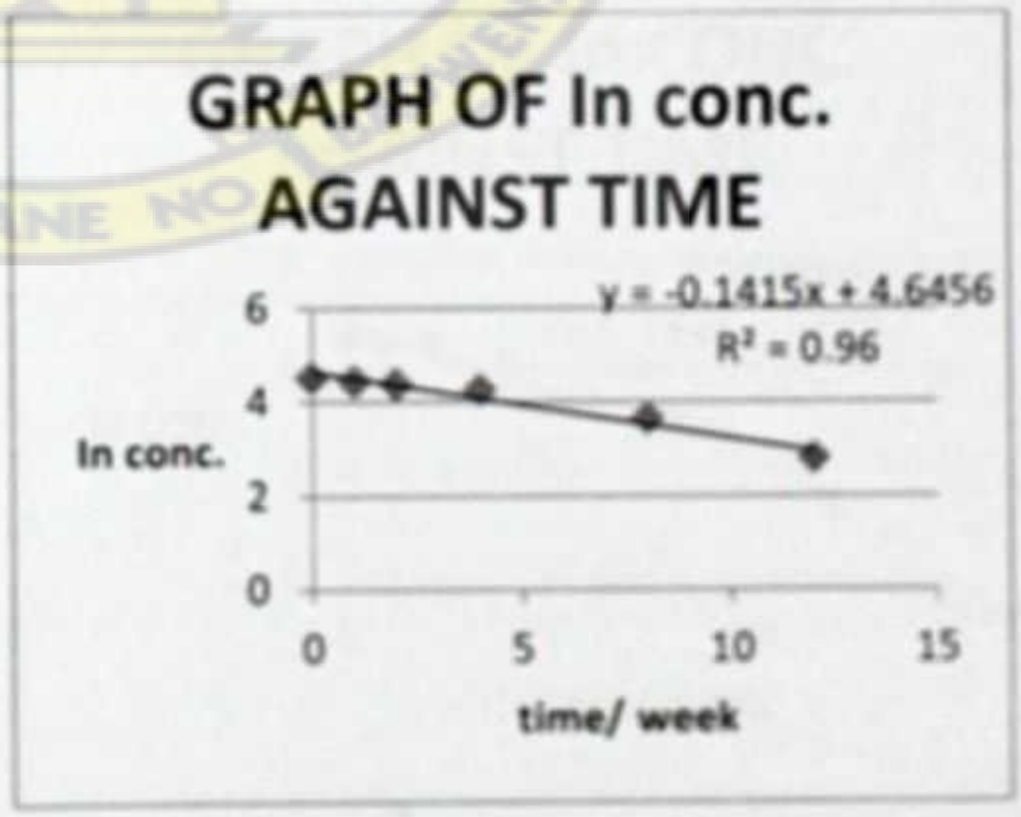
FLUCLOX/ 100MG DRIED STARCH



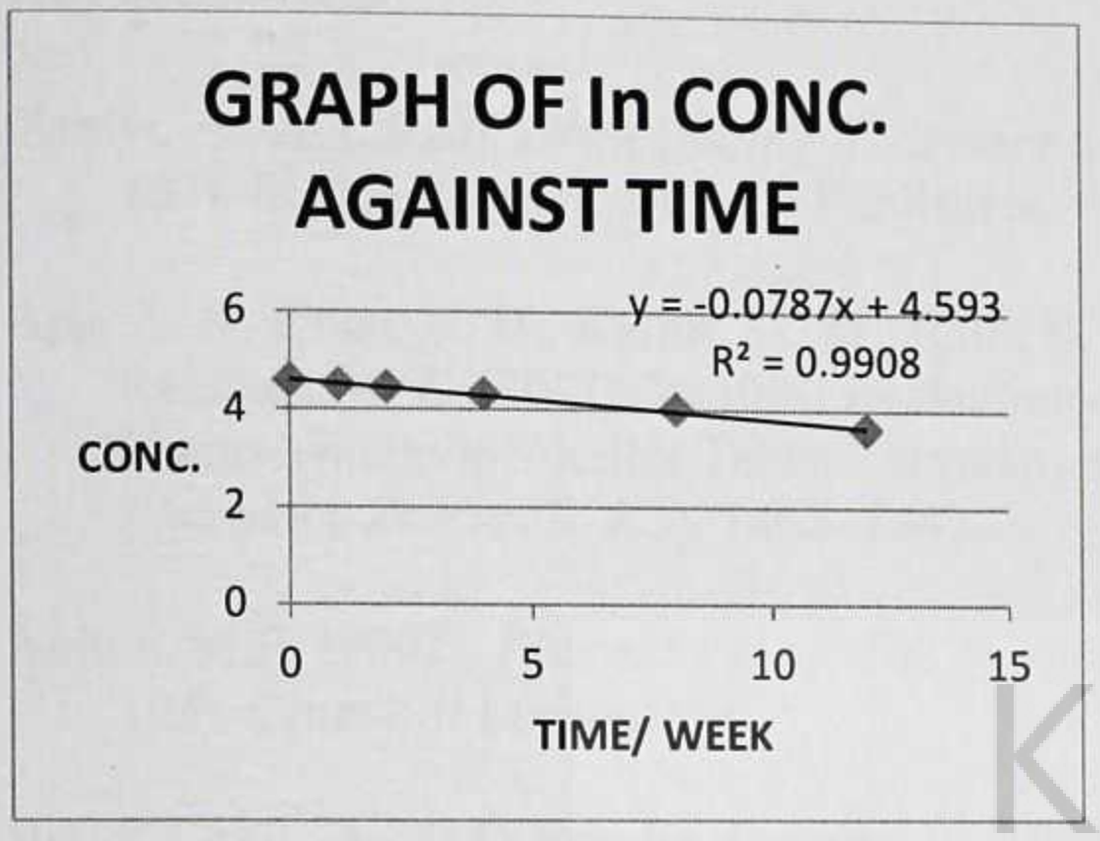
PURE FLUCLOXACILLIN



