VISUAL INSPECTION, DISSOLUTION TEST AND SPECTROPHOTOMETRIC EVALUATION OF SOME BRANDS OF TETRACYCLINE AVAILABLE IN PHARMACIES IN BENIN CITY

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CERTIFICATION

We hereby certify that this project work titled **"V**ISUAL INSPECTION, DISSOLUTION TEST AND SPECTROPHOTOMETRIC EVALUATION OF SOME BRANDS OF TETRACYCLINE AVAILABLE IN PHARMACIES IN BENIN CITY**"** was carried out by IZEVBIGIE EMMANUEL from the Department of Pharmaceutical chemistry, Faculty of Pharmacy, University of Benin, Benin City, done in partial fulfillment of the requirement for the award of Bachelor of Pharmacy and Doctor of Pharmacy degree of the University of Benin, Benin City.

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DEDICATION

“I DID ALL THESE THROUGH CHRIST THAT STRENGTHENETH ME.”[PHILIPPIANS 4:13].

This work is dedicated to Almighty GOD, Lord of all the worlds and the uncreated creator whose mercy and grace over me knows no bound.

Also, to my parents, Dr. and Mrs. IZEVBIGIE., who have excellently strove for my success in both worlds and supported me from pregnancy till this day.

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ABSTRACT

Background: .The tetracycline class of antimicrobials demonstrates a broad spectrum of activity against various pathogens, encompassing Gram-positive and Gram-negative bacteria, as well as atypical organisms.However, their use for bacterial infections has been restricted in recent years due to the emergence of resistant organisms employing efflux and ribosomal protection mechanisms.However tetracycline has been used for therapeutic reasons in both humans and animals

Aim: The primary aim is to perform a quantitative and qualitative analysis on various brands of tetracycline found in pharmacies in Benin city.

Method: Visual inspection was performed according to World health Organisation Visual Inspection of Medicines Template while the dissolution test was performed using the United State Pharmacopoeia modified method of spectrometry according to Ahmed *et* al was used and absorbance was taken at 362nm.

Results: The visual inspection showed that 90% of the brand inspected met the required standard according to the USP. The dissolution test showed that the percentage content in 20 40 and 60 minutes was within the range of 91.31 - 99.53, 92.62 - 99.53 and 97.83 - 99.57 respectively .the spectrometry test shows that all the brands met up to the 90 - 125% (USP).

Conclusion: The conclusion of this research shows that the analysed tetracycline brands follow the official monographs, except for the color deviation that was noted in the T7 tablets.

CHAPTER ONE

1.1 Introduction/Literature Review

The advancements in analytical chemistry, particularly in instrumental methods of chemical analysis, are evident in their inclusion in pharmacopoeia monographs and industry standards. Chromatographic techniques, such as high-performance liquid chromatography (HPLC), thin layer chromatography (TLC), and gas chromatography (GC), consistently play a crucial role. Standardization of equipment requires precise and detailed descriptions of analysis conditions. Other significant methods include ultraviolet–visible (UV–vis) and infrared (IR) spectrophotometry, atomic absorption spectrophotometry (AAS), nuclear magnetic resonance (NMR), mass spectrometry (MS), and spectrofluorometry. Electromigrational methods like capillary electrophoresis (CE), capillary zone electrophoresis (CZE), and micellar electrokinetic capillary chromatography (MEKC), along with voltamperometric methods, are also used for determining non-steroidal anti-inflammatory drugs (NSAIDs). Flow injection analysis (FIA) is gaining popularity due to its full automation, minimizing side reactions, and enhancing sensitivity and selectivity (Gouda *et al*., 2013).

The tetracycline class of antimicrobials demonstrates a broad spectrum of activity against various pathogens, encompassing Gram-positive and Gram-negative bacteria, as well as atypical organisms. These compounds are bacteriostatic, functioning by binding to the bacterial 30S ribosomal subunit and hindering protein synthesis. Tetracyclines have been effectively utilized to treat diverse infectious diseases, including community-acquired respiratory tract infections, sexually transmitted diseases, and acne management. However, their use for bacterial infections has been restricted in recent years due to the emergence of resistant organisms employing efflux and ribosomal protection mechanisms (Zhanel *et al*., 2004).

Tetracycline antibiotics constitute a primary group employed for veterinary, human therapeutic, and agricultural purposes. Among various antibiotics, special attention is given to tetracyclines due to significant environmental concerns, posing ecological risks and potential harm to human health. Their widespread use has led to evidence indicating the omnipresence of tetracycline

antibiotics in various ecological compartments. After administration, over 70% of these antibiotics are excreted in an active form into the environment through urine and feces from both humans and animals. Their highly hydrophilic nature and low volatility contribute to their prolonged persistence in aquatic environments (Daghrir *et al*., 2013).

The history of tetracyclines involves the collective efforts of numerous dedicated researchers, scientists, clinicians, and business executives spanning over 60 years. Initially discovered as natural products from actinomycetes soil bacteria, tetracyclines were first documented in scientific literature in 1948. Recognized for their extensive antibacterial activity, they were successfully introduced into clinical use in the late 1940s to early 1950s. The subsequent development of second-generation semisynthetic analogs and more recent third-generation compounds demonstrates the ongoing evolution of the tetracycline scaffold. These derivatives exhibit increased potency and efficacy against tetracycline-resistant bacteria, coupled with improved pharmacokinetic and chemical properties. Their efficacy against a wide range of microbial pathogens and applications in mammalian models for inflammation, neurodegeneration, and other biological systems suggest that tetracyclines will continue to be valuable therapeutics in infectious diseases and potential treatments for inflammation-related mammalian cell diseases (Nelson *et al*., 2011).

There is a growing global concern surrounding the issue of counterfeit medications. Specifically, counterfeit antimicrobial drugs pose a significant threat to public health, leading to severe consequences for patients such as increased mortality and morbidity, as well as the emergence of drug resistance. Moreover, physicians treating these patients experience a loss of confidence in the medications being used and often encounter high levels of bacterial resistance. The problem of fake and suboptimal medications has exacerbated with the rise of the World Wide Web, as a considerable portion of drugs sold through online pharmacies is found to be counterfeit. Several initiatives by the World Health Organization (such as the International Medical Products Anti-Counterfeiting Taskforce) are anticipated to address this critical public health issue (Kelesidis *et al*., 2007).

1.1.1 Antimicrobial Resistance and Tetracyclines

Throughout history, humans have engaged in a prolonged struggle against microorganisms, particularly bacteria, which have caused substantial morbidity and mortality in various human populations worldwide. In the early 1940s, penicillin emerged as a potent antimicrobial agent effective against bacteria, widely used to combat infectious diseases. However, the excessive utilization of penicillin led to a decline in its effectiveness, as bacteria developed diverse resistance mechanisms (Tenover 2006).

Antimicrobial resistance (AMR) is characterized by the ability of microorganisms to endure and remain viable in the presence of antimicrobial agents. Various types of antimicrobial agents, including antibiotics, disinfectants, and food preservatives, are employed to diminish the growth, inhibit the multiplication, or even exterminate microorganisms. Natural, semi-synthetic, and synthetic agents with distinct mechanisms can induce significant alterations on the metabolic and physiological levels, such as modifications in cell wall synthesis (e.g., β-lactams and glycopeptides), inhibition of protein synthesis (e.g., Macrolides and tetracyclines), interference with metabolic pathways (e.g., sulfonamides), and disruption of DNA replication and translation (e.g., Fluoroquinolones) (Tenover 2006; Khameneh *et al*., 2016).

Bacterial resistance has become a major concern in healthcare organizations. The widespread use of antibiotics globally provides increased opportunities for bacteria to develop more complex resistance against these drugs. Consequently, the emergence of new modified bacterial strains reduces the effectiveness of treatments, leading to profound consequences such as increased morbidity, mortality, and clinical complications (Tenover 2006; Morgan *et al*., 2011; Khameneh *et al*., 2016).

The prolonged and extensive use of antibiotics has resulted in the evolution of antimicrobial resistance (AMR) within bacteria. This natural genetic adaptation to resist antibiotics has reached alarming levels in the 21st century, presenting AMR as a serious global health challenge that requires early intervention. Bacterial resistance to antibiotics compromises the efficacy of antibiotic use in healthcare, and compelling evidence indicates that the inappropriate use of antibiotics will inevitably contribute to the development of resistance (Tenover 2006).

Presently, due to their excessive use, bacteria have developed resistance to tetracyclines (specifically of the efflux pump type), contrasting with the earlier compounds. Medicinal chemists, aiming to enhance the structure and bolster antibacterial efficacy, have successfully

incorporated an alkaline group at C-9 of the minocycline skeleton, originating from total synthesis as exemplified by Tigecycline (patented by Pfizer and Wyeth, available for therapeutic use since 2005). In the quest for novel molecules, it is crucial not only to investigate drug binding specifically to bacterial ribosomes but also to comprehend how the tetracycline skeleton can function as a chelator and ionophore (Lambs *et al*., 1988).

