

**STABILITY STUDIES OF METRONIDAZOLE SUSPENSION UNDER VARYING  
ENVIRONMENTAL AND STORAGE CONDITIONS USING PET BOTTLES**



**BY**

**AGBONTAEN BLESSING UHUNOMA IWINOSA**

**PHA1808328**

**DEPARTMENT OF PHARMACEUTICS AND PHARMACEUTICAL TECHNOLOGY**

**FACULTY OF PHARMACY**

**UNIVERSITY OF BENIN**

**BENIN CITY**

**FEBRUARY, 2025**

**STABILITY STUDIES OF METRONIDAZOLE SUSPENSION UNDER VARYING  
ENVIRONMENTAL AND STORAGE CONDITIONS USING PET BOTTLES**



**BY**

**AGBONTAEN BLESSING UHUNOMA IWINOSA**

**PHA1808328**

**A PROJECT SUBMITTED TO THE DEPARTMENT OF PHARMACEUTICS AND  
PHARMACEUTICAL TECHNOLOGY IN PARTIAL FULFILMENT OF THE  
REQUIREMENT FOR THE AWARD OF DOCTOR OF PHARMACY (PHARM. D)  
DEGREE OF THE UNIVERSITY OF BENIN, BENIN CITY, EDO STATE.**

**FEBRUARY, 2025.**

**CERTIFICATION**

We hereby certify that this work was carried out by **AGBONTAEN BLESSING UHUNOMA** in the department of Pharmaceutics and Pharmaceutical Technology. Faculty of Pharmacy, University of Benin, Nigeria as a project in partial fulfilment of the requirements for the award of Doctor of Pharmacy degree by the University.

**PROF.(MRS.) FLORENCE EICHIE**  
(Supervisor)

\_\_\_\_\_  
**DATE**

**PROF SLYVESTER ERAGA**  
(Head of Department)

\_\_\_\_\_  
**DATE**

**AGBONTAEN BLESSING UHUNOMA.**  
(Student)

\_\_\_\_\_  
**DATE**

## **DEDICATION**

I dedicate this work to the Almighty God for His endless grace and guidance and to myself for the strength and determination to complete this journey.

## ACKNOWLEDGEMENT

First and foremost, I am grateful to God for the strength and inspiration throughout this journey. My neverending appreciation goes to my loving parents, Dr Agbontaen for his support and wonderful advice and Mrs Egheosa Agbontaen who have been my pillar of support and motivation. My heartfelt appreciation goes to my Big Mum, Mrs Bose Okundaye for her endless support and care throughout this pharmacy school. I am very grateful for your love.

I want to thank my project Supervisor, Prof (Mrs.) Florence Eichie for her guidance and mentorship. God bless you greatly ma.

I would also like to acknowledge Prof Eraga for his guidance, you are appreciated Sir. And to my amazing friends Eliana Ella, Godsplan, Feh, Jennifer, Shawna, Glorious, Amy, Dani, Naomi, Roy, Peter, Osaode, I want to say thank you for the steady encouragement, support and well wishes.

## TABLE OF CONTENT

|   |      |
|---|------|
| COVER PAGE  |      |
| TITLE PAGE  | ii   |
| CERTIFICATION   | iii  |
| DEDICATION  | iv   |
| ACKNOWLEDGEMENT   | v    |
| TABLE OF CONTENTS   | vi   |
| ABSTRACT  | vii  |
| <b>CHAPTER ONE</b>  |      |
| 1.0 INTRODUCTION  | 1    |
| 1.1 ENVIRONMENTAL FACTORS AND STORAGE CONDITIONS                                      | 2    |
| 1.2 PLASTICS  | 4    |
| 1.3 PACKAGING OF PHARMACEUTICALS  | 6 16 |
| 1.4 DRUG-PLASTIC INTERACTION  | 7 17 |
| 1.5 METRONIDAZOLE   | 8 18 |
| 1.6 OVERVIEW OF PET MATERIALS AND ITS IMPACT ON METRONIDAZOLE                         | 12   |
| 1.7 METRONIDAZOLE AND ITS PACKAGING CONSIDERATONS                                     | 16   |
| <b>CHAPTER TWO</b>  |      |
| 2.0 MATERIALS AND METHODS   | 33   |
| 2.1 MATERIALS   | 33   |
| 2.2 METHOD  | 34   |
| 2.2.1 EVALUATION OF METRONIDAZOLE SUSPENSION BY POLYETHYLENE<br>TEREPHTHALATE BOTTLES | 35   |
| 2.2.2 PREPARATION OF STANDARD CALIBRATION CURVE.                                      | 36   |
| 2.2.3 ANALYTICAL METHOD AND STATISTICAL ANALYSIS.                                     | 36   |
| <b>CHAPTER THREE</b>  |      |
| 3.0 RESULTS AND DISCUSSION  | 38   |
| 3.1 RESULTS OF METRONIDAZOLE CONCENTRATION VS. TIME                                   | 40   |
| <b>CHAPTER FOUR</b>   |      |
| 4.1 CONCLUSION  | 50   |

|                    |    |
|--------------------|----|
| 4.2 RECOMMENDATION | 50 |
| REFERENCE          | 51 |
| APPENDIX           | 55 |

## ABSTRACT

### **Background:**

Metronidazole, a widely used antimicrobial agent, is susceptible to environmental factors that may compromise its stability and efficacy. This study investigates the stability of metronidazole suspension stored in polyethylene terephthalate (PET) bottles under different environmental conditions. Given the potential interaction between PET packaging and liquid pharmaceuticals, it is crucial to assess how temperature, light exposure, and pH affect drug degradation.

**Aim:** The purpose of this study is to evaluate the stability of metronidazole suspension under various environmental conditions to determine the optimal storage practices and highlight the impact of temperature, light exposure, and packaging materials on drug degradation and drug concentrations.

### **Method:**

Metronidazole suspensions were formulated and stored under various conditions, including refrigerator (4°C), room temperature (25°C), elevated temperature (55°C), sunlight exposure (38°C), and acidic and basic pH environments. Spectrophotometric analysis at 277 nm was conducted weekly for 10 weeks to determine the concentration of metronidazole remaining in each sample. The percentage of the drug remaining was calculated for each condition to evaluate the extent of degradation.

### **Result:**

By week 10, samples stored under elevated temperature (55°C) and sunlight exposure (38°C) showed significant degradation, with metronidazole content reduced to 27.33% and 41.90%, respectively. At room temperature (RT) it reduced to 76.34%, at acidic pH (AP) to 69.04%, and basic pH (BP) to 58.62%. Refrigerated storage (4°C) and dark storage provided optimal stability, maintaining over 94% of the initial drug concentration.

### **Discussion:**

The results indicate that temperature and light are critical factors affecting the stability of metronidazole in suspension. Elevated temperatures accelerate degradation, likely due to increased hydrolysis and photodegradation. In contrast, refrigeration effectively preserves the drug's stability, reducing the risk of degradation. These findings emphasize the importance of storage conditions for maintaining the efficacy of metronidazole.

### **Conclusion:**

Refrigeration and storage in light-protected environments are recommended for maintaining the stability of metronidazole suspension. Further studies on alternative packaging materials and the long-term effects of extreme pH conditions could provide additional insights into improving drug formulation and packaging.

# CHAPTER ONE

## 1.0. INTRODUCTION AND LITERATURE REVIEW

The stability and efficacy of pharmaceutical formulations are significantly influenced by their storage conditions and the materials used in their packaging. The pharmaceutical industry frequently uses polyethylene terephthalate (PET) bottles because of its advantageous qualities, which include strength, clarity, and chemical resistance (Chandran et al., 2019). However, environmental factors including temperature, exposure to light, and length of storage can affect the quality of the drugs contained in PET bottles as well as the integrity of the bottles themselves.

The degradation of polyethylene terephthalate (PET) under various environmental conditions has received a lot of attention due to the increasing usage of PET in packaging materials, particularly for beverages and medications. Understanding how environmental factors, like temperature, UV radiation, moisture, and storage conditions, impact the structural integrity and chemical characteristics of PET is crucial for ensuring the safety and effectiveness of products kept in PET bottles. The usage of drugs like metronidazole, which come in polyethylene terephthalate (PET) packaging, raises further concerns about the potential for chemical migration and degradation, which could compromise both the material's durability and the medication's efficacy. This study of the literature looks at the interaction between PET and various storage and environmental conditions, with a focus on the effects on product safety and package stability.

Metronidazole, an antibiotic and antiprotozoal agent, is frequently formulated as an oral suspension for the treatment of various infections. Inadequate storage conditions can jeopardize

the stability of metronidazole suspensions. For example, it has been demonstrated that exposure to high temperatures and extended storage times increases the migration of chemicals like bisphenol A (BPA) and antimony from PET bottles into their contents, which may compromise the medication's safety and effectiveness.

Furthermore, poor storage conditions may cause shrinkage and decreased stress crack resistance in PET bottles, among other physical changes that could jeopardize the integrity of the container and, in turn, the quality of the pharmaceutical product. Given these factors, it is imperative to look at how environmental factors and storage conditions affect PET bottles that contain metronidazole suspension. The stability, effectiveness, and safety of the drug throughout its shelf life will be ensured by following best practices for handling and storage based on an understanding of these impacts.

## **1.1 Environmental factors and storage conditions**

They have a vital role in sustaining the quality, efficacy, and safety of pharmaceutical goods. Factors such as temperature, humidity, and light exposure can dramatically alter the stability of drugs, potentially leading to diminished potency or the production of dangerous degradation products.

### **1.1.1 Temperature**

One major issue with medication storage is temperature variations. A certain temperature range, usually between 15°C and 25°C (59°F and 77°F), is necessary for the preservation of many drugs. The stability of drugs may be jeopardized by exposure to temperatures outside of this range. For example, excessive heat can break down the active chemicals in medications, making

them less effective or even dangerous. According to a study, in order to prevent degradation, drugs should not be exposed to temperatures more than 30°C (86°F).

### **1.6.2 Humidity**

Pharmaceutical items, especially those in solid dose forms like pills and capsules, can potentially be impacted by humidity levels. The active components may deteriorate due to hydrolysis brought on by too much moisture. On the other hand, extremely low humidity might make some compositions fragile. Wherever medications and supplies are kept, proper environmental control is crucial, including preserving the right humidity levels.