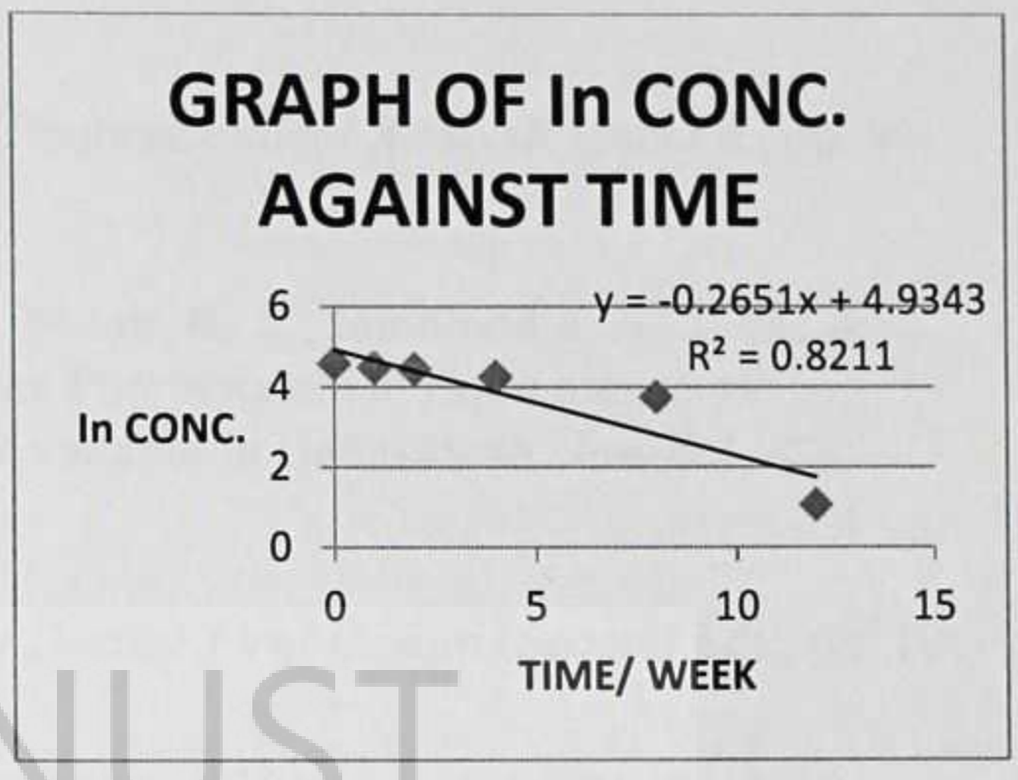
FLUCLOX/ 50MG DRIED STARCH



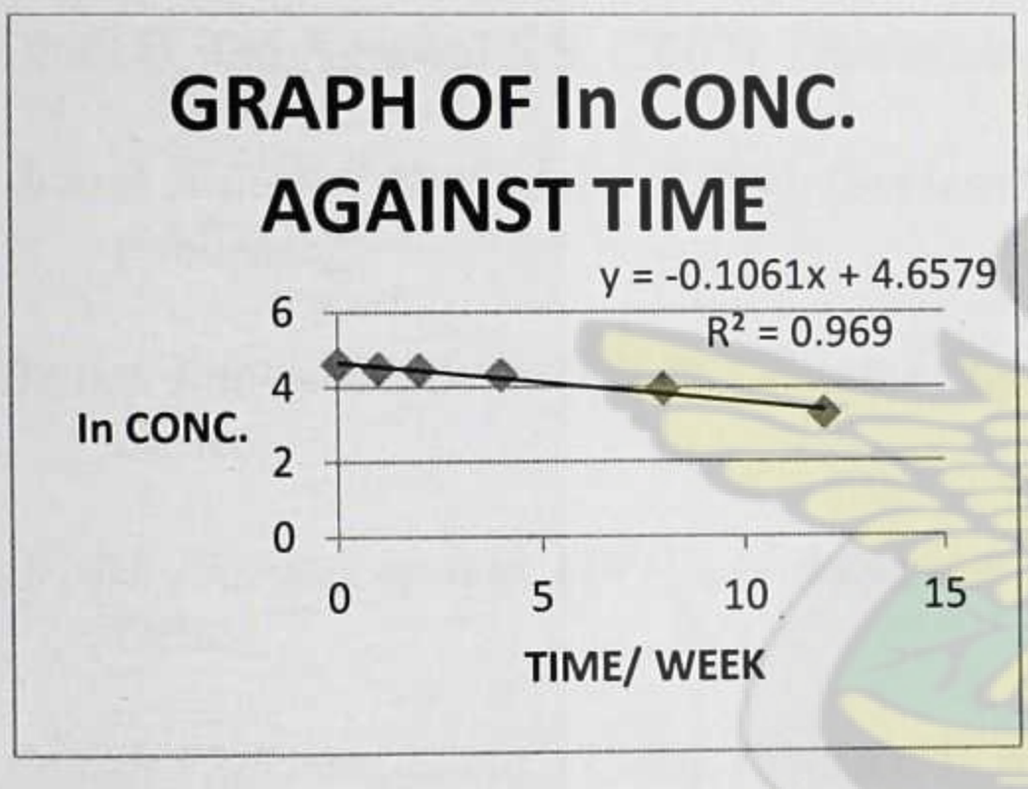
FLUCLOX/ 200MG UNDRIED STARCH