The reported levels of bacterial resistance to tetracyclines have exhibited considerable variation depending on geographic location and the year of isolation (Kucers *et al*., 1987). Nevertheless, by the mid-1970s, resistance to tetracycline had become prevalent among Enterobacteriaceae, staphylococci, streptococci, and bacteroides (Levy 1984). In certain areas, resistance rates were alarmingly high. For instance, in a Boston Hospital in 1969, 38% of S. aureus, 61% of E. coli, 62% of Klebsiella spp., 58% of Enterobacter spp., 91% of Proteus spp., and 97% of Serratia spp. demonstrated resistance to tetracyclines (Sabath 1969). Similarly elevated rates of resistance were also documented in Bacteroides fragilis and H. influenzae in the early 1980s, both in the United States and Europe (Kucers *et al*., 1987).

The emergence of resistance to tetracyclines in human clinical isolates has significantly curtailed the continued efficacy of these drugs, contributing to a decline in their use for the treatment of human infections in most countries (Chopra *et al*., 1992; Finch *et al*., 1997; Kucers *et al*., 1987). Fortunately, resistance has not yet become a widespread issue in situations where tetracyclines remain the preferred drugs, as evidenced by the apparent absence of resistance in Brucella melitensis (Agalar *et al*., 1999) and Coxiella burnetii (Maurin *et al*., 1999), and low rates or sporadic reports of resistance in periodontal bacteria (Lacroix *et al*., 1993), Helicobacter pylori (Midolo *et al*., 1996), and possibly Chlamydia trachomatis (Somani *et al*., 2000). However, the landscape may change, particularly in light of recommendations for tetracyclines as first-line agents for acne, given recent reports of resistance rates as high as 25% in cutaneous propionibacteria (Jones *et al*., 1996). It is noteworthy that resistance in propionibacteria is attributed to mutations rather than the acquisition of tet genes (Ross *et al*., 1998).

Furthermore, the ongoing development of the next generation of antibacterial tetracyclines is anticipated to exhibit high specificity for bacterial species, incorporating new groups and rings onto the classical skeleton (Charest *et al*., 2005). The mechanism of action of tetracyclines is categorized into two groups: "Typical," acting as bacteriostatic, and "atypical," acting as

bactericidal. Typical tetracyclines selectively bind to bacterial ribosomal subunits, while those lacking ribosomes as their primary target are deemed atypical. Additionally, these atypical mechanisms of action exhibit considerable toxicity to both prokaryotes and eukaryotes, including mammalian cells. Although all tetracyclines employed in therapy thus far have a broad-spectrum effect against microbial agents, researchers are actively working on a platform to introduce exclusively novel tetracyclines with a narrow-spectrum tailored for infectious diseases (Nelson *et al*., 2011).

1.1.2 Classification of Tetracyclines

Historically, tetracyclines have been categorized into three generations based on their origin and synthesis. The first generation includes those obtained through biosynthesis, such as Tetracycline, Chlortetecycline, Oxytetracycline, and Demeclocycline. The second generation comprises derivatives of semi-synthesis, including Doxycycline, Lymecycline, Meclocycline, Methacycline, Minocycline, and Rolitetracycline. Tigecycline, obtained through total synthesis, is considered the third generation, although some researchers argue that it should be classified separately as a member of a new family of antibacterials known as Glycylcyclines (Fuoco *et al*., 2012).

This paper introduces a novel perspective on the nomenclature and classification of tetracycline-structure-based drugs. Future tetracycline derivatives currently undergoing advanced clinical trials (Phase III of Pharmaceutical Trials Protocol) will soon be introduced for therapeutic use. Tetracyclines obtained through total synthesis, exemplified by Tigecycline, are classified as third generation if they exhibit broad-spectrum activities against both Gram-positive and Gram-negative bacteria. Aminomethylcycline derivatives are considered akin to Glycylcyclines. Over the last five years, a significant number of medicinal chemists worldwide have developed and patented more than 310 tetracycline-like compounds, particularly in the USA. Harvard University and Tetraphase have introduced pentacycline antibacterials, azatetracycline, and flurocycline, representing structural modifications such as the incorporation of five rings in Doxycycline and the insertion of heteroatoms into the D ring, along with alkylaminotetracycline antibacterials (Fuoco *et al*., 2012; Xiao *et al*., 2012).

All these compounds are the logical outcomes of modifications around the four rings of tetracyclines, which trace back historically to the pioneering work of Golub and McNamara. Three decades ago, they introduced the first eight compounds known as chemically modified tetracyclines (CMTs) in the scientific literature. In 1983, Golub and McNamara presented a groundbreaking concept concerning the therapeutic utility of tetracyclines, putting forth two key ideas. Firstly, tetracyclines, distinct from other antibiotics, possess the ability to inhibit collagenase—an exclusive collagenolytic metallo-neutral protease produced by host tissues, frequently implicated in periodontal destruction. Secondly, this newfound property of the drugs could offer a novel approach to treating diseases, such as periodontal diseases and certain medical disorders (e.g., non-infected corneal ulcers), characterized by excessive collagen destruction. In such cases, tetracyclines seem to inhibit collagenase activity through a mechanism unrelated to the drug's antibacterial efficacy. Notably, all chemically modified tetracyclines have undergone modifications involving the removal of the dimethylamino group from the C4 position on the A ring (Golub *et al*., 1983; McNamara *et al*., 1986).

To comprehensively categorize these new compounds, understanding the chemical properties enabling tetracycline-structure-based drugs to function as a "chameleonic" entity is crucial. As discussed earlier, tetracyclines can be regarded as broad-spectrum antibiotics effective against bacteria, fungi, viruses, and cancer cells. In this context, tetracyclines stand as an exemplary instance of multi-target drugs and the first extensively documented in the literature. Furthermore, due to their chemical similarities, chemical physics properties, and application as anti-cancer drugs, it is apt to classify anthracyclines like Doxorubicin alongside tetracyclines (Golub *et al*., 1983; McNamara *et al*., 1986).

1.1.3 Structural Activity Relationship (SAR) of Tetracyclines

The diagram presented below illustrates the rigid structure of tetracyclines (TCs), including the numbering of the four rings, groups, and the upper and lower sides, as commonly referred to. Numerous chemical alterations applied to both first and second-generation tetracyclines have resulted in compounds with varying levels of activity, some exhibiting antibacterial properties and others being inactive.

For a tetracycline to demonstrate antibacterial activity, it must feature a linearly arranged DCBA naphthacene ring system with a C1-C3 diketo substructure in the A-ring and an exocyclic C2 carbonyl or amide group. Those tetracyclines that function as inhibitors of protein synthesis in bacteria necessitate the presence of the amino group at position C4, along with keto-enolic tautomers at positions C1 and C3 of the A ring. The antibacterial activity is particularly dependent on a C4-dimethylamino group, with the natural 4S isomer contributing to optimal efficacy, while conversion to the 4R isomer diminishes Gram-negative activity (Doerschuk *et al*., 1955).

Furthermore, the lower peripheral region demands a C10-phenol and C11-C12 keto-enol substructure in association with a 12a-OH group. These substituents, along with their respective tautomeric equilibriums, are crucial for recognition and bonding in ribosomal subunits, and any chemical modification results in the loss of bioactivity. Although modification of the amide at C2 is feasible, it comes with a reduction in potency. Positions C5 to C9 can be chemically altered to influence their bioactivity, serving the upper peripheral regions and generating derivatives with varied antibacterial efficacy.

While groups R1, R2, and R3 can be modified to enhance selectivity for the biological target in antifungal tetracyclines, such modifications do not impact antibacterial activity. The D ring is the most flexible for alterations. All modifications of groups R4, R5, and R6 are permissible, providing high bacterial specificity and significant changes in pharmacokinetics due to modifications in log P (Fuoco *et al*., 2012).