### **1.6.3 Light Exposure**

Certain drugs can undergo photodegradation when exposed to light, particularly ultraviolet (UV) light, which can result in decreased effectiveness or the production of hazardous byproducts. To mitigate this risk, sensitive pharmaceuticals are often packaged in opaque or amber-colored containers that block or limit light exposure.

### **1.6.4 Storage Practices**

Maintaining the quality of medications requires careful adherence to suggested storage procedures. This entails keeping medications out of direct sunlight, in suitable packaging, and in conditions with regulated humidity and temperature. Monitoring storage conditions on a regular basis is also crucial. For instance, in Malawi, to guarantee adherence to suggested storage conditions, temperature readings in pharmacies and spaces used for storing medications were automatically recorded every 15 minutes.

## **1.2 PLASTICS**

Plastics are versatile materials composed of polymers that can be molded, extruded, or pressed into different shapes. The adaptability of plastics in manufacturing makes them suitable for a wide range of applications. They are lightweight, durable, flexible, and cost-effective to produce, which has led to their widespread use in modern society. While most plastics are derived from fossil fuels like oil and natural gas, recent advancements have introduced renewable alternatives made from materials like corn and cotton derivatives (Sharma et al., 2018; Manjula et al., 2020).

### **1.2.1 Etymology**

The term "plastic" originates from the Greek word *plastikos*, meaning "capable of being shaped or molded," which in turn comes from *plastos*, meaning "molded" (Liddell & Scott, 2011). The term plasticity specifically refers to the ability of materials to deform and take on various shapes, which allows plastics to be molded, extruded, or compressed into different products such as films, fibers, plates, and containers (Sundararajan et al., 2019).

### **1.2.2 Structure of Plastics**

Plastics are primarily composed of organic polymers, which are long chains of carbon atoms, often with attached oxygen, nitrogen, or sulfur atoms. These chains are made up of repeating units called monomers, and the polymer structure typically consists of several thousand monomers (Ebbing & Gammon, 2016). The properties of plastics depend on the type of polymer used and the arrangement of the monomers.

### **1.2.3 Properties and Classification of Plastics**

Plastics can be classified based on the chemical structure of the polymer backbone and side chains. Major categories include acrylic, polyester, silicone, polyurethane, and halogenated plastics. Additionally, plastics can be classified according to the manufacturing processes, such as condensation, polymerization, or cross-linking (Joanne & Steffanie, 2007). Key physical properties include hardness, density, tensile strength, thermal resistance, and glass transition temperature. Plastics can also be categorized based on their resistance to environmental factors like solvents, oxidation, and radiation (Kent, 2008).

Thermoplastics are one of the most commonly used types of plastic. These are polymers that become soft and moldable when heated and solidify upon cooling, making them recyclable. Some examples include polyethylene (used in packaging and containers), polypropylene (used in food containers and medical devices), and polyvinyl chloride (PVC), which is used in pipes and flooring (Baeurle et al., 2006). These plastics offer advantages like recyclability, energy-efficient processing, and good chemical resistance. However, they are less suited for high-temperature applications due to their low melting points.

On the other hand, thermosetting plastics, such as vulcanized rubber and fiberglass, irreversibly harden upon heating. These materials cannot be remolded once they have been set, but they provide advantages such as high thermal stability, corrosion resistance, and strength (Anne Marie Helmenstine, 2022).

## **1.3 PACKAGING OF PHARMACEUTICALS**

Packaging refers to the science and art of enclosing or protecting products for distribution, storage, and use (Soroka, 2002). In the pharmaceutical industry, packaging plays a critical role in protecting the product, maintaining its integrity, and ensuring safety.

### **1.3.1 Purpose of Packaging**

The primary purposes of packaging include physical protection from mechanical damage, providing a barrier against contaminants (like oxygen and moisture), and ensuring proper containment (Lee et al., 1998). Packaging also transmits information about the product, such as instructions, expiration dates, and handling guidelines (Severin, 2007). Furthermore, packaging can serve as a marketing tool, helping to attract consumers and communicate brand identity (Johnston, 1997).

### **1.3.2 Types of Packaging**

Packaging is categorized into three main types: primary, secondary, and tertiary packaging. Primary packaging is in direct contact with the product, such as bottles or blister packs. Secondary packaging groups several primary packages for storage and sale, while tertiary packaging is used for bulk transportation and storage, typically in the form of pallets or large containers.

### **1.3.3 Ideal Packaging Requirements**

Ideal packaging should protect the product from external environmental conditions, preserve its quality, and ensure that it does not alter the product's appearance or characteristics. Additionally,

it must comply with regulatory requirements, such as those set by organizations like NAFDAC, and be tamper-evident to ensure safety (Severin, 2007).

#### **1.3.4 Plastic Packaging**

Plastic packaging involves the use of plastics to enclose and protect products. Plastics are favored for packaging due to their versatility, cost-effectiveness, and durability (Sharma et al., 2018). They are lightweight, resistant to corrosion, and can be made tamper-proof, which makes them ideal for products like pharmaceuticals and food.

#### **1.3.5 IMPORTANCE OF PLASTICS AS PACKAGING MATERIALS**

Plastics offer numerous benefits, including durability, safety, hygiene, and ease of manufacturing. Their light weight reduces transportation costs, while their strength and flexibility allow for diverse packaging shapes and sizes. Additionally, plastic packaging is compatible with a wide range of materials and processes, making it ideal for mass production (Sundararajan et al., 2019).

### **1.4 DRUG-PLASTIC INTERACTION**

Drug-plastic interactions refer to the potential effects that materials used in plastic containers may have on pharmaceutical products. These interactions can alter the stability of the drug or release harmful substances from the plastic (Fitzpatrick et al., 2017).

#### **1.4.1 Categories of Drug-Plastic Interactions**

Drug-plastic interactions can be classified into four main categories: leaching, chemical reactivity, permeation, and sorption (Fitzpatrick et al., 2017). Leaching occurs when components

of the plastic leach into the drug solution, while chemical reactivity involves chemical reactions that modify the plastic. Permeation refers to the movement of gases or liquids through the plastic, which may affect the drug, and sorption describes the loss of drug components to the plastic container. Understanding these interactions is critical for ensuring the stability and safety of pharmaceutical products.

## 1.5 METRONIDAZOLE

Metronidazole is an **antimicrobial** and **antiprotozoal** agent used in the treatment of various infections caused by **anaerobic bacteria** and **protozoa**. It is frequently prescribed for conditions such as **bacterial vaginosis**, **trichomoniasis**, **giardiasis**, and **Clostridium difficile** infections. Metronidazole works by disrupting microbial DNA, inhibiting replication, and leading to cell death. This drug is often used as part of a multi-drug regimen for **Helicobacter pylori eradication** and in the treatment of **intestinal amoebiasis** (Atherly et al., 2013; Saha et al., 2018).

### 1.5.1 Classification of Metronidazole

Metronidazole belongs to the **nitroimidazole** class of drugs, which are characterized by the presence of an **imidazole ring** and a **nitro group** in their chemical structure. These compounds exhibit **antimicrobial** and **antiprotozoal** properties through their ability to interact with microbial DNA. Metronidazole is specifically classified as an **anaerobic antimicrobial** and is commonly used in the treatment of infections caused by **anaerobes** and **protozoa** (Berg et al., 2015; Hazen, 2018).

### 1.5.2 Mechanism of Action (MOA)

The pharmacodynamics of metronidazole are based on its selective activation within the target organism under **anaerobic conditions**. The drug is a **prodrug**, which means it requires activation inside the microbial cell. The reduction of the nitro group by **nitroreductase** enzymes generates a reactive intermediate that binds to microbial DNA, causing **strand breakage** and inhibiting DNA replication (Barkley et al., 2014). This results in **bactericidal** and **antiprotozoal** effects (Cannon, 2017).

Metronidazole's mechanism is **anaerobic-specific** since **aerobic** organisms lack the necessary enzymes to activate the drug effectively (Brown et al., 2012). This selective activation makes metronidazole highly effective against **anaerobic bacteria** and **protozoa** such as *Trichomonas vaginalis*, *Giardia lamblia*, and *Entamoeba histolytica* (Haas et al., 2014). The drug is less effective against **aerobic bacteria**, as they do not possess the nitroreductase system necessary for its reduction and activation (Spinks et al., 2013).

### 1.5.3 Clinical Use

Metronidazole is commonly used for the treatment of infections caused by **anaerobic bacteria** and **protozoa**, with the following primary clinical indications:

#### 1. **Bacterial Infections:**

- **Anaerobic infections:** Metronidazole is highly effective against **anaerobic infections** such as **bacterial vaginosis**, **pelvic inflammatory disease**, **intra-abdominal abscesses**, and **colorectal infections** (Van den Broek et al., 2018). It is often used in combination with other antibiotics to provide broader coverage.

- **Clostridium difficile:** Metronidazole is one of the first-line treatments for **C. difficile** infections, especially in less severe cases (McFarland et al., 2015).
- **Helicobacter pylori:** In combination with other drugs, metronidazole is used in the treatment of **H. pylori** infections, which are implicated in the pathogenesis of **peptic ulcers** (Graham et al., 2011).

## 2. Parasitic Infections:

- **Giardiasis:** Caused by *Giardia lamblia*, this parasitic infection is commonly treated with metronidazole (Giglio et al., 2012).
- **Amebiasis:** Metronidazole is a treatment of choice for infections caused by *Entamoeba histolytica*, which can result in severe gastrointestinal disease (Stark et al., 2017).
- **Trichomoniasis:** Metronidazole is highly effective for treating **trichomoniasis**, a sexually transmitted infection caused by *Trichomonas vaginalis* (Van der Pol et al., 2014).

## 3. Topical Use:

- **Rosacea:** In addition to systemic administration, metronidazole is available in topical formulations for the treatment of **rosacea**, a chronic inflammatory skin condition (Bamberger et al., 2013).