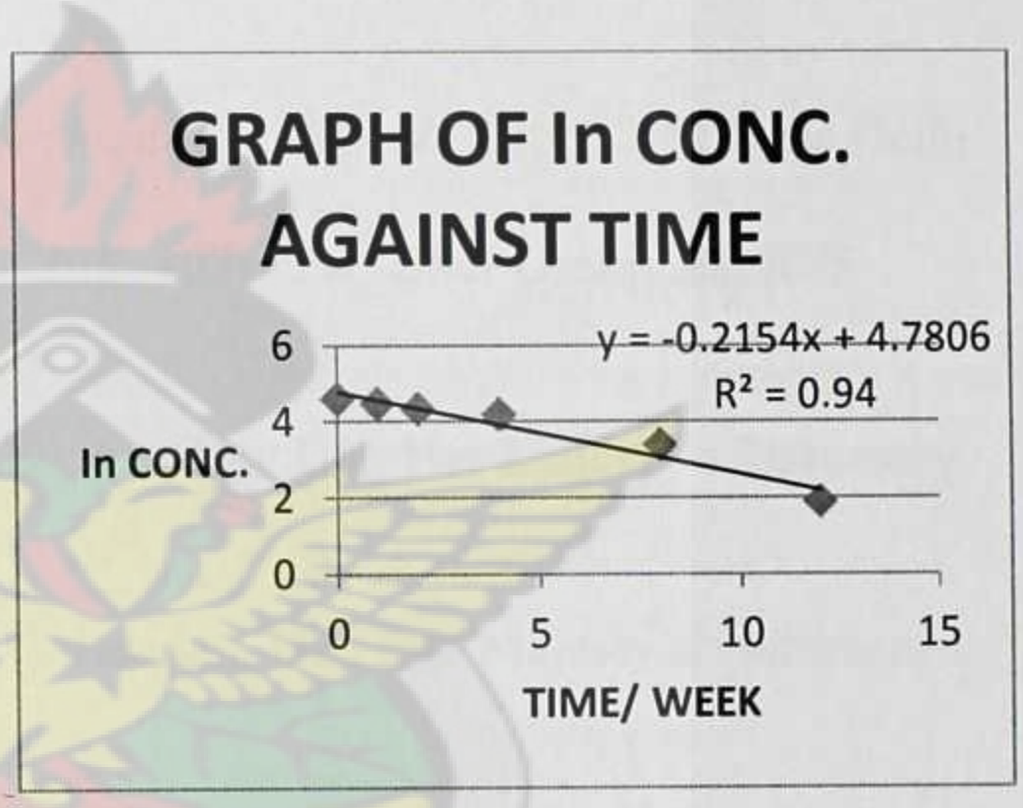
FLUCLOX/ 200MG SODIUM CMC



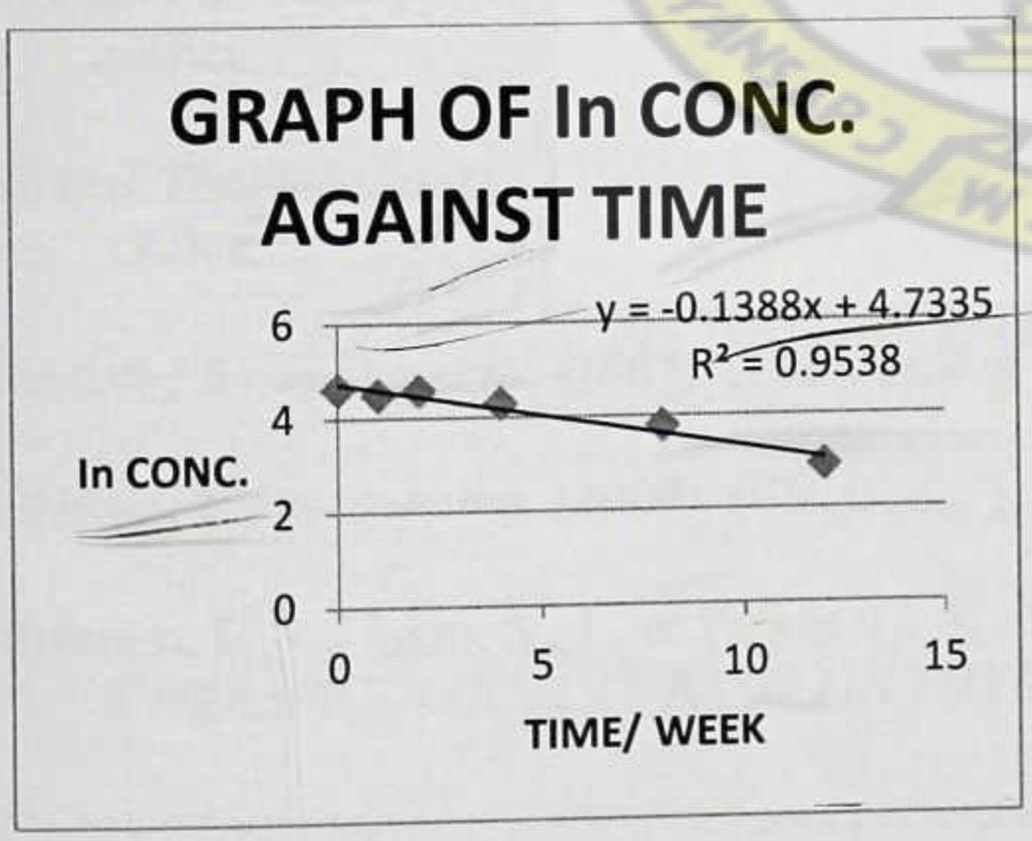
FLUCLOX/ 100MG UNDRIED STARCH