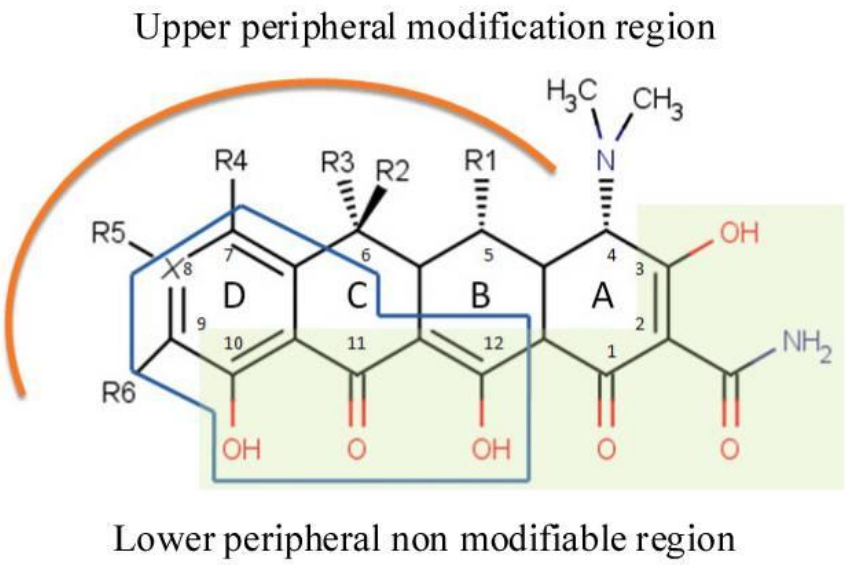

Figure 1: Structure activity relationship of Tetracyclines (TCs) (Fouco *et al*., 2012).

1.1.4 Mechanism of Action of Tetracyclines

The entire tetracycline family exhibits biological and chemical dynamism, showcasing multiple mechanisms of action against various targets, including ribosomes and cell membranes. The diverse mechanisms of action of tetracycline involve the interaction of different functional groups within the molecular structure, such as hydroxyl and amine groups. Hydroxyl groups serve as sources of reactive oxygen species (ROS), inducing significant damage to macromolecules like DNA, RNA, and proteins. This oxidative stress leads to the eventual demise of bacterial cells. Notably, antibacterial tetracyclines operate differently compared to their antifungal and antitumor counterparts (Fouco *et al*., 2012).

Initially, tetracyclines surpass eukaryotic cells to form complexes at different positions with calcium and magnesium ions present in the blood, altering the electronic balance equilibrium and sequestering divalent ions. Subsequently, tetracyclines employ both typical and atypical mechanisms against bacteria, either by binding to ribosomal subunits to inhibit protein synthesis or by directly inducing bacterial cell death (Fouco *et al*., 2012).

The typical mechanism of action involves preventing the association of aminoacyl-tRNA with bacterial ribosomes, thus inhibiting bacterial protein synthesis. Tetracyclines interact with targets

in gram-positive and gram-negative bacteria by traversing one or more membrane systems. Understanding the mechanism of action requires insights into the uptake and ribosomal binding process. In gram-negative bacteria, tetracycline crosses the outer membrane through channels formed by outer membrane proteins F and C, as part of positively charged cation-tetracycline coordination complexes. The Donnan potential of the outer membrane attracts the cationic metal ion-antibiotic complex, accumulating it in the periplasm. In this location, the metal ion-tetracycline complex may dissociate, releasing uncharged tetracycline, a weakly lipophilic molecule that efficiently diffuses through the lipid bilayer regions of the inner (cytoplasmic) membrane. Similarly, electroneutral and lipophilic forms of tetracycline are transferred across the cytoplasmic membrane of gram-positive bacteria. This uptake process is energy-dependent. Within the cytoplasm, tetracycline molecules form chelates under specific conditions when internal pH and divalent metal ion concentrations exceed those outside the cell. The magnesium-tetracycline complex, an active drug species, binds to the bacteria's ribosome. The bacteriostatic effects of antibiotics are elucidated by understanding the reversible interaction of tetracycline with the ribosome (Tariq *et al*., 2018).

1.1.5 Therapeutic Uses of Tetracyclines

- **As antibiotics**: Tetracyclines, a class of broad-spectrum antibiotics, find applications in the management and treatment of a diverse range of infectious diseases. These drugs exhibit efficacy against conditions such as rickettsial infections, ehrlichiosis, anaplasmosis, leptospirosis, amebiasis, actinomycosis, nocardiosis, brucellosis, melioidosis, tularemia, chlamydial infections, pelvic inflammatory disease, syphilis, traveler's diarrhea, early Lyme disease, acne, legionnaire's disease, and Whipple disease. They are effective against various pathogens including Borrelia recurrentis, Mycobacterium marinum, Mycoplasma pneumoniae, Staphylococcus aureus (including methicillin-resistant S. aureus [MRSA]), Vibrio vulnificus, and vancomycin-resistant enterococcus (VRE) in susceptible strains. Additionally, tetracyclines can be employed for meningococcal prophylaxis (Shutter *et al*., 2019).

  Beyond their primary applications, tetracyclines also show promise in addressing conditions such as rosacea, bullous dermatoses, sarcoidosis, Kaposi sarcoma, pyoderma gangrenosum, hidradenitis suppurativa, sweet syndrome, a1-antitrypsin deficiency,

panniculitis, pityriasis lichenoides chronica, rheumatoid arthritis, scleroderma, as well as certain cancers and cardiovascular diseases like abdominal aortic aneurysm and acute myocardial infarction (Sapadin *et al*., 2006).

- **Inhibition of Metalloproteinases**: Matrix metalloproteinases (MMP), a family of zinc-dependent proteases produced by inflammatory and connective tissue cells, play crucial roles in various physiological and pathophysiological processes (Golub *et al*., 1998). Elevated metalloproteinase activity is a common feature in human conditions associated with inflammation (Arvelo *et al*., 2006). Tetracyclines hinder metalloproteinase activity by binding to zinc or calcium ions within the enzyme structure (Golub *et al*., 1991). The three main classes of metalloproteinases, namely collagenases, gelatinases, and stromelysins, have been extensively studied in the context of tetracycline influence, with a focus on their impact in periodontal diseases. Neutrophils primarily produce collagenases, and tetracyclines selectively affect collagenases produced by neutrophils without impeding fibroblasts' enzyme production necessary for normal gingival connective tissue remodeling (Roy *et al*., 2011). Additionally, tetracyclines exhibit inhibitory effects on enzymes like alpha-amylases and phospholipases within the hydrolase group, including phospholipase A2, a key enzyme in the biosynthesis of inflammatory mediators (Khanapure *et al*., 2007).

- **Anti-Inflammatory Activity**: Tetracyclines demonstrate a broad anti-inflammatory effect, initially observed in conditions like rosacea, where bacterial factors are not directly implicated in pathogenesis (Bahrami *et al*., 2012). Even at doses below the minimal inhibitory concentration, tetracyclines indirectly alleviate inflammation by inhibiting bacterial breakdown products that stimulate inflammatory processes. They reduce the production of pro-inflammatory cytokines by neutrophils, such as interleukin-1β (IL-1β), IL-8, and tumor necrosis factor-α. Moreover, tetracyclines hinder leukocyte migration during the early stages of inflammation by binding intracellular calcium, crucial for microtubule formation enabling cell movement (Webster *et al*., 2007; Lourenço 2020). Tetracyclines also mitigate nitric oxide (NO) production by inhibiting inducible nitric oxide synthase (iNOS) activity, preventing the formation of highly

cytotoxic peroxynitrite radicals responsible for inhibiting collagen synthesis and increasing MMP expression (Trachtman *et al*., 1996). NO is implicated in vessel permeability, edema, and erythema development associated with rosacea (Orylska-Ratynska *et al*., 2022).

- **Antioxidant Effect**: Tetracyclines exhibit antioxidant properties by scavenging reactive oxygen species (ROS) and preventing oxidative damage to cell structures (Griffin *et al*., 2010). The phenolic ring is crucial for retaining ROS, forming stable phenolic radicals upon free radical attachment, which do not undergo further interactions (Kraus *et al*., 2005).

- **Anti-Apoptotic Effect**: Tetracyclines display an anti-apoptotic effect by influencing apoptotic pathways, particularly in psychiatric and neurodegenerative diseases. In vitro studies on animal models reveal a decrease in caspase expression, and tetracyclines can accumulate in mitochondria, potentially altering membrane potential and interfering with mitochondrial DNA-encoded protein synthesis (Chen *et al*., 2000; Sagar *et al*., 2010; Riesbeck *et al*., 1990). The proposed mechanisms include the inhibition of cytochrome c release and alterations in mitochondrial membrane potential (Sagar *et al*., 2010). Studies on minocycline, a tetracycline derivative, have provided significant insights into these anti-apoptotic properties, with positive results observed in animal models, though clinical trials present challenges due to variations in drug doses and the complexity of reproducing certain disease symptoms in animal models (Orylska-Ratynska *et al*., 2022; Romero-Miguel *et al*., 2021).