### 1.5.4 Pharmacology of Metronidazole

The pharmacokinetics of metronidazole involve rapid absorption after oral administration, with peak plasma concentrations occurring within 1 to 2 hours and a bioavailability of approximately 80% (Seyler et al., 2014); wide distribution throughout the body, including therapeutic concentrations in the liver, lungs, kidneys, and cerebrospinal fluid, with the ability to cross the

placenta and be excreted in breast milk (Barkley et al., 2014); metabolism primarily in the liver via cytochrome P450 enzymes through oxidation and glucuronidation, producing inactive metabolites (Brown et al., 2012); and renal excretion, with 50-60% of the drug eliminated unchanged in urine and a half-life of 6 to 8 hours, which may be prolonged in patients with renal or hepatic impairment (Atherly et al., 2013; Harris et al., 2017). Special considerations include the need for dose adjustments in patients with liver disease due to reduced metabolism and increased plasma concentrations (Barkley et al., 2014); potential delays in elimination in individuals with renal dysfunction, requiring dosage modifications (Atherly et al., 2013); and its classification as pregnancy category B, indicating general safety during pregnancy, though caution is advised in the first trimester, as well as during breastfeeding due to its excretion in breast milk (McFarland et al., 2015).

### **1.5.5 Adverse Effects of Metronidazole**

Metronidazole is generally well-tolerated, but side effects, particularly with prolonged therapy or high doses, may include gastrointestinal symptoms such as nausea, vomiting, diarrhea, and a metallic taste (Berg et al., 2015); neurological effects like headaches, dizziness, and seizures, which can be severe in rare cases (Barkley et al., 2014); hypersensitivity reactions such as skin rashes and pruritus, with rare severe cases including Stevens-Johnson syndrome and toxic epidermal necrolysis (Hazell, 2017); a disulfiram-like reaction when alcohol is consumed, leading to flushing, nausea, headaches, and abdominal cramps (Brown et al., 2012); hematological issues like leukopenia and thrombocytopenia, particularly with prolonged use

(Van den Broek et al., 2018); and, although rare, hepatotoxicity, especially in patients with pre-existing liver disease or those on long-term therapy (Stark et al., 2017).

Metronidazole is a potent antimicrobial and antiprotozoal drug with a well-established mechanism of action. While generally safe and effective for the treatment of anaerobic infections, appropriate monitoring is necessary, particularly in patients with liver or renal dysfunction. Its **adverse effects** are generally dose-dependent and can be managed by adjusting the dose or discontinuing therapy when necessary.

## **1.6 Overview of PET Materials and Its Impact on Metronidazole Degradation**

Polyethylene terephthalate (PET) is widely used for packaging due to its durability and chemical resistance. However, its degradation under environmental conditions can influence the stability of drugs like **metronidazole**, particularly when stored in PET containers. Structural factors such as **crystallinity, surface morphology, polymer flexibility, and molecular weight** affect PET degradation (Sevilla et al., 2023). As PET breaks down, changes in **polymer permeability and chemical leaching** can create conditions that accelerate metronidazole degradation.

### **Enzymatic Degradation of PET and Its Influence on Metronidazole Stability**

The enzyme PETase from *Ideonella sakaiensis* degrades PET by hydrolyzing ester bonds, producing **terephthalic acid and ethylene glycol** (Sevilla et al., 2023). This degradation process increases **polymer porosity**, potentially allowing external factors such as **moisture, oxygen, and light** to penetrate PET packaging more easily. These factors contribute to **oxidative hydrolysis**

**and photodegradation of metronidazole**, leading to the formation of degradation byproducts that reduce its efficacy.

### **Environmental Conditions Affecting PET and Metronidazole Degradation**

Several environmental factors influence both PET degradation and metronidazole stability:

- **Temperature:** PET degradation increases at **higher temperatures**, promoting chain scission and oxidation (Sevilla et al., 2023). Elevated temperatures also **accelerate metronidazole hydrolysis**, particularly under humid conditions, leading to the breakdown of its nitroimidazole structure.
- **pH Levels:** PETase enzymes function best at pH 7–8 (Sevilla et al., 2023), and **metronidazole stability is also pH-dependent**, with increased degradation in alkaline or highly acidic conditions.
- **Moisture Content:** PET hydrolysis is facilitated by moisture (Sevilla et al., 2023), and excess moisture also accelerates **metronidazole hydrolysis**, breaking down its active compound.
- **Oxygen Availability:** Increased PET degradation under aerobic conditions (Sevilla et al., 2023) correlates with **oxidative degradation of metronidazole**, leading to inactive degradation products.

### **Effect of UV Exposure on PET and Metronidazole**

UV exposure significantly affects PET degradation by inducing **photo-oxidation**, which weakens the polymer structure and enhances permeability (Chaisupakitsin et al., 2019). This increased permeability allows greater exposure of **metronidazole to UV light**, leading to

**photodegradation** of its active compound. Metronidazole degradation under UV exposure results in **loss of antimicrobial activity** due to the breakdown of the nitro group in its structure.

### **Long-term Sunlight Exposure and PET-Metronidazole Degradation**

Extended sunlight exposure causes **photo-degradation, hydrolysis, aldehyde formation, and chain scission** in PET, leading to reduced mechanical stability (Chaisupakitsin et al., 2019). This degradation process allows:

- **UV penetration**, further accelerating metronidazole photodegradation.
- **Increased hydrolysis**, contributing to faster breakdown of metronidazole in humid conditions.
- **Potential contamination** from PET degradation byproducts such as aldehydes, which may interact with metronidazole, altering its chemical composition.

### **Environmental Factors Affecting PET and Metronidazole Stability Under Sunlight**

- **Higher temperatures** accelerate both PET hydrolysis and metronidazole degradation (Chaisupakitsin et al., 2019).
- **Moisture content** enhances hydrolysis in both PET and metronidazole, leading to breakdown of ester bonds and nitroimidazole rings, respectively.
- **UV intensity and oxygen levels** promote PET oxidation and metronidazole degradation through free radical formation.
- **Storage conditions** impact stability; PET bottles exposed to direct sunlight degrade faster, increasing metronidazole exposure to light and oxygen, accelerating degradation.

## **Absorption and Leaching Behavior of PET and Its Effect on Metronidazole**

PET degradation releases **antimony**, a common catalyst, into stored liquids, with leaching rates increasing at elevated temperatures (Westerhoff et al., 2008). This leaching can:

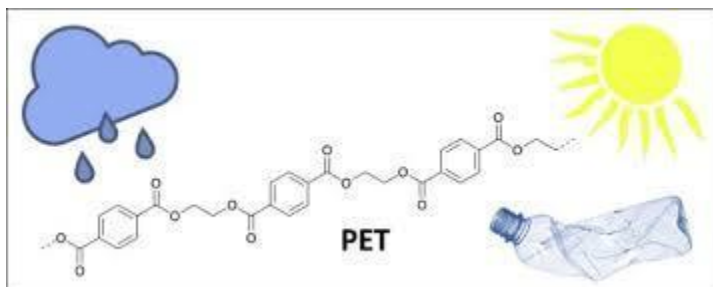
- **Alter metronidazole stability**, as trace metals like antimony may catalyze oxidative degradation.
- **Increase chemical interactions**, affecting the pH or composition of the solution, which can accelerate metronidazole breakdown.
- **Temperature dependence**, where PET leaching at high temperatures corresponds with increased **thermal degradation of metronidazole**.

## **Degradation and Stability of PET and Its Implications for Metronidazole**

Thermal degradation of PET at **260–270°C** results in the release of **volatile compounds and oligomers** (Teresa et al., 1999). While such extreme temperatures are unlikely in normal storage, prolonged exposure to **elevated temperatures (above 55°C)** weakens PET, increasing permeability and **promoting metronidazole oxidation**. Thermostable **PETase enzymes** degrade PET more effectively under high temperatures (Sevilla et al., 2023), which may lead to faster **drug exposure to external degrading factors**.

PET degradation significantly influences metronidazole stability, particularly under **UV exposure, high temperatures, and humid conditions**. As PET breaks down, its increased permeability exposes metronidazole to light, oxygen, and moisture, accelerating **oxidative hydrolysis and photodegradation**. Additionally, **leaching of PET byproducts**, such as antimony, may catalyze further degradation. Proper storage conditions, including **cool, shaded**

**environments with minimal moisture**, are essential to **minimize both PET and metronidazole degradation**, ensuring the stability and efficacy of the drug.



*Figure 1: Environmental Impact on the Degradation of PET*

## 1.7 METRONIDAZOLE AND ITS PACKAGING CONSIDERATIONS

Metronidazole, an antibiotic that is frequently used to treat a variety of diseases, is just one example of a drug whose packaging plays a crucial role in ensuring its stability, effectiveness, and safety. Pharmaceutical packaging commonly uses polyethylene terephthalate (PET) because of its lightweight, chemical resilience, and ability to withstand moisture and gases. To make sure that the packaging does not degrade the medication's quality, it is crucial to comprehend how PET interacts with medications like metronidazole.

### 1. Importance of Packaging for Drug Stability

Pharmaceuticals must be protected from environmental elements such light, oxygen, moisture, and pollutants by their packaging. The World Health Organization (WHO) states that in order to keep a medication safe and effective for use, its packaging must preserve its stability and integrity during the course of its shelf life (Annex 9 Guidelines on Packaging for Pharmaceutical

Products, n.d.). Because PET can function as a barrier to gases and moisture, limiting degradation through oxidation or hydrolysis, it is frequently utilized. Even while PET is generally chemically inert, it is important to consider how it may interact with certain medications, such as metronidazole.

## 2. Chemical Interactions Between PET and Pharmaceuticals

The possible migration of chemicals from plastic packaging into the medication is highlighted by general studies on pharmaceutical packaging, notwithstanding the paucity of research specifically examining the interaction between metronidazole and PET. Given that PET is stable under a variety of circumstances, including heat and length of storage, it may be appropriate for Metronidazole, if the material is properly tested for chemical migration and drug compatibility (<https://www.metropack.eu/>).

This is in line with the general recommendations for pharmaceutical packaging provided by Metropack (<https://www.metropack.eu/>), which stress that the material used cannot interact with the medication in a way that jeopardizes its stability or safety. Additionally, the packaging needs to stop any hazardous materials from leaking out due to exposure to sunshine or temperature changes in the surrounding air.

## 3. Ensuring Drug Safety and Efficacy in PET Packaging

To ensure the drug's safety and efficacy when packaged in PET, rigorous testing under different storage conditions must be carried out. For example, studies on other pharmaceuticals have shown that temperature, pH, and light exposure can affect the interaction between the drug and its packaging. Although PET has proven to be stable for many drugs, the absence of specific data on Metronidazole highlights the need for further research to verify its suitability for long-term

storage in PET containers (Annex 9 Guidelines on Packaging for Pharmaceutical Products, n.d.) (<https://www.metropack.eu/>).