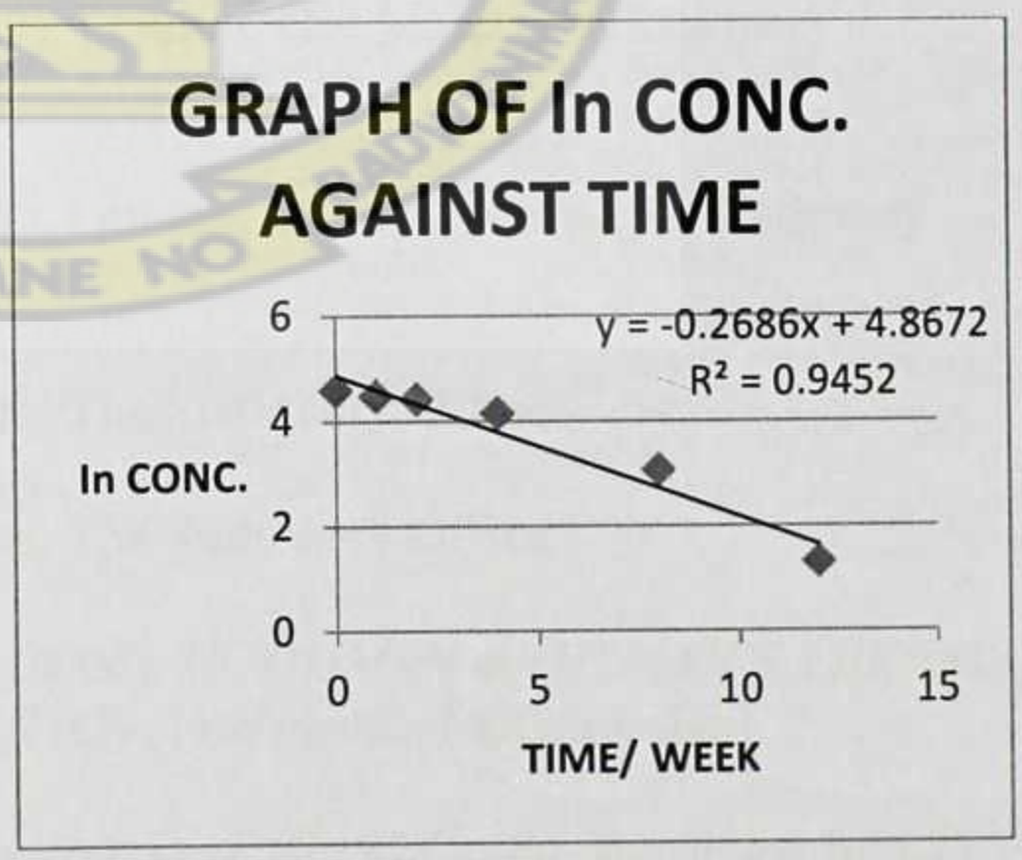
FLUCLOX/ 100MG SODIUM CMC



FLUCLOX/ 50MG UNDRIED STARCH



FLUCLOX/ 50MG SODIUM CMC



REFERENCES

0laniyi, A., A. (2010). *Drug Quality Assurance and Pharmaceutical Analysis* (second., pp. 93-102). Ibadan, Nigeria: Mosuro Publishers.