1.1.6 Assay of Tetracyclines

Tetracycline hydrochloride, chemically defined as (4S,4aS,5aS,6S,12aS)-4-Dimethylamino-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide monohydrochloride, is a widely utilized antibiotic with potent activity against both Gram-positive and Gram-negative bacteria (Sweetman 2009). Various techniques have been developed for the quantitative determination of tetracycline hydrochloride in pharmaceutical formulations and biological samples, encompassing fluorimetry (Zhao *et al*., 2010),

electrochemical methods (Gholivand *et al*., 2013), atomic absorption spectrophotometry (Abdulghani *et al*., 2013), liquid chromatography (Li *et al*., 2013; Gavilán *et al*., 2015), capillary electrophoresis (Nozal *et al*., 2004), and chemiluminescence (Townshend *et al*., 2005; Lau *et al*., 2004). UV-Visible spectrophotometry remains a convenient and cost-effective method for the analytical determination of tetracycline in pharmaceutical formulations. Several spectrophotometric and colorimetric procedures for tetracycline determination in bulk materials and dosage forms are documented in the literature (Ni *et al*., 2010; Hadi *et al*., 2014; Thanasarakhan *et al*., 2011). Recently, there has been a growing interest in developing methods for determining tetracycline antibiotics in environmental samples and food products. The widespread use of tetracycline antibiotics, owing to their low cost, high antimicrobial activity, and relative safety, extends beyond medicine to include applications in animal husbandry (Udalova *et al*., 2015).

A straightforward, precise, and rapid visible spectrophotometric method has been established for determining tetracycline hydrochloride in pure form, pharmaceutical preparations, and environmental water samples. The method relies on the oxidation of tetracycline hydrochloride by sodium hypochlorite in an alkaline medium, producing a red-colored product with an absorption peak at 400 nm. Beer's Law was valid in the concentration range of 2-24 μg/ml, with a molar absorptivity of 1.346×10^4 L.mol^-1.cm^-1. The method exhibited a relative standard deviation of less than 2%, and the accuracy, as indicated by the average recovery, was 100 ± 0.85%. The optimal conditions for color development are detailed, and the proposed method has been effectively applied to determine tetracycline hydrochloride in pharmaceutical preparations, chicken meat, and wastewater samples (Ahmed *et al*., 2018).

1.2 Spectrophotometry and Principles, Instrumentation and Applications of Different Spectrophotometric Techniques

In nature, every compound possesses the inherent ability to interact with light, specifically by either absorbing, transmitting, or reflecting electromagnetic radiation at distinct wavelengths. This characteristic lends itself to quantitative measurement through spectrophotometric techniques. Spectrophotometry, a method centered on evaluating the interaction of light with materials, becomes applicable when light encounters a material capable of reflecting,

transmitting, scattering, or absorbing it. In such interactions, the material may emit absorbed light at a different frequency, a phenomenon attributed to gained energy from the incident light, such as electroluminescence, or from the material's temperature, known as incandescence (Germern *et al*., 2014).

Spectroscopy and spectrophotometry, encompassing various methods, play a pivotal role in identifying and quantifying compounds across research, industrial, and chemical laboratories. For instance, UV-visible spectrophotometry is foundational in fields like chemistry and pharmacy, relying on the application of the Beer-Lambert-Bouguer Law for sample analysis. In biochemistry and molecular biology, spectrophotometric analysis is indispensable for determining the concentration of biomolecules like DNA, RNA, or proteins (Trumbo *et al*., 2013). Clinical laboratories extensively employ both manual and automated spectrophotometric methods for analyzing blood, urine, and body fluid samples (Rand 1972).

Among the diverse spectrophotometric methods, two primary techniques stand out: absorption spectrophotometry, relying on the absorption of radiation at specific wavelengths to generate an absorption spectrum, and UV-visible spectrophotometry, concerned with the reflectance of specific spectra within the UV and visible range of the electromagnetic radiation spectrum (Dadi *et al*., 2022).

A spectrophotometric instrument generally comprises four crucial components: a light/radiation source, a collimator, a monochromator, and a detector. The monochromator includes a fixed entrance slit, a dispersing element (such as a prism or diffraction grating), and a moving exit slit (Kruschwitz 2018).

1.2.1 UV-Visible Spectrophotometry

1.2.1.1 Principle

The fundamental principle of UV-visible spectrophotometry is rooted in the Law of Absorption, famously known as Beer-Lambert law. This law establishes a relationship between the thickness of the absorbing material and the concentration of the sample solution. According to this law, the amount of light absorbed is directly proportional to both the concentration of the absorbing substance and the thickness of the absorbing material (Upadhyay *et al*., 1993).

1.2.1.2 Instrumentation

The UV-visible spectrophotometer comprises a light source, sample holders, a monochromator, and a detector (De Caro *et al*., 2015).

- **Light Source**: UV light sources include hydrogen and deuterium lamps, while visible light typically utilizes tungsten filament lamps.
- **Sample Holders**: UV and visible ranges employ cuvettes made from quartz or ordinary glass, with quartz or silica cells in the UV region and glass cells in the visible region, each having a standard path length of 1 cm.
- **Monochromators**: These components convert polychromatic radiation into monochromatic radiations with narrow bands.
- **Detectors**: Commonly used detectors in the UV and visible range include photovoltaic cells, phototubes, and photomultipliers. A block diagram illustrating the main components of a UV-Visible spectrophotometer is shown in Figure 3 (Upadhyay *et al*., 1993).

1.2.1.3 Applications

UV-visible Spectrophotometry finds diverse applications:

- **Pharmaceutical Analysis**: Applied extensively in determining drug concentrations in pharmaceutical formulations. For instance, it is utilized in analyzing etravirine in bulk and pharmaceutical formulations (Murali *et al*., 2014).
- **Vaporization Studies**: Involved in determining the vaporization of low-volatile compounds in the vapor phase (Verevkin *et al*., 2018).
- **Nucleic Acid Identification**: Used for identifying pure analytes, especially nucleic acids, aiding in the discovery of genetic materials in various organisms, as depicted in Figure 5 (Li *et al*., 2013).
- **Quantification and Identification:** Widely employed in the pharmaceutical industry for quantifying and identifying organic compounds (Dadi *et al*., 2022).
- **Biochemical Analysis:** Essential in biochemistry for determining micromolar concentrations in blood, urine, and body fluids. It is also employed in studying biochemical processes (Dadi *et al*., 2022).

- **Color Index Assessment**: Applied in assessing the Color Index of Transformer Insulating Oil (Leong *et al*., 2018).
- **Protein-Nanoparticle Interaction**: Utilized in a case study combining UV-visible spectroscopy and chemometrics to determine the interaction of human serum albumin (HSA) and gold nanoparticles (AuNPs) (Wang *et al*., 2014).

1.2.2 Infrared Spectrophotometry

1.2.2.1 Principle

Infrared spectrophotometry operates on the principle that when a molecule absorbs light of higher wavelength, vibrational transitions occur in the molecules, leading to the formation of an IR spectrum. These vibrational transitions are a result of electronic transitions when a substance absorbs light energy (Dadi *et al*., 2022).

1.2.2.2 Instrumentation

Similar to UV-visible spectrophotometry, an IR spectrometer comprises a light source, sample holder, monochromator, and detector (Dadi *et al*., 2022).

- **Light Source**: In the near IR-region, xenon and tungsten lamps are typically used as light sources (Mahesar *et al*., 2019).
- **Sample Holders**: Quartz cuvettes are commonly used as sample holders in the IR region (Dadi *et al*., 2022).
- **Monochromator**: Gratings serve as monochromators in the IR region.
- **Detectors**: Common detectors in IR spectrophotometry are based on indium gallium arsenide (InGaAs) semiconductor materials (Dadi *et al*., 2022).

1.2.2.3 Methodology

The energy in the IR spectrophotometer is a function of wavelength and reaches a maximum at a wavelength (μm) equal to \(2897/T\), where \(T\) is the absolute temperature (K). This energy, providing a short-wavelength limit of the spectrum (~ 2 μm), decreases as the wavelength gets longer (Colthup 2003). The radiation energy falling onto the samples induces excitations followed by molecular vibrations, producing an IR spectrum detected by the detectors. The detectors then convert radiation energy into an electrical signal, which is subsequently amplified

and processed to yield a spectrum providing information about various functional groups present in the sample (Dadi *et al*., 2022).