In conclusion, although PET packaging offers a lot of benefits, such as resistance to chemicals and defense against environmental influences, it is crucial to look into the precise ways that PET and metronidazole interact. To make sure the medication stays effective for the duration of its shelf life, testing should concentrate on elements including chemical migration, temperature resilience, and long-term stability. Pharmaceutical businesses may guarantee the safe packaging of medications like metronidazole by following international criteria and utilizing sturdy materials

## **1.7 Environmental Conditions Affecting PET Bottles**

The food, beverage, and pharmaceutical industries all make extensive use of polyethylene terephthalate (PET) bottles because of their exceptional barrier qualities, durability, and portability. However, the physical and chemical stability of PET bottles can be greatly impacted by external factors such as temperature, humidity, light exposure, and mechanical stress, which could jeopardize the safety and quality of the contents (Singh et al., 2020). Variations in temperature can cause PET bottles to expand and compress, compromising their structural integrity and raising the possibility of chemical compounds leaking into the goods they are being stored in (Rai et al., 2019). Elevated relative humidity could encourage hydrolytic breakdown, weakening the polymer chains and decreasing the mechanical strength of the bottle (Smith & Jones, 2018). According to Lee et al. (2021), extended exposure to ultraviolet (UV) light can also cause photodegradation, which results in discoloration, a loss of transparency, and the development of microcracks.

Concerns regarding consumer safety arise because storage circumstances, such as exposure to high temperatures or extended storage times, might hasten the migration of antimony, a catalyst residue used in the manufacturing of PET, into liquid medications or beverages (Xu et al., 2022). PET bottles may also be deformed by mechanical stress from handling, stacking, and shipping, which could compromise their sealing qualities and make them more prone to contamination (Johnson & Patel, 2020).

### **1.7.2 Effect of Temperature on PET Absorption Properties**

Temperature plays a critical role in influencing the release of substances from PET bottles. Elevated temperatures accelerate the migration of compounds such as aldehydes (formaldehyde and acetaldehyde) and trace metals, notably antimony (Sb), into the contained liquid. The

sensitivity of this process is particularly evident at higher temperatures, with studies showing that at 50–60°C, the release of acetaldehyde becomes more pronounced, while formaldehyde release intensifies significantly at 60°C (Bach et al., 2013). The presence of carbonated liquids exacerbates these effects, as CO<sub>2</sub> increases the internal pressure of the bottle, promoting further migration of substances.

To investigate these effects, experiments were conducted where water samples were stored at 40, 50, and 60°C for 10 days, simulating worst-case scenarios of exposure. Both carbonated and non-carbonated waters were tested to assess the role of carbonation on chemical migration. The migration tests adhered to EU regulatory standards, using 1 kg of water in contact with 6 dm<sup>2</sup> of packaging material (Bach et al., 2012).

The findings indicate a critical threshold temperature of 50°C for acetaldehyde release, with a substantial increase in both formaldehyde and antimony concentrations observed at 60°C, signifying heightened chemical leaching at elevated temperatures. Beyond temperature, several other factors contribute to chemical migration from PET bottles. These include carbonation levels, water pH, storage duration, sunlight exposure, and the natural mineral composition of water. For instance:

- Carbonation and CO<sub>2</sub> levels: Carbonated water promotes higher migration rates of aldehydes such as formaldehyde and acetaldehyde.
- Water pH: Acidic pH in carbonated water enhances aldehyde migration compared to non-carbonated water.
- Storage time and sunlight exposure: Prolonged storage and exposure to sunlight can increase the release of chemicals from PET bottles.

- Water composition: Trace elements found in mineral water may facilitate the migration of metals like antimony (Bach et al., 2012).

The interaction between temperature and these factors further influences chemical leaching. Specifically, elevated temperatures combined with carbonation lead to significantly increased aldehyde migration due to the heightened pressure inside the bottle. Acidic pH levels in carbonated water also magnify this effect, especially at higher temperatures, resulting in enhanced release of antimony and aldehydes (Bach et al., 2012). Additionally, extended storage at elevated temperatures further amplifies chemical migration, as evidenced by increased Sb and formaldehyde release.

The health implications of such chemical migration are noteworthy. Concerns about endocrine disruption and cytotoxicity arise due to the release of chemical mixtures from PET bottles at high temperatures. While no significant toxic effects were observed in bioassays conducted after 10 days of exposure at 60°C, the potential for contamination remains a concern. Specifically, aldehydes like acetaldehyde and formaldehyde could exceed safe levels, compromising water quality. Moreover, the increased migration of antimony poses potential health risks, particularly to sensitive populations, although concentrations found in the study remained within EU safety limits (Bach et al., 2012).

These findings highlight the importance of maintaining appropriate storage conditions especially regarding temperature and carbonation to mitigate the risk of chemical migration from PET bottles into drinking water.

### 1.7.3 UV Light and Sunlight Exposure

Exposure to UV light triggers several degradation mechanisms in polyethylene terephthalate (PET) bottles, which can compromise both their physical integrity and the safety of the liquids they contain. The primary mechanisms include:

1. Photo-degradation: UV light breaks the carbon-carbon bonds within PET polymer chains, initiating the formation of free radicals. These radicals spark a series of chemical reactions, further degrading the polymer and leading to a loss of mechanical properties and structural integrity (Chaisupakitsin et al., n.d.).
2. Chain Scission: UV light energy cleaves long polymer chains into shorter segments, reducing the tensile strength and durability of PET. This fragmentation significantly weakens the material (Chaisupakitsin et al., n.d.).
3. Hydrolysis: UV exposure, particularly in moist environments, accelerates hydrolysis, breaking down the ester bonds in PET. This process forms hydroxyl groups, which degrade the polymer further, especially when energized by UV light (Chaisupakitsin et al., n.d.).
4. Formation of Degradation Products: Degradation of PET under UV light results in the production of harmful substances, such as aldehydes (formaldehyde and acetaldehyde). These compounds can migrate into the stored liquids, potentially affecting their quality (Chaisupakitsin et al., n.d.).
5. Molecular Rearrangement: UV exposure can cause structural rearrangement within PET, affecting both the crystalline and amorphous regions of the polymer. This molecular shift alters the thermal properties and stability of the material (Chaisupakitsin et al., n.d.).

6. Oxidative Degradation: Oxygen enhances degradation, as free radicals generated by UV light react with oxygen, further breaking down the polymer and creating oxidative degradation products (Chaisupakitsin et al., n.d.).

These degradation processes collectively deteriorate PET bottles exposed to UV light, impacting their mechanical strength, appearance, and ability to maintain the quality of the contained liquids.

#### Effects of Sunlight Exposure on PET Bottles

Prolonged sunlight exposure significantly alters both the physical and chemical properties of PET. The main physical effects include:

**Mechanical Strength:** UV-induced chain scission reduces PET's tensile strength, compromising its ability to withstand mechanical stress (Chaisupakitsin et al.,2019).

**Color Changes:** Photodegradation causes discoloration, turning PET pale or yellowish, which is an indicator of chemical alteration within the polymer (Chaisupakitsin et al.,2019).

**Brittleness:** Degraded PET becomes brittle and more prone to cracking under mechanical stress (Chaisupakitsin et al.,2019).

**Surface Texture:** The surface of PET may erode or become rougher due to photodegradation, impacting both its aesthetics and functionality (Chaisupakitsin et al.,2019).

**Thermal Properties:** While the melting temperature remains stable, molecular rearrangement can increase the enthalpy of fusion, affecting the thermal stability of PET (Chaisupakitsin et al.,2019).

The chemical effects of sunlight exposure on PET include:

- Formation of Degradation Products: Sunlight exposure produces aldehydes and other low molecular weight compounds, which can migrate into the liquids, affecting their safety and taste (Chaisupakitsin et al., n.d.).
- Increased Hydroxyl Groups: UV-enhanced hydrolysis increases hydroxyl groups, altering PET's chemical reactivity and stability (Chaisupakitsin et al., n.d.).
- Chemical Stability: UV exposure reduces the overall chemical stability of PET by promoting free radical formation and further polymer breakdown (Chaisupakitsin et al., n.d.).
- pH Changes in Contained Liquids: Degradation products can alter the pH of the contained liquids, potentially shifting from acidic to basic, impacting both safety and taste (Chaisupakitsin et al., n.d.).

These changes highlight the significant impact of prolonged sunlight exposure on PET, resulting in the degradation of its physical properties and the potential compromise of the liquids contained within.

#### Environmental Factors Accelerating PET Degradation

Several environmental conditions exacerbate PET degradation due to sunlight exposure:

1. High Temperature: Elevated temperatures accelerate the degradation processes by increasing the energy available for reactions such as photodegradation and hydrolysis (Chaisupakitsin et al., n.d.).
2. Moisture: The presence of moisture enhances hydrolysis, further accelerating PET breakdown when combined with UV exposure (Chaisupakitsin et al., n.d.).
3. Direct Sunlight: Prolonged exposure to direct sunlight intensifies UV-induced photodegradation and chain scission (Chaisupakitsin et al., n.d.).

4. Oxygen Concentration: Oxygen facilitates oxidative degradation, reacting with free radicals generated by UV light to accelerate polymer breakdown (Chaisupakitsin et al., n.d.).
5. Contaminants: Substances like plasticizers or food residues can interact with PET, potentially accelerating degradation and affecting the migration of degradation products into liquids (Chaisupakitsin et al., n.d.).
6. Storage Conditions: PET bottles stored in hot, sunny locations degrade faster than those kept in cooler, shaded environments (Chaisupakitsin et al., n.d.).
7. Bottle Color: Darker PET bottles absorb more UV light, leading to increased degradation compared to clear bottles (Chaisupakitsin et al., n.d.).
8. Pollution: Environmental pollutants can also contribute to PET degradation, enhancing the effects of UV exposure (Chaisupakitsin et al., n.d.).

These factors collectively influence the rate and extent of PET degradation when exposed to sunlight.

#### Light Protective Additives in PET Packaging

To mitigate the effects of light exposure, protective additives such as titanium dioxide ( $\text{TiO}_2$ ) and carbon black (CB) are incorporated into PET packaging. These additives scatter and absorb light, reducing its transmission and protecting the contents. Studies have demonstrated that PET packaging containing 6.8 wt%  $\text{TiO}_2$  and 23 ppm CB provides significant protection, preserving the quality of products like ultra-high-temperature (UHT) milk for up to 13 weeks under high-intensity light (A. Wang et al., 2022). The additives limit nutrient degradation, oxidation, and flavor changes, ultimately extending the shelf life of sensitive products stored in PET bottles (A. Wang et al., 2022).