Apu, A. S., Khan, N. H., Karim, M. M., Balo, N. G., Munir, K. S., Jamaluddin, A. T. M., & Rahman, M. Z. (2011). In Vitro Evaluation of the Pharmaceutical Equivalence of Phenoxymethylpenicillin Tablet Formulations Available in Bangladesh. *Journal of Pharmacy Research*, *4*(5), 1445- 1447.

Aulton. M.E. (2002). *Pharmaceutics, The Science of Dosage form Design* (Second Edi., pp. 101-105). Churchill Livingstone.

Beloff-Chain, A., & D'accadia, Dentice, F. (1952). A chemical method of determining penicillin in culture fluids. *Analyst*, (917), 423-425.

Bhatt.B. and Agrawal.S.S. (2007). *Pharmaceutical Technology (Capsules)* (pp. 2-4). New Delhi.

British National Formulary. (2009). (September., pp. 295- 297). UK: BMJ Group and RPS publishing.

British Pharmacopoeia. (1968). (Volume One., p. 229). London, UK: Her Majesty's Stationery Office.

British Pharmacopoeia. (1973). (volume one., p. 117). London, UK: Her Majesty's Stationery Office.

British Pharmacopoeia. (1980). (volume 1., p. 343). London: Her Majesty's Stationery Office.

British Pharmacopoeia. (1988). (volume two., p. A70). London: Her Majesty's Stationery Office.

British Pharmacopoeia. (1993). (volume two., p. 912). London, UK: Her Majesty's Stationery Office.

British Pharmacopoeia. (2007). (Electronic.). London: The Stationery Office.

British Pharmacopoeia. (2009). (Electronic.). London: The Stationery Office.

Brunton, L., L., Lazo, S., J., & Parker, L., K. (Eds.). (2006). *GOODMAN & GILMAN'S THE PHARMACOLOGICAL BASIS OF THERAPEUTICS* (11th elect.). McGraw- Hill.

Codex Alimentarius. (2009).*Sodium carboxymethyl cellulose (Cellulose gum)*. Retrieved July 23, 2012, from http://www.codexalimentarius.net/gsfaonline/additives/details.html?id=51

Deshpande, A. D., Baheti, K. G., & Chatterjee, N. R. (2004). Degradation of β -lactam antibiotics. *Current Science*, *87*(12).

Excipients for pharmaceuticals.pdf. (n.d.). Retrieved July 23, 2012, from http://www.apitco.org/Profiles/Exipients for pharmaceuticals.pdf

Gujral, Singh, R., Haque, Manirul, S., & Shanker, P. (2009). A Sensitive Validated Spectrophotometric Method for the Determination of Flucloxacillin Sodium. *E-Journal of Chemistry*, *6*, S398- S405.

HPLC validation PE.pdf. (n.d.). Retrieved July 23, 2012, from http://www.standardbase.com/tech/HPLC validation PE.pdf

Hoefler, A. C. (n.d.). Sodium carboxymethylcellulose(chemistry, functionality and applications). Retrieved July 19, 2012, from http://class.fst.ohio-state.edu/fst621/Additive classes/cmctlk.pdf

Husain, S. W., Ghoulipour, V., & Sepahrian, H. (2004). CHROMATOGRAPHIC BEHAVIOUR OF ANTIBIOTICS ON THIN LAYERS OF AN INORGANIC ION-EXCHANGER. *ACTA Chromatographica*, (14), 102-109.