1.2.2.4 Applications

IR Spectroscopy is versatile in various applications:

- **Identification of Compounds:** Assists in identifying chemical compounds and functional groups in organic molecules, enabling the analysis of aliphatic, aromatic, saturated and unsaturated hydrocarbons, amino acids, ether and hydroxyl groups, halogens, nitrogen, phosphorous, silicon, sulfur-oxy compounds, etc. A case in point is the IR spectra of 1-hexene shown in Figure 8 (Johnson *et al*., 2019).
- **Characterization of Nanoparticles**: Plays a crucial role in characterizing nanoparticles, particularly in studying the physicochemical characteristics of drug nanocarriers and identifying functional groups on the surface of developed nanoparticles involved in drug targeting systems (Yaneva *et al*., 2018).
- **Surface Biology Research**: Contributes significantly to surface biology research by studying the surface interaction of drugs and antibodies with cell surface proteins and other biological molecules, aiding in optimizing sensitivity between interacting molecules (Hamers *et al*., 2011).
- **Rate of Reactions**: Provides valuable insights into enzymatic reactions involving various functional groups, allowing the assay of enzymatic activity. For example, the study of pyruvate kinase enzymatic activity with its substrate phospho-enol pyruvate using IR spectroscopy (Kumar *et al*., 2010).
- **Structural Determination of Minerals:** An essential tool in determining the structure of minerals, as demonstrated in a case study on the alterations of chondrules in NWA 2086 CV3 meteorite (Kereszturi *et al*., 2015).

1.2.3 Fourier Transform Infrared Spectroscopy (Ftir)

1.2.3.1 Principle

FTIR operates based on the principles of IR spectroscopy, but it differs significantly in instrumentation from traditional IR spectroscopy (Dadi *et al*., 2022).

1.2.3.2 Instrumentation

The FTIR spectrometer includes components such as a light source, sample holder, monochromator, and detector, resembling an IR spectrophotometer. The major distinction lies in the interferometer, a crucial component making FTIR more advanced. The interferometer comprises a compensator plate, beam splitter, fixed mirror, and scanning mirror, all linked to a detector. The superiority of FTIR over conventional infrared instruments is attributed to its enhanced spectral quality, data collection speed, reproducibility, and ease of maintenance (Titus *et al*., 2019). See Figure 10 for the instrumentation details (Titus *et al*., 2019).

1.2.3.3 Applications

FTIR spectroscopy serves as a potent analytical tool for identifying chemical constituents and elucidating structures in various real-world samples. It finds application in characterizing the unpredictability in the fuel stability of biodiesel and antioxidant samples. FTIR facilitates the identification of organic and inorganic compounds in samples through spectral analysis (Shameer *et al*., 2019). Moreover, it is instrumental in identifying functional groups in polymers and co-polymers. For instance, in the case of poly-3-hydroxybutyrate (PHB), FTIR reveals peaks corresponding to C═O stretching and the adsorption band in the ester group (Sindhu *et al*., 2015). FTIR with attenuated total reflectance (ATR) has been employed in forensic analysis for biochemical information on postmortem interval estimation and assessing the immobilization of active substances in biomedical materials (Kowalczuk *et al*., 2019).

1.2.4 Raman Spectroscopy

1.2.4.1 Principle

Raman spectroscopy, based on the Raman effect described by C.V. Raman in 1928, involves the scattering of light. When monochromatic radiation interacts with sample molecules, the scattered light's frequency differs from that of the incident light, resulting in inelastic scattering (Zhang *et al*., 2017).

1.2.4.2 Instrumentation

Modern Raman spectroscopy instrumentation comprises a light source, prism or grating, and detectors. Mercury lamps were historically used as light sources, but lasers, particularly diode or Nd:YAG lasers, have become standard due to their stable and intensive beams. Grating monochromators are used in dispersive Raman spectrophotometers, while Michelson interferometers are employed in non-dispersive Raman spectrophotometers. Detectors have

evolved from thermoelectrically cooled photomultiplier tubes to more sensitive charge transfer devices like CCDs and CIDs (Dadi *et al*., 2022).

1.2.4.3 Applications

Raman spectroscopy plays a crucial role in cell therapy development, aiding in understanding biochemical and functional characteristics of therapeutic cells. It is also a powerful tool in the molecular diagnosis of cervical cancer, offering insights into biomolecular structures. In agriculture and food systems, Raman spectroscopy is employed for early detection of plant diseases and identifying food adulteration. Additionally, it proves valuable in the biopharmaceutical industry for particle size measurement, contaminant identification, and protein-related analyses (Rangan *et al*., 2020; Ramos *et al*., 2015; Weng *et al*., 2019; Dadi *et al*., 2022; Buckley *et al*., 2017).

1.2.5 Spectro-Fluorometry

1.2.5.1 Principle

Fluorescence, where a molecule emits radiation of longer wavelength after absorbing radiations, is the phenomenon exploited in spectro-fluorometry. It is a short-lived phenomenon providing information about events taking less than 10^-7 seconds (Dadi *et al*., 2022).

1.2.5.2 Instrumentation

Spectrofluorometers differ from spectrophotometers by having two monochromators—one before and one after the sample holder. The sample holder incorporates a temperature maintenance device since fluorescence is maximum between 25 and 30°C (Dadi *et al*., 2022).

1.2.5.3 Applications

Spectro-fluorometry proves valuable in identifying the 3-D structure of proteins, aiding in computational drug discovery. In the food industry, it assists in determining various components, adulterants, additives, and contaminants. Additionally, it is a rapid and sensitive method for quality control in food processing. Common applications include qualitative and quantitative analyses, as well as studies on protein structure (Dadi *et al*., 2022; Misra 2019; Román-Pizarro *et al*., 2018; Karoui 2016).

1.2.6 Atomic Absorption Spectrophotometry (AAS)

1.2.6.1 Principle

AAS operates on the principle that when sample molecules are volatilized, the produced atoms absorb specific wavelengths of light, generating a characteristic atomic spectrum (Dadi *et al*., 2022).

1.2.6.2 Instrumentation

Key components of an atomic absorption spectrophotometer include atomizers (commonly electrothermal atomizers), a light source (hollow cathode lamp), and detectors. Computer systems aid in isolating and quantifying wavelengths of interest and controlling instrument operation (Butcher *et al*., 1998).

1.2.6.3 Applications

AAS proves sensitive and highly selective for determining trace and ultra-trace levels of elements in various samples, such as soil, sediments, plants, and body fluids. It finds applications in toxicological investigations, food quality determination, and water quality assessment (Dadi *et al*., 2022; Calatayud *et al*., 2005).

1.2.7 Nuclear Magnetic Resonance Spectroscopy (NMR)

1.2.7.1 Principle

NMR relies on the excitation of atomic nuclei in sample molecules when subjected to a strong magnetic field and a radiofrequency transmitter. This excitation results in spectral lines in the NMR spectrum (Günther 2013).

1.2.7.2 Instrumentation

NMR spectrometers consist of a radiation source (radiofrequency transmitter), a superconducting magnet, a receiver for the absorbed signal (free induction decay), and a computer system for data processing. The magnet produces a magnetic field ranging from 1 to 10 Tesla, with advanced instruments exceeding 3.5 Tesla (Dadi *et al*., 2022).

1.2.7.3 Applications

In biochemistry, NMR is crucial for metabolic research and the study of intact biological specimens like the heart and kidney. In pharmacy, NMR spectroscopy aids in visualizing single atoms and molecules, facilitating structure elucidation and quantification of various organic molecules. Chemistry applications include the unambiguous identification of novel compounds.

NMR is also employed for quantitative determination of metabolite concentrations and in the petroleum industry for identifying and quantifying hydrocarbons (Fan *et al*., 2016; Misra 2019; Chachaty 1987; Upadhyay *et al*., 1993; Ure *et al*., 2019; Dadi *et al*., 2022).

1.3 Background of Study

Tetracycline antibiotics constitute a primary group employed for veterinary, human therapeutic, and agricultural purposes. Among various antibiotics, special attention is given to tetracyclines due to significant environmental concerns, posing ecological risks and potential harm to human health. Their widespread use has led to evidence indicating the omnipresence of tetracycline antibiotics in various ecological compartments. The effectiveness of pharmaceutical formulations is contingent on the drug's quality and consistency (Daghrir *et al*., 2013). Spectroscopy and spectrophotometry, encompassing various methods, play a pivotal role in identifying and quantifying compounds across research, industrial, and chemical laboratories (Rand 1972). The spectrophotometric evaluation of various brands of tetracycline available in Benin City pharmacies seeks to assess their quality, ensuring therapeutic efficacy and patient safety.

1.4 Problem Statement

A rising worldwide apprehension revolves around the problem of fraudulent pharmaceuticals. Particularly, counterfeit antimicrobial medications present a substantial risk to public health, resulting in severe repercussions for individuals, including heightened mortality and morbidity, along with the emergence of drug resistance. Additionally, healthcare providers attending to these patients face a decline in trust regarding the efficacy of the medications employed and frequently confront elevated levels of bacterial resistance. The issue of spurious and substandard drugs has intensified with the proliferation of the World Wide Web, given that a significant portion of drugs distributed via online pharmacies is identified as counterfeit (Kelesidis *et al*., 2007). In the pharmaceutical market, variations in the quality of tetracycline formulations may exist among different brands. These variations could impact the drug's efficacy and patient outcomes. Therefore, there is a need to systematically evaluate and compare the

spectrophotometric characteristics of multiple tetracycline brands in the local market most especially pharmacies around Edo state.