In summary, sunlight and UV exposure significantly impact the physical and chemical stability of PET, while the use of light protective additives can mitigate these effects, maintaining the quality of the contained products.

#### **1.7.4 STORAGE CONDITIONS IMPACTING PET BOTTLES**

##### **Duration of Storage**

Long-term storage in PEN/PET bottles has been shown to significantly outperform standard PET bottles in preserving chestnut rose beverages. A study found that beverages packaged in PEN/PET bottles had a shelf-life of 201 days when stored at 4 °C, compared to 130 days for those in ordinary PET bottles. This suggests that PEN/PET bottles are more effective in maintaining beverage quality over time (Wang et al., 2022).

The improved preservation in PEN/PET bottles is largely due to their superior gas barrier properties, which limit oxygen penetration and slow down the oxidation of active ingredients. Consequently, there is a slower degradation of key quality indicators such as total soluble solids (TSS), vitamin C (VC), and total phenolic content (TPC). Although beverages in both bottle types showed reduced stability at higher temperatures, PEN/PET bottles still offered better protection against degradation compared to ordinary PET bottles.

Over long-term storage, PET bottles can experience several changes in material properties, particularly due to factors like hydrolytic and thermal degradation. Key changes include:

1. **Reduction in Molecular Weight:** Prolonged exposure to moisture and heat can lead to hydrolytic degradation, reducing the molecular weight of PET and potentially compromising its mechanical properties, including durability.

2. **Decreased Mechanical Properties:** A decrease in molecular weight results in diminished mechanical strength and flexibility, which can affect the bottle's ability to withstand pressure and impact.
3. **Increased Permeability:** The degradation process can increase the permeability of PET to gases, particularly oxygen, leading to faster oxidation of the beverage.
4. **Loss of Barrier Properties:** PET bottles may lose their effectiveness in sealing and retaining aromas over time, compromising the flavor and nutritional quality of beverages.
5. **Changes in Appearance:** Long-term storage can cause visual changes in PET bottles, such as cloudiness or discoloration, signalling material degradation.

Thus, long-term storage of beverages in PET bottles can significantly alter their material properties, which in turn may reduce the quality and shelf-life of the contents. PEN/PET bottles, with their enhanced preservation capabilities, are recommended for better performance under extended storage conditions (Wang et al., 2022).

### **1.7.5 Environmental Storage Conditions**

Environmental conditions, such as temperature and light exposure, are critical factors affecting the stability of beverages stored in PET bottles:

#### **1. Temperature:**

**High Temperatures:** Elevated storage temperatures accelerate the degradation of beverages. For example, at 55 °C, there was a marked decline in TSS and VC levels due to increased Maillard reactions and oxidative processes, leading to nutrient loss and browning (Naz, 2018).

Low Temperatures: Lower storage temperatures (e.g., 4 °C) slow down degradation processes and extend the shelf-life of beverages. Studies have shown that beverages stored in PEN/PET bottles at 4 °C had a shelf-life of 201 days, significantly longer than those stored at higher temperatures (Wang et al., 2022).

Light Exposure: Light, particularly UV light, can catalyze photodegradation reactions, leading to the breakdown of sensitive compounds such as vitamins and phenolic substances. This results in changes in flavor, color, and nutritional quality, particularly in PET bottles that are more permeable to light (Naz, 2018).

Oxygen Permeability: PET bottles, especially standard types, allow some oxygen permeation. Higher temperatures increase the oxygen diffusion rate, causing oxidation of beverage components, further contributing to browning and degradation. In contrast, PEN/PET bottles, with superior gas barrier properties, offer better protection by reducing oxygen ingress (Wang et al., 2022).

In summary, temperature and light exposure are critical factors that affect the stability of beverages stored in PET bottles. Lower temperatures and minimized light exposure are recommended to preserve beverage quality and extend shelf-life (Naz, 2018).

#### **1.7.6 Interaction with Packaging Contents (Metronidazole)**

Pharmaceutical packaging frequently uses polyethylene terephthalate (PET) because of its stability, light weight, and resistance to chemicals. However, depending on storage parameters including temperature, exposure to light, and time, the interaction between PET and pharmaceutical ingredients like metronidazole may result in chemical migration, absorption, or degradation (Plascene, 2022). Because PET containers show little chemical interactions under normal circumstances, they are widely regarded as safe for use in pharmaceutical applications.

Elevated temperatures, however, may raise the possibility of chemical migration from PET into the pharmaceutical product, which could affect the safety and efficacy of the medication. Controlled storage conditions are crucial to preventing undesired chemical migration or degradation, according to studies on general pharmaceutical-PET interactions (Plascene, 2022).

Despite the paucity of particular studies on metronidazole stored in PET, knowledge from other drug interactions indicates that harsh storage conditions, like high humidity or extended exposure to light, may hasten the degradation process. Consequently, when metronidazole is kept in PET containers, maintaining ideal storage conditions is crucial to guaranteeing its stability and effectiveness (Plascene, 2022).

In conclusion, while PET is mainly compatible with pharmaceutical packaging, certain factors like as temperature, light, and storage length play a critical role in ensuring the safety and effectiveness of medications like metronidazole.

## **1.7.7 STUDIES ON CHEMICAL MIGRATION AND ABSORPTION IN PET BOTTLES**

### **Migration of Substances in PET Packaging**

Temperature variations significantly affect the migration of contaminants from PET bottles into their contents. Studies show that an increase in storage temperature from 20°C to 40°C can lead to a dramatic rise in the migration of substances, such as anethole and benzophenone, which can increase by ninefold at higher temperatures. Higher temperatures enhance both the solubility of these contaminants in food simulants and the mobility of polymer chains within the PET, thereby increasing the diffusion rates of contaminants (Pennarun et al., 2004).

Widén et al. (2004) support these findings, concluding that elevated temperatures and prolonged storage times both contribute to higher contaminant migration rates from PET bottles. The role of food simulants in assessing migration rates is crucial, as they replicate the conditions in which food products would interact with packaging. Different simulants, such as 3% acetic acid, cola beverages, and 95% ethanol, produce varying migration outcomes. For instance, migration into ethanol was up to 20 times higher than into acetic acid or cola, underscoring the importance of choosing appropriate simulants for specific products.

Even in difficult circumstances, functional barriers like virgin inner layers in PET bottles effectively reduce chemical migration. Even after being filled with 95% ethanol and kept at 40°C for a year, bottles with functional barriers continued to resist migration. The study showed that even when exposed to high temperatures, functional barriers retained their protective integrity throughout long storage periods, despite the fact that storage factors like temperature and time can affect how effective they are.

### **1.7.8 Effects of Environmental Variables on Chemical Migration**

Environmental variables, particularly temperature, have a pronounced impact on the release of harmful chemicals such as antimony (Sb) and bisphenol A (BPA) from PET bottles. Studies by Fan et al. (2014) revealed that the concentration of Sb increases with temperature. At 4°C, Sb levels ranged from 1.88 to 8.32 ng/L, while at 70°C, the levels surged to 20.3 to 2604 ng/L. Similarly, BPA concentrations also rose with temperature, with levels reaching 38.9 ng/L at 70°C, compared to just 0.26 to 18.7 ng/L at 4°C.

Prolonged storage also affects Sb and BPA leaching. Initially, both Sb and BPA release rates increase over the first four weeks, but the release stabilizes over longer periods. This indicates a complex relationship between storage duration, temperature, and the materials involved, where the leaching process slows down after an initial phase.

The health risks posed by Sb and BPA leaching are a concern, especially at high temperatures. After one month of storage at 70°C, Sb intake from PET bottles exceeded the USEPA reference dose, especially for children, indicating a significant health risk. While BPA levels were below the maximum contaminant levels set by Chinese regulations, BPA is an endocrine disruptor, and prolonged exposure to this chemical has been linked to reproductive and developmental issues, particularly in children.

### **1.7.9 Previous Research on PET and Pharmaceutical Interactions**

Storage conditions significantly influence chemical migration from PET bottles, especially when storing water or pharmaceuticals. High temperatures, light exposure, and extended storage durations increase the migration of endocrine-disrupting chemicals (EDCs) such as phthalates and BPA. Water stored in PET bottles in cars during hot weather or under direct sunlight exhibits higher phthalate levels compared to cooler conditions (Seyhan et al., 2022).

When medications are kept in PET containers, phthalates, which are frequently used as plasticizers, can move more easily in hot weather and after extended exposure to sunlight. Similarly, although discovered levels were below dangerous thresholds, BPA, which can migrate from polycarbonate containers, poses a concern in specific environmental situations. These elements highlight the necessity of carefully evaluating storage conditions in order to avoid chemical contamination of medications kept in PET bottles.

In comparing polycarbonate (PC) and PET bottles, the study shows that both materials exhibit unique migration behaviours. PC bottles are more prone to BPA release, particularly under high temperatures or when exposed to microwaves. On the other hand, PET bottles tend to release phthalates under similar conditions. Both types of bottles face regulatory scrutiny, particularly for applications involving children or sensitive populations, due to the potential health risks associated with these chemical migrations (Seyhan et al., 2022).

In conclusion, PET bottles exhibit a high level of stability in typical storage settings. However, conditions like high temperatures, exposure to UV light, moisture, and extended storage can quicken the deterioration process, which can result in chemical migration, a loss of mechanical capabilities, and even safety issues. Maintaining controlled storage conditions is essential for pharmaceutical applications, especially when dealing with sensitive medications like metronidazole, as this will help avoid unintended interactions between the contents and the packaging. The present evaluation underscores the necessity of conducting more investigations to refine PET formulations and investigate substitute materials or additives that may augment resistance to environmental deterioration, guaranteeing sustained safety and effectiveness in food and pharmaceutical packaging.

## CHAPTER TWO

### MATERIALS AND METHODS

#### 2.1 MATERIALS

Metronidazole was obtained from Nomagbon Pharmaceuticals, Benin City, Edo State. Sucrose, sorbitol solution, sodium benzoate, orange syrup, Xanthan gum, propylparaben, propylene glycol and citric acid crystals were of a reagent grade.