Jun, H., Wakai, J., Yasuda, H., & Uno, T. (1985). Spectrophotometric Assay of Penicillins by Reaction with 1,2,4- Triazole and Mercury (II) Chloride. *Analytical Sciences*, *1*(April), 73-76.

MHRA. (2007). *DRUG ALERT (REF: MDR 09-08/07)* (p. 8).

Moats, A, W. (1983). Detection and semiquantitative estimation of penicillin G and cloxacillin in milk by thin-layer chromatography. *Journal of Agricultural and Food Chemistry*, *6*(31), 1348-1350.

Musa, H., Gambo, A., & Bhatia, P. G. (2011). STUDIES ON SOME PHYSICOCHEMICAL PROPERTIES OF NATIVE AND MODIFIED STARCHES FROM DIGITARIA IBURUA AND ZEA MAYS, *3*(1), 3-6.

Nabi, S. A., Khan, M. A., & Khowaja, S. N. (2006). THIN-LAYER CHROMATOGRAPHIC SEPARATION OF PENICILLINS ON STANNIC ARSENATE – CELLULOSE LAYERS. *ACTA Chromatographica*, (16), 164-172.

Nikam, D. S., Bonde, C. G., Surana, S. J., Venkateshwarlu, G., & Dekate, P. G. (2009). Development and Validation of RP-HPLC Method for Simultaneous Estimation of Amoxicillin trihydrate and Flucloxacillin sodium in capsule dosage form, *1*(3), 935-939.

Penicillin Antibiotics (Classification, Side Effects and Uses). (n.d.). Retrieved July 24, 2012, from http://www.boybdream.com/articles.php?articleid=138596

Penicillin Development. (n.d.). Retrieved July 17, 2012, from http://www.news-medical.net/health/Penicillin-Developments.aspx

Remington. J.P. (2005). *The Science and Practice of Pharmacy*. (D. Troy & H. J. Matthew, Eds.) (21st ed., pp. 269,1025, 1027 and 1073). Philadelphia, USA: Lippincot Williams and Wilkins.

Rosenberg, J. (n.d.). Alexander Fleming Discovers Penicillin. Retrieved July 24, 2012, from http://history1900s.about.com/od/medicaladvancesissues/a/penicillin_2.htm

Sodium carboxymethylcellulose- FAO. (1984). Retrieved July 17, 2012, from http://www.fao.org/ag/agn/jecfa-additives/specs/Monograph1/additive-396-m1.pdf

Sweetman, C. S. (Ed.). (2009). *Martindale: The Complete Drug Reference* (36th ed., pp. 213 and 277). China: Everbest Company Ltd.

Validation of analytical procedures: text and methodology. (2005). Retrieved July 17, 2012, from http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf

Vogel, A. I. (1979). *Vogel's Textbook on Macro and Semimicro Qualitative Inorganic Analysis*. (G. Svehla, Ed.) (fifth., p. 286). New York, USA: Longman Inc.

Vogel, A. I. (1989). *Vogel's textbook of quantitative chemical analysis 5th edition.pdf*. (.Jeffrey.G.H, Bassett.J., J. Mendham, & R. . Denney, Eds.) (5th ed., pp. 139 and 840).

Zhou, Q., Ruan, Z., Yuan, H., Juan, B., & Xu, D. (2007). RP-HPLC analysis of flucloxacillin in human plasma validation and application to a bioequivalence study. *Die Pharmazie*, *62*, 101-104.

con014961.pdf. (n.d.). Retrieved July 24, 2012, from http://www.mhra.gov.uk/home/groups/l-unit1/documents/websiteresources/con014961.pdf

con2033471.pdf. (n.d.). Retrieved July 24, 2012, from http://www.mhra.gov.uk/home/groups/l-unit1/documents/websiteresources/con2033471.pdf

newsletter19.pdf (application/pdf Object). (2003). Retrieved July 23, 2012, from http://www.ienica.net/newsletters/newsletter19.pdf