1.5 Justification Of Study

Ensuring the quality and consistency of pharmaceuticals is paramount for public health. The assessment of tetracycline formulations through spectrophotometry provides a rapid and reliable means of identifying any disparities in drug content among different brands. This study holds significance in safeguarding patient health, supporting regulatory efforts, and contributing to the overall quality assurance of pharmaceutical products in Benin City.

1.6 Aim and Objectives of the Study

The primary aim of the study is to conduct a spectrophotometric analysis of various brands of tetracycline available in Benin City pharmacies.

The objectives include;

1. To compare the spectrophotometric profiles of different tetracycline brands to identify any significant variations.
2. To assess the conformity of tetracycline formulations to established pharmacopeial standards.
3. To provide insights into the quality and consistency of tetracycline products in the local pharmaceutical market.
4. To contribute valuable data for regulatory bodies and healthcare practitioners, aiding in informed decision-making regarding the prescription and use of tetracycline antibiotics.

CHAPTER TWO

2.0 Materials and Methods

2.1 Materials and Apparatus

Distilled water, Dissolution apparatus, UV-VIS spectrophotometer, 15ml PTFE tubes, 100ml Volumetric flask, Pipette, Petri dish, Spatula, Stirrer, Beaker, Measuring Cylinder, Filter paper, Conical flask, Weighing balance, Methanol.

2.1.1 Selection of Sample

Ten (10) brands of tetracycline hydrochloride capsules (250mg) (coded T1-T10) were selected from community pharmacies around Benin city used for this study.

2.2 Methods

2.2.1 Visual inspection

Visual inspection was performed on each brand of tetracycline according to WHO Visual Inspection of Medicines Template (WHPA, 2018). The parameter inspected included the drug packaging, expiry dates, batch number, manufacturing date, drug composition, name of manufacturer, efficiency of packaging materials (primary and secondary), color presentation and others.

2.2.2 Dissolution testing

A study was performed using the Rotating Paddle to ascertain the invitro release using dissolution apparatus (USP Type 11). All brands were evaluated using900ml of distilled water maintained at 37 ± 0.5°C. 10ml aliquots were withdrawn at definite time intervals of 20, 40 and 60 minutes, and replaced with fresh dissolution medium after each withdrawal. All withdrawn samples were then assayed using the T80 Double Beam UV-Visible spectrophotometer at 276nm. The percentages of the drug dissolved from the capsules were then calculated.

2.2.3 UV-VIS spectrophotometric assay

2.2.3.1 Preparation of Standard

0.1g of tetracycline hydrochloride (TCH) was weighed into in a 100ml volumetric flask, dissolved with methanol and made up to mark. 10ml of the stock solution was then transferred into another 100 ml volumetric flask and made up to mark with methanol to obtain 100ppm. Calculated aliquots of standard solution of TCH were transferred into a series of 25 ml volumetric flasks containing 1 ml of 1 N sodium hydroxide and 1 ml of 0.1% sodium hypochlorite solution and diluted to the mark with distilled water to obtain a series of red colored standard solutions.

2.2.3.2 Preparation of Sample

100 mg of tetracycline hydrochloride was accurately weighed into a 100ml volumetric flask. 60ml of methanol was added and the flask was shaken for 20 mins, then made up to mark with methanol. The flask was stoppered and its content mixed vigorously and filtered. 5ml of solution of the sample was thereafter transferred into a 25 ml volumetric flask containing 1 ml of 1 N sodium hydroxide and 1 ml of 0.1% sodium hypochlorite solution. The solution was diluted to the mark with methanol to obtain red-colored product. The absorbance of each red-colored product was measured at 362 nm using the V-750 UV/Visible NIR spectrophotometer.

CHAPTER THREE

3.0 Results

3.1 Visual inspection

All brands passed the test on visual inspection except T7 which presented with golden yellow color different from the characteristic pale-yellow color for standard tetracycline.

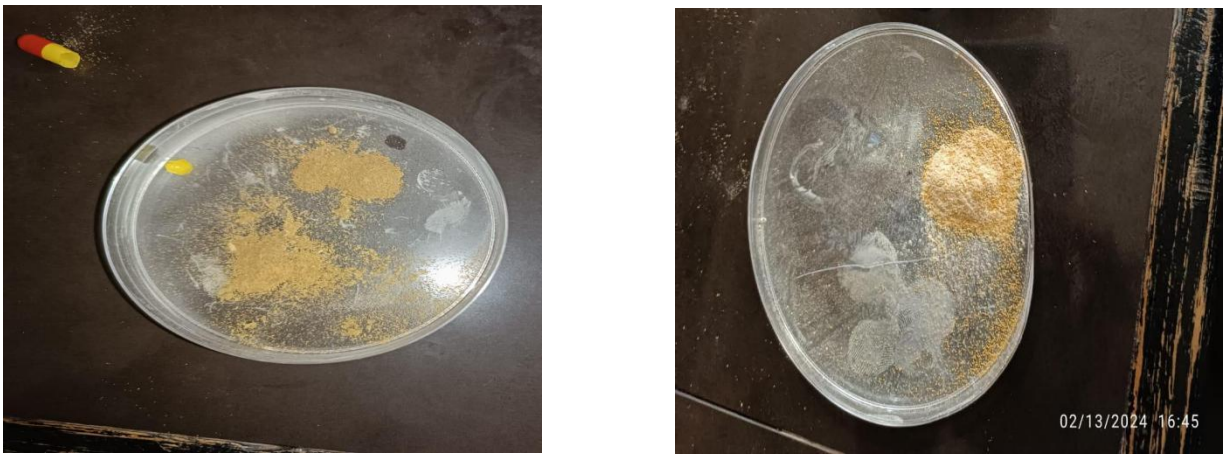

Fig 3.1: Figure a and b showing coloration of T7 (left) and another brand (right)

3.2 Dissolution test

The results of the dissolution testing showed that all brands used for the study passed the dissolution as the test compounds had more than 90% of their content dissolved within the first 60mins of the procedure.

Table 3.1: Dissolution test result for different brands of tetracycline hydrochloride

Brand Code	Time (mins)	Absorbance	% Dissolved
	20	0.21	91.31 ± 1.02
T1	40	0.223	96.96 ± 2.49
	60	0.229	99.57 ± 2.23
	20	0.211	91.75 ± 2.81
T2	40	0.213	92.62 ± 3.03
	60	0.219	95.23 ± 2.39
	20	0.213	92.62 ± 3.02
T3	40	0.227	98.70 ± 1.83
	60	0.228	99.14 ± 2.08
	20	0.228	99.14 ± 1.88
T4	40	0.229	99.57 ± 2.04
	60	0.229	99.57 ± 3.40
	20	0.215	93.49 ± 2.48
T5	40	0.227	98.70 ± 2.15
	60	0.227	98.70 ± 1.99
	20	0.217	94.36 ± 1.63
T6	40	0.225	97.83 ± 1.88
	60	0.225	97.83 ± 1.69
	20	0.214	93.05 ± 2.95
T7	40	0.221	96.09 ± 3.17
	60	0.223	96.96 ± 2.84
	20	0.227	98.70 ± 1.83
T8	40	0.229	99.57 ± 1.80
	60	0.229	99.57 ± 1.83
	20	0.229	99.57 ± 1.94
T9	40	0.224	97.40 ± 2.08
	60	0.229	99.57 ± 1.88
	20	0.224	97.40 ± 2.39
T10	40	0.224	97.40 ± 1.79
	60	0.225	97.83 ± 2.46

Table 3.2: Disolution test result for different brands of tetracycline hydrochloride

BRAND	TIME	ABS	% DISSOLVED
	20	0.21	91.311654
T1	40	0.223	96.9642802
	60	0.229	99.5731846
	20	0.211	91.7464714
T2	40	0.213	92.6161062
	60	0.219	95.2250106
	20	0.227	98.7035498
T3	40	0.228	99.1383672
	60	0.228	99.1383672
	20	0.229	99.5731846
T4	40	0.229	99.5731846
	60	0.229	99.5731846
	20	0.215	93.485741
T5	40	0.227	98.7035498
	60	0.227	98.7035498
	20	0.217	94.3553758
T6	40	0.225	97.833915
	60	0.225	97.833915
	20	0.214	93.0509236
T7	40	0.221	96.0946454
	60	0.223	96.9642802
	20	0.213	92.6161062
T8	40	0.229	99.5731846
	60	0.229	99.5731846