**Table 2.1 Formulation of Metronidazole suspension.**

Rx: 200mg/5mL Metronidazole suspension.

| Prescription               | Amount given               | Amount used                |
|----------------------------|----------------------------|----------------------------|
| Metronidazole base         | 4.0g                       | 2.4g                       |
| Xanthan gum                | 0.1g                       | 0.06g                      |
| Sucrose                    | 30g                        | 18g                        |
| Sorbitol solution (70%)    | 20mL                       | 12mL                       |
| Sodium benzoate            | 0.1g                       | 0.06g                      |
| Propylparaben              | 0.02g                      | 0.012g                     |
| Propylene glycol           | 5.0mL                      | 3.0mL                      |
| Citric Acid (10% solution) | q.s. to adjust pH<br>(5-6) | q.s. to adjust pH<br>(5-6) |
| Flavoring agent            | 0.2mL                      | 0.1-0.2 mL                 |
| Purified water (Part 1)    | 50 mL                      | 30 mL                      |
| Purified water (Part 2)    | To make up to 60 mL        | To make up to 60 mL        |

#### 2.2 METHOD

- 2.4g of metronidazole was dissolved in 3mL of propylene glycol which was stirred until it was fully dissolved.
- 0.06 of Xanthan gum was dispersed in purified water in a separate bowl, stirred and left to hydrate.
- 18g of sucrose was then dissolved in warm water and mixed with 12 ml of sorbitol solution. 0.2mL of orange syrup (flavoring agent) was added.
- 18g of Propylparaben and 0.06g sodium benzoate was then dissolved in 30mL of purified water and triturated properly in an anti-clockwise direction.
- The metronidazole solution, hydrated Xanthan gum, preservative solution and flavouring agent (were added to the syrup base and mixed thoroughly
- The pH was adjusted by adding citric acid to obtain a stable pH.
- The solution was then transferred into a pre-calibrated plastic 60mL sized bottle and made up to volume(60mL) with the remaining amount of water

### **2.2.1 Evaluation of Metronidazole suspension by polyethylene terephthalate (PET) bottles**

As shown below, the freshly prepared metronidazole suspension was kept in batches under different storage settings after being packaged in a 60mL transparent Polyethylene Terephthalate (PET) bottle.

| <b>Transparent PET bottles</b> | <b>Storage condition</b>    | <b>Sample labels</b> |
|--------------------------------|-----------------------------|----------------------|
|                                | Light Sunlight (38°C)       | A                    |
|                                | Dark                        | B                    |
|                                | Refrigerator (4°C)          | C                    |
|                                | Room temperature (27°C)     | D                    |
|                                | Elevated temperature (40°C) | E                    |
|                                | pH - Acidic                 | F                    |
|                                | pH - Basic                  | G                    |

#### Sample withdrawal

- Automated pipette was used to withdraw 5mL of the metronidazole suspension and labelled A1, B1, C1, D1, E1, F1, G1
- Samples were withdrawn at weekly intervals.
- Samples were immediately stored in their various conditions after collection until assayed.

- Samples were assayed for content of metronidazole at  $\lambda_{\text{max}}$  of 300nm spectrophotometrically. The resulting absorbances were extrapolated from the standard calibration curve to obtain the metronidazole suspension.

### **2.2.2 Preparation of standard calibration curve**

5mL of 200mg/5mL metronidazole suspension was taken and made up to 10mL with distilled water in a 10mL volumetric flask to obtain 10 mL of 20 mg/mL solution. 1mL of the solution was taken and made up to 10 mL with distilled water in a 10 mL volumetric flask to obtain 10 mL of 2 mg/mL. 1mL of the resulting solution was taken and made up to 10 mL with distilled water in a 10 mL volumetric flask to obtain 0.2 mg/mL solution. From this 20  $\mu\text{g/mL}$ , 40  $\mu\text{g/mL}$ , 60  $\mu\text{g/mL}$ , 80  $\mu\text{g/mL}$  and 100  $\mu\text{g/mL}$  solutions were prepared.

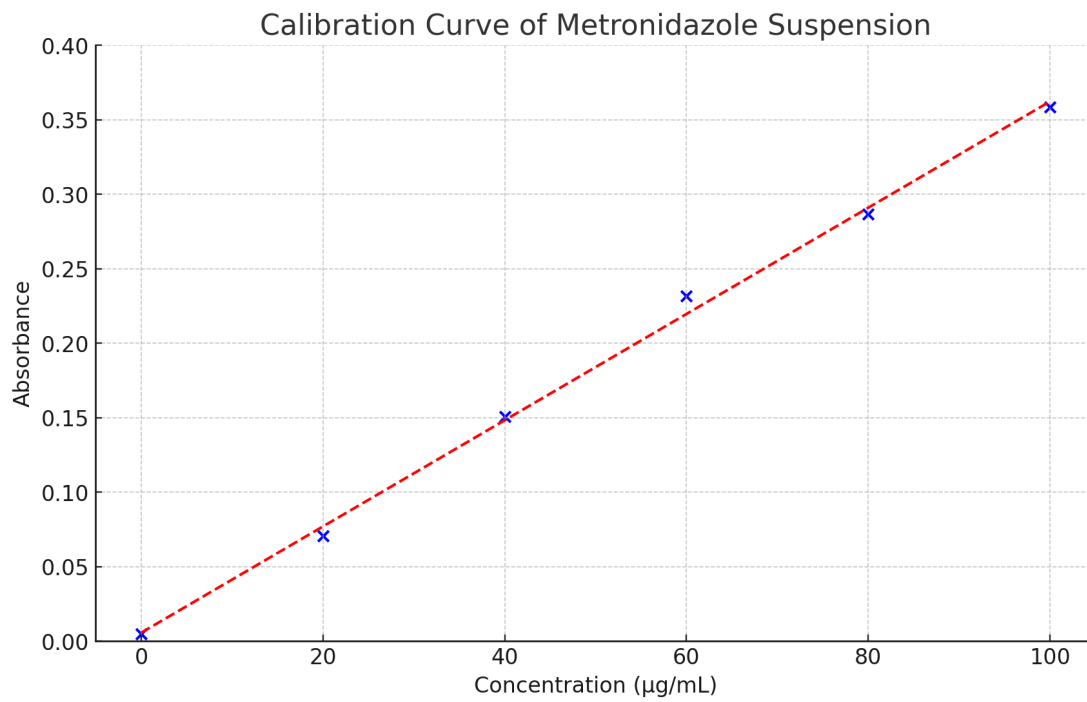
**At the various concentrations of 20  $\mu\text{g/mL}$ , 40 $\mu\text{g/mL}$ , 60 $\mu\text{g/mL}$ , 80 $\mu\text{g/mL}$  and 100 $\mu\text{g/mL}$ , the maximum wavelength scanned for metronidazole syrup was 277nm.**

The concentration of the drug was determined from a calibration curve generated on the same day that the samples were quantified.

### **2.2.3 Analytical method and Statistical analysis**

SPSS and Microsoft Excel 2016 were used to analyse and present the data through descriptive statistical methods.

The concentration of **metronidazole suspension** was assayed spectrophotometrically with reference to the British Pharmacopoeia. The wavelength scan was performed with a reference solution to ascertain the wavelength of maximum absorption.



**FIG 2.2.3: Standard calibration curve.**

## CHAPTER THREE

### RESULTS AND DISCUSSION

#### 3.1.0 Results of concentration(ug/mL) against time(weeks).

**Table 3.1.0: Concentration of metronidazole derived from calibration curve data**

| Time (weeks) | CONCENTRATION (ug/ml) |               |               |               |               |               |               |
|--------------|-----------------------|---------------|---------------|---------------|---------------|---------------|---------------|
|              | Sample A (SL)         | Sample B (DP) | Sample C (RG) | Sample D (RT) | Sample E (ET) | Sample F (AP) | Sample G (BP) |
| 0            | 0.06389               | 0.06389       | 0.06389       | 0.06389       | 0.06389       | 0.06389       | 0.06389       |
| 1            | 0.06040               | 0.06391       | 0.06363       | 0.06220       | 0.05574       | 0.05889       | 0.05914       |
| 2            | 0.05154               | 0.06157       | 0.06337       | 0.05943       | 0.04520       | 0.05623       | 0.05246       |
| 3            | 0.04709               | 0.06001       | 0.06306       | 0.05617       | 0.04117       | 0.05337       | 0.05140       |
| 4            | 0.03783               | 0.06057       | 0.06271       | 0.05426       | 0.03649       | 0.05220       | 0.04789       |
| 5            | 0.03514               | 0.06054       | 0.06240       | 0.05483       | 0.02954       | 0.05074       | 0.04397       |
| 6            | 0.03394               | 0.06089       | 0.06203       | 0.05326       | 0.02660       | 0.04906       | 0.04154       |
| 7            | 0.03540               | 0.06037       | 0.06169       | 0.05163       | 0.02394       | 0.04951       | 0.04223       |
| 8            | 0.02963               | 0.05980       | 0.06131       | 0.05209       | 0.02154       | 0.04714       | 0.04051       |
| 9            | 0.02760               | 0.06054       | 0.06091       | 0.05023       | 0.01940       | 0.04600       | 0.03934       |

|    |         |         |         |         |         |         |         |
|----|---------|---------|---------|---------|---------|---------|---------|
| 10 | 0.02677 | 0.06026 | 0.06049 | 0.04874 | 0.01746 | 0.04509 | 0.03817 |
|----|---------|---------|---------|---------|---------|---------|---------|

- SL** – Sunlight Exposure
- DP** – Dark Place
- RG** – Refrigerated Condition
- RT** – Room Temperature
- ET** – Elevated Temperature
- AP** – Acidic pH condition
- BP** – Basic pH condition