	20	0.215	93.485741
T9	40	0.224	97.3990976
	60	0.229	99.5731846
	20	0.224	97.3990976
T10	40	0.224	97.3990976
	60	0.225	97.833915

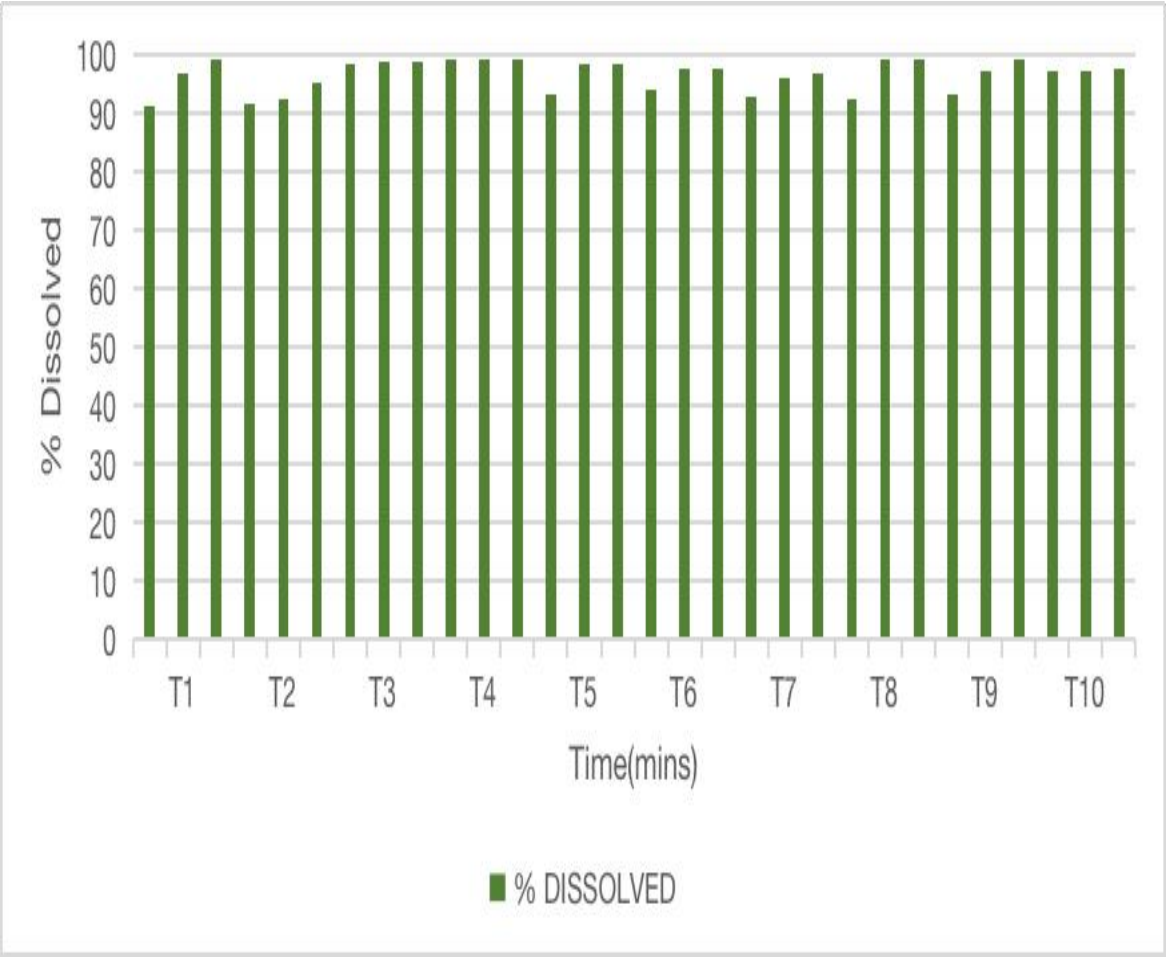

Fig 3.2: A graph depliciting the dissolution of various tetracycline compounds.

3.2 Assay test

The assay/spectrophotometric test showed that the best wavelength of absorbance of tetracycline in methanol is 362nm because it gave the highest peak at that range.

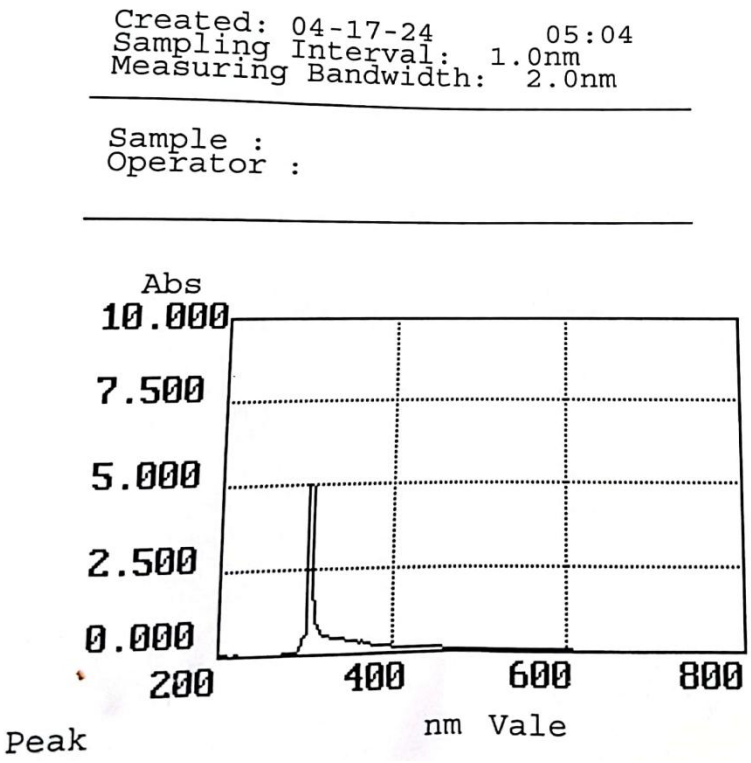

Fig3.3: Absorbance against wavelength of standard tetracycline.

Table 3.3: Absorbance against wavelength of standard tetracycline

NO	WL (nm)	Abs	WL (nm)	Abs
1	362	0.357	282	0.052
2	341	0.486	276	0.042
3	297	5.00	274	0.036
4	270	0.044	268	0.023
5	259	0.041	264	0.028

Table 3.4: Concentration and Absorbance of Standard Tetracycline.

Concentration (µg/ml)	Absorbance
0	0
10	0.038
20	0.072
40	0.14
80	0.285
100	0.357

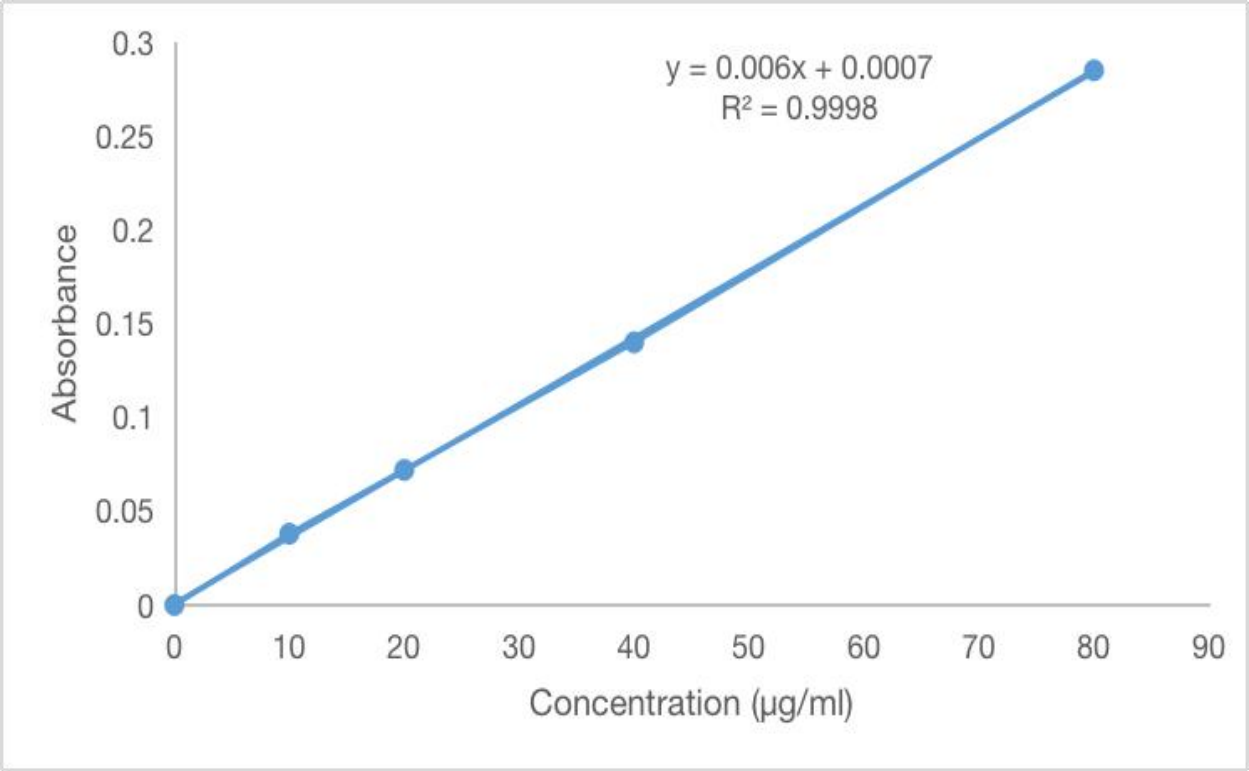

Fig 3.4: Graph of Concentration against Absorbance

Table 3.5: Table of Absorbance and Concentration of the various Tetracycline brands

S/N	ABSORBANCE	CONCENTRATION [mcg/ml]
T1	0.332	92.03
T2	0.338	94.00
T3	0.333	92.3
T4	0.345	95.63
T5	0.331	91.75
T6	0.328	90.91
T7	0.325	90.08
T8	0.357	98.97
T9	0.332	92.03
T10	0.329	91.19

CHAPTER FOUR

4.0 Discussion

4.1 Visual inspection

4.1.1 Drug Packaging

The visual inspection of all brands of tetracycline drug packagings used supports their compliance with WHO's Visual Inspection of Medicines Template. The results indicate that the packaging materials used for different brands were up to standard and represented 100% efficient protection of the drug product.

The pharmacopoeia states that the inspected parameters included the drug packaging, expiry dates, batch number, manufacturing date, drug composition, manufacturer's name, and the effectiveness of the packaging materials (primary, secondary)., among others (USP/NF, 2023). Apart from T7, all the remaining tested tetracycline brands passed the visual packaging quality inspection tests, signifying that the companies provide good quality packaging.

4.1.2 Colour

The visual inspection of the samples showed that tetracycline hydrochloride capsules except brand T7 had the shade of pale yellow color as is usual in tetracycline hydrochloride. On the other hand, the color scheme of the T7 brand featured golden-yellow tones as opposed to the usual.

The observed color difference on T7 brand might well be the sign of inappropriate product formulation, quality or breach in good manufacturing practice (Egbuna, Chukwuebuka 2019). Tetracycline is usually pale-yellow in color, and any color deviation could indicate a formulation or raw quality material issue or product degradation problem.

4.2 Dissolution test

4.2.1 Dissolution Test Results for Different Brands of Tetracycline Hydrochloride

The dissolution test outcome proved all the tetracycline hydrochloride brands tested to be within the acceptable 90% drug release conditions within the first 60 minutes of testing. This finding is particularly significant because it shows that the drug formulations were efficiently able to dissolve the active components in the aqueous media thus, which is the principal factor of a drug formulation in terms of its bioavailability and therapeutic efficacy.

The data shown in Tables 1 and 2 of the Result Document demonstrate the dissolution profile of the various tetracycline types. Each brands of T1 through T10 indicates a more than 90% drug dissolution within the first 60 minutes of the test. The in vitro release profile characterized by consistency across the different brands suggests that the manufacturers have developed potent formulations and processes for manufacturing to ensure the required in vitro release attributes of the tetracycline products.

The slight variations noted in the dissolution profiles among the various brands can be explained by the formulation composition, the manufacturing process used and the quality of the raw materials. However, albeit some brands that exhibited minor differences yet all of the brands had

good enough dissolution performance within the given time frame and thereby satisfying the pre-established dissolution standard criteria.

The stable and constantly converting nature of these brands observed during the analyses indicate the quality and probability of tetracycline products absorption. The fact that this medicine can be delivered to the body in consistent and reliable quantities is what makes the tetracycline formulations applicable to being able to achieve the desired results.

4.3 Assay test

4.3.1 Absorbance changes against wavelength of pure Tetracycline

The absorbance spectrum for the standard tetracycline hydrochloride solution was determined using UV-Vis spectrophotometric analysis and the maximum peak (highest) was found at 362 nm. This result agrees with the literature since it has been established that Tetracycline hydrochloride has measures its maximum absorbancy in the UV region with a specific wavelength between 360-370 nm as per the most common spectrum.

Figure 3.3 above is a diagram showing the absorbance data of the reference solution of tetracycline when measured at different wavelengths. These wavelengths are between 264 nm and 362 nm. The absorbance point of maximum absorption (0.357) was at 362 nm, confirming that the suitable and sensitive detection indeed fell at this wavelength for quantitation of tetracycline.

The wavelength of 362 nm is selected by titration procedures for the assay, which is a commonly used well-established approach in the spectrophotometric determination of tetracycline. This specific wavelength provides a good compromise between sensitivity and specificity because there are very few chances of the drug microparticle sample to be affected by other constituents of the formulation.

4.2.3 Absorbance and concentration of the various tetracycline brands

The data obtained from a number of experiments showed that there was a very strong linear relationship between the absorbance and the concentration of the standard tetracycline hydrochloride solution, as is evident in both the Table 4 and the subsequent graph. The straight-line regression analysis result gave the highest value of the correlation coefficient ($R^2 = 0.9998$) which proved the confidence and precision of spectrophotometric method for the determination of tetracycline in the quantitative form

The data depicted in the table of "Absorbance and the Concentration of the Various Tetracycline Brands" is the result of the absorbance measurement of the various tetracycline products and the calculation of their drug concentrations. The concentrations of drug ranged from 91.19 µg/mL to 98.97 µg/mL, but majority of the products had a range that was relatively close of each other.3.2.2Absorbance against wavelength of standard tetracycline

The above result indicates that the drug is present in the proper amount in each of the trial formulation that fall in the acceptable range. The uniformity of drug concentrations among various brands of the same medicine intensively promotes the level of quality and confidence of the production process of the pharmaceutical companies.

The high linearity of the standard curve and the good drug content results of the various tetracycline trademarks suggest the compatibility and robustness of the UV-Vis spectrophotometric method to be used in the quantitative analysis of tetracycline in pharmaceutical formulations. This analytical approach can be equivalently used for the application of routine QC and QA practices.

CHAPTER FIVE

5.1 CONCLUSION

The present study was carried to extensively assess different brands of tetracycline hydrochloride readily available in Benin City pharmacies in Nigeria. The research included visual examination, dissolution testing, and spectrophotometric analysis of all tetracycline mixtures.

The visual examination of all tested brands' drug packaging demonstrated compliance with the set WHO standards, suggesting that the packaging materials were in good condition and effective in safeguarding the drug products. Nevertheless, T7 possesses a deviation from the characteristic pale-yellow color of tetracycline hydrochloride as the these tablets are colorful yellow. The discrepancy might indicate these two brands' different T7 formulations, therefore, more detailed study may be needed.

The dissolution test outcomes were highly satisfactory since the pharmacopeial criteria were not only met but exceeded, as all brands had a drug release of more than 90% in the very first sixty minutes. This result proves that the formulations were able to release the active ingredient rapidly in the aqueous solution, a pre-requisite for delivering therapeutic efficacy with good bioavailability.

The spectrophotometric testing indeed demonstrated that the 362 nm wavelength would be the best choice for a quantitative determination of tetracycline owing to the most distinct absorbance line for the standard solution. The linear regression analysis of the standard curve revealed a very high correlation coefficient (R^2=0.9998), which indicates the specificity and reliability of the spectrophotometric process for the determination of tetracycline.

The analysis results of different tetracycline brand showed the drugs concentrations ranged 91.19 to 98.97. All the contents were in the acceptable range according to the national standard. The conformity in the drug concentrations from one brand to another strengthens the role of the quality and the trustworthiness of the manufacturing process by the respective pharmaceutical companies.

The conclusion of this research shows that the analysed tetracycline brands follow the official monographs, except for the color deviation that was noted in the T7 tablets. Satisfactory results

of dissolution profiles and an accurate spectrophotometric assay prove the quality and consistency of the available tetracycline medications in the local pharmaceutical market.

5.2 Contribution to Knowledge

Such conclusions thus contribute to the body of knowledge in this field and help the regulatory bodies and medical practitioners in making competent decisions concerning tetracycline antibiotics prescription and use. The next step of investigation may be required to identify the reason of color difference observed at T7 brand and also to maintain the quality and safety of tetracycline in the market.

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