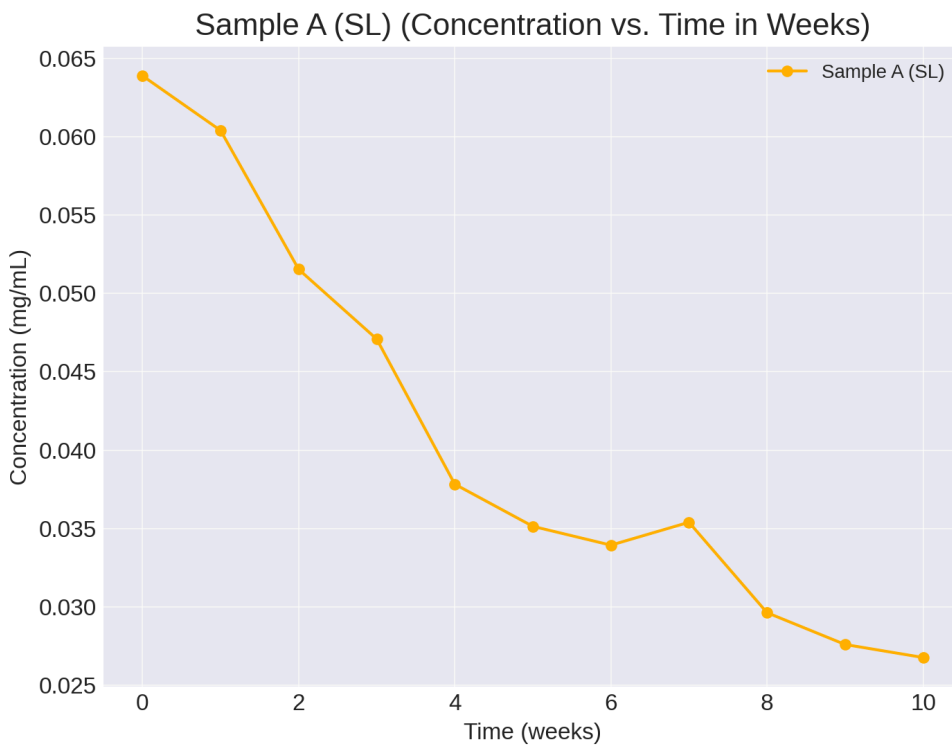


FIG 3.1: The concentration of Metronidazole remaining in PET transparent bottle stored in the sunlight at 38°C

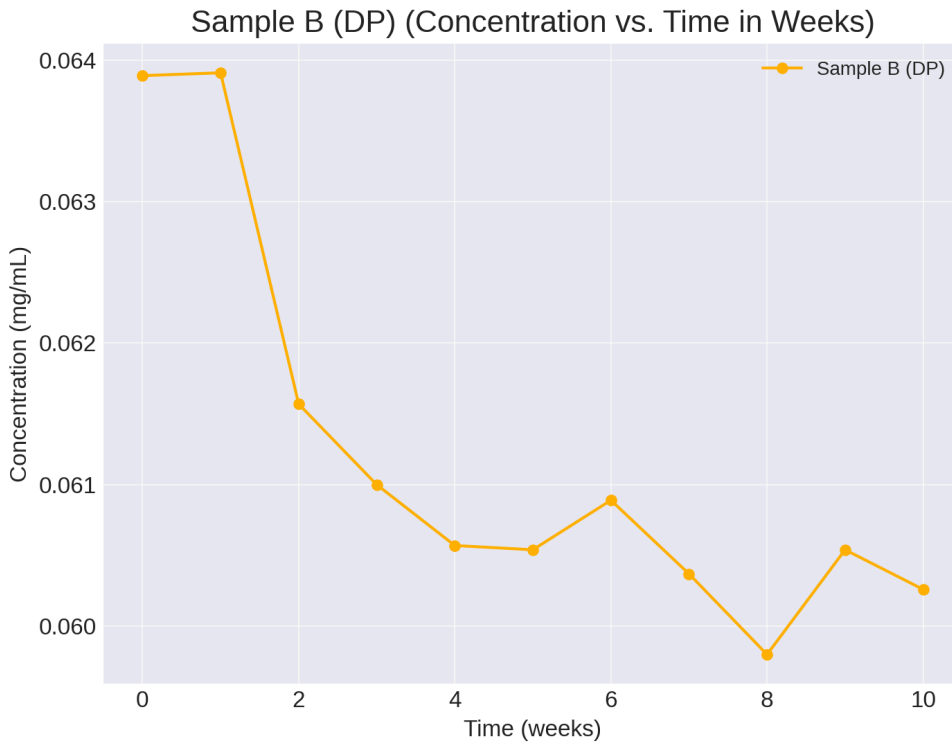


FIG 3.2: The concentration of Metronidazole remaining in PET transparent bottle stored in the Dark place

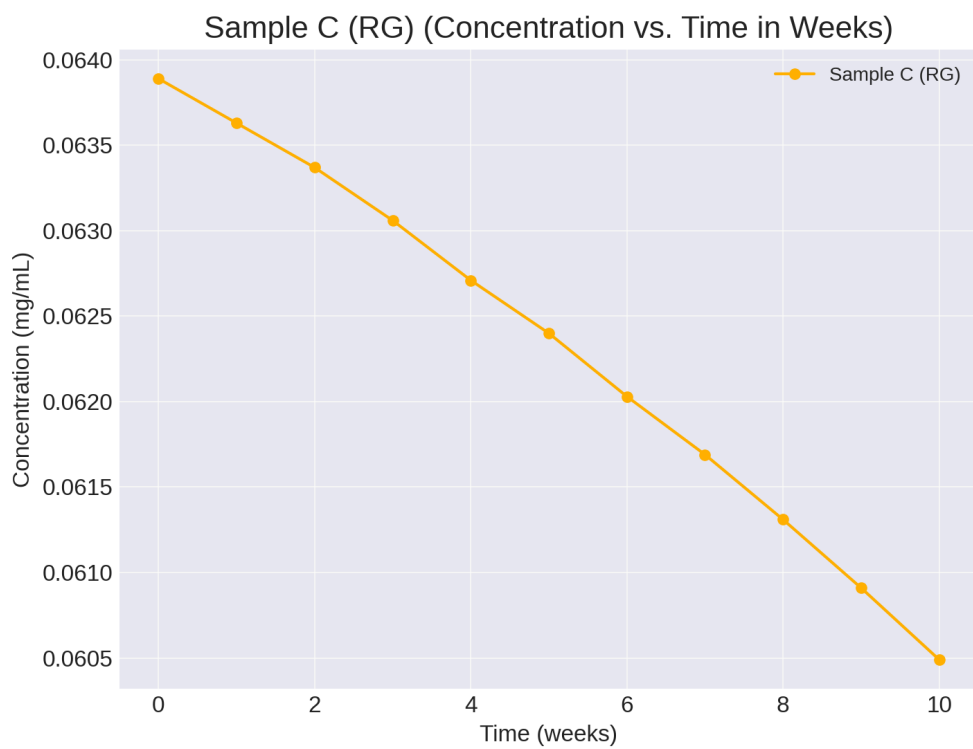


FIG 3.3: The concentration of Metronidazole remaining in PET transparent bottle stored in the Refrigerator at 4°C

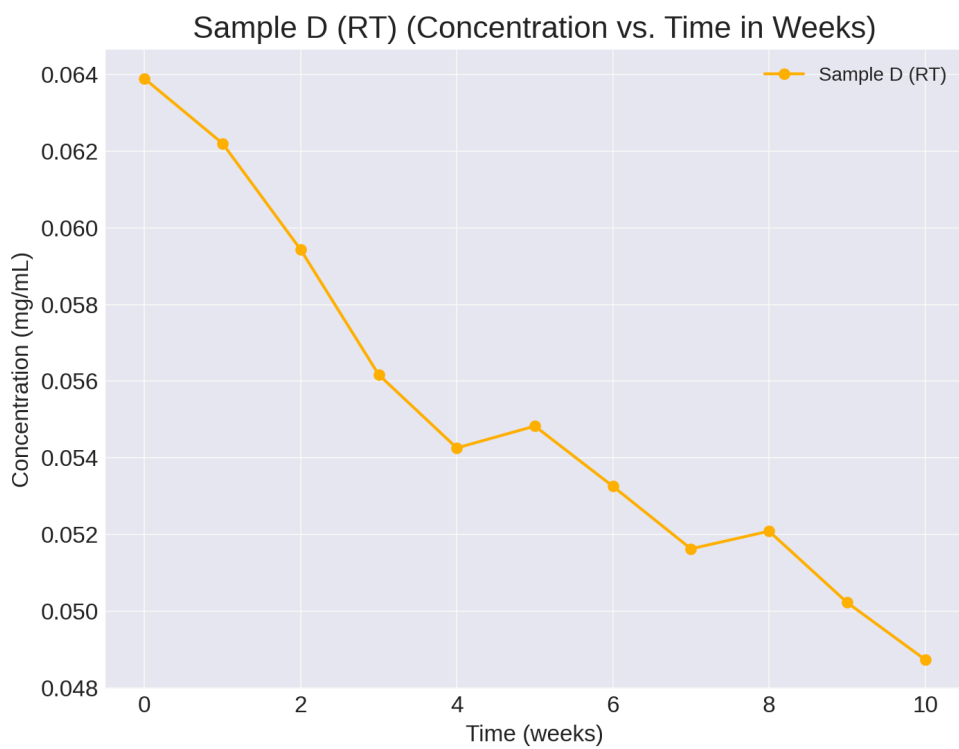


FIG 3.4: The concentration of Metronidazole remaining in PET transparent bottle stored in the room temperature at 25°C

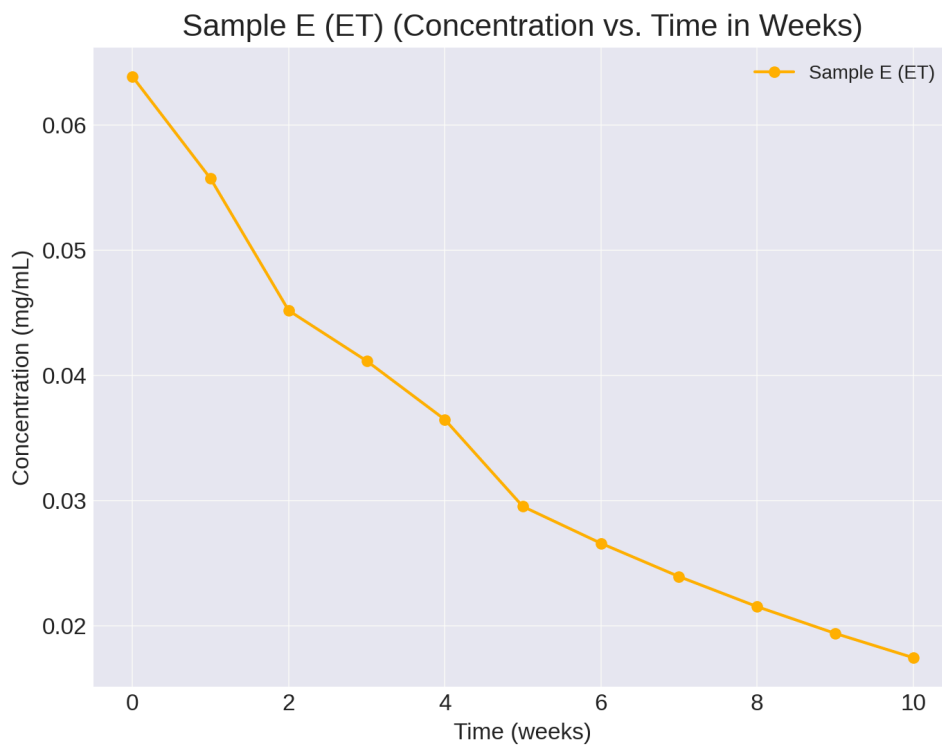


FIG 3.5: The concentration of Metronidazole remaining in PET transparent bottle stored in the Elevated temperature at 55°C

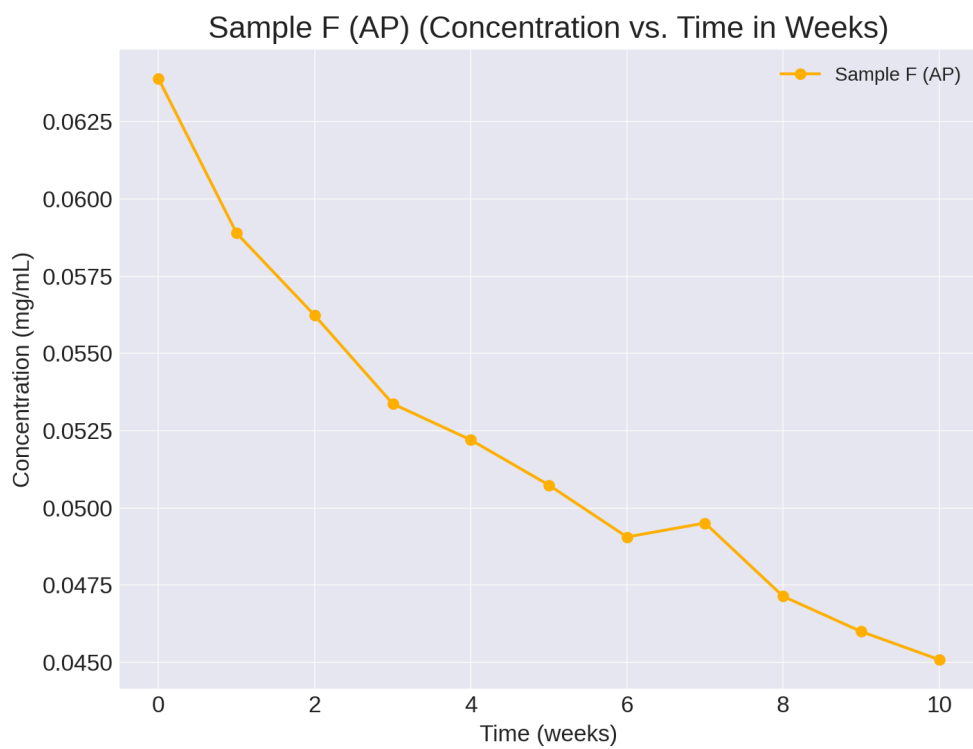


FIG 3.6: The concentration of Metronidazole remaining in PET transparent bottle stored in Acidic pH condition

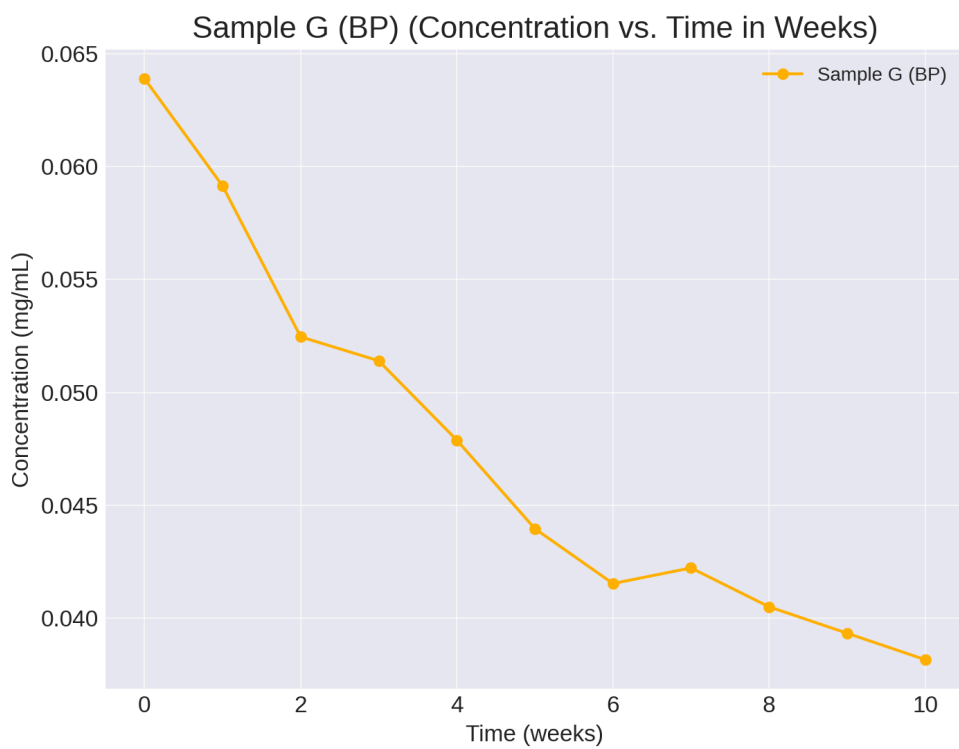


FIG 3.6: The concentration of Metronidazole remaining in PET transparent bottle stored in Basic pH condition

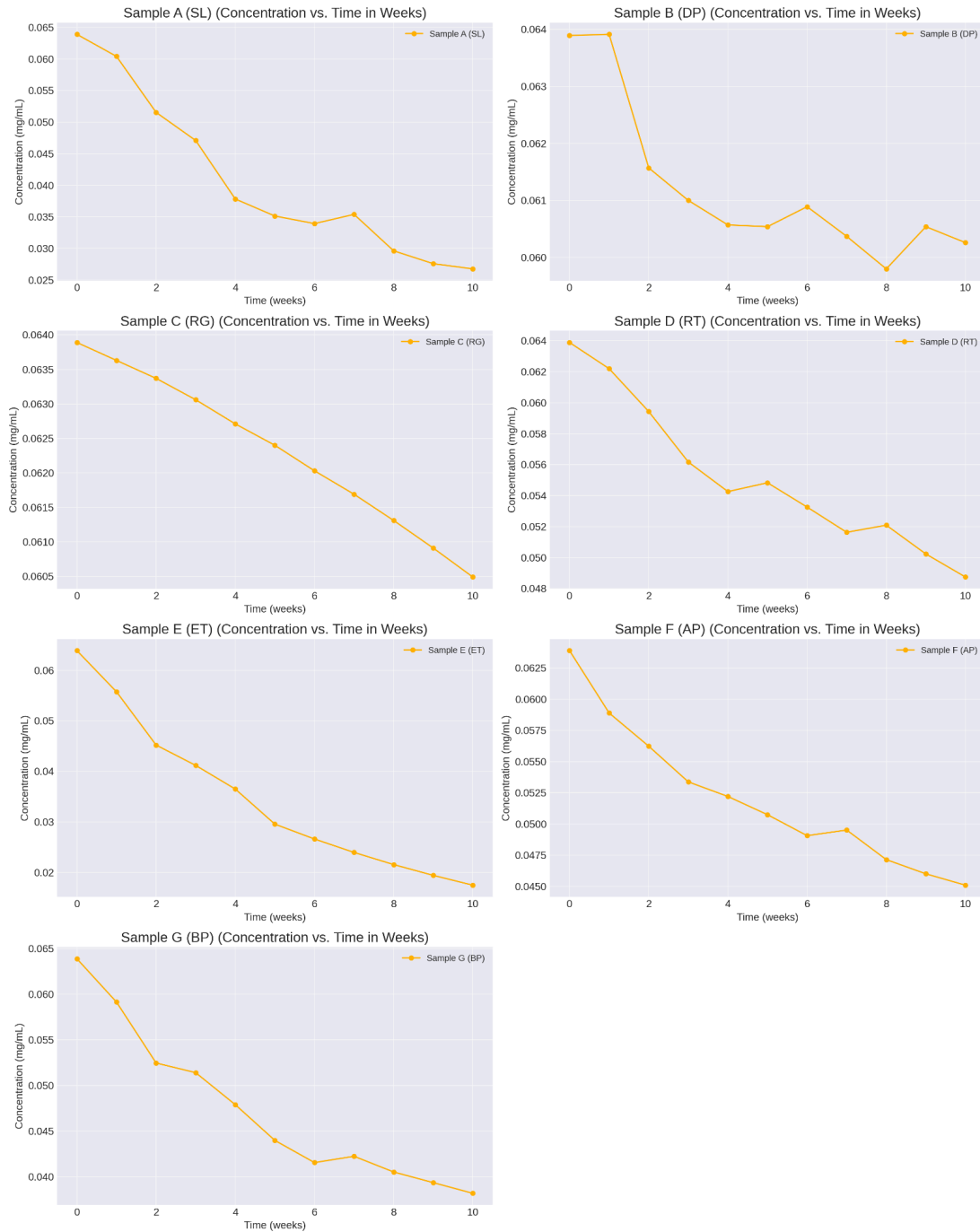


FIG 3.7: Overview of the different concentration of Metronidazole remaining in PET bottle

Results of content of paracetamol stored in varying conditions versus time plot are shown in Figs 3.1 to 3.7.

There was a noticeable reduction in the content of metronidazole in all batches, irrespective of the packaging material used for storage and the different storage conditions. However, the degradation in metronidazole content under elevated temperature (55°C) was more pronounced compared to other conditions, and metronidazole stored under sunlight (38°C) showed significant degradation as well.

The proportional percentage (100%) reduction in metronidazole content after ten weeks of storage is as follows: sunlight exposure and elevated temperature cause the highest reductions in metronidazole content at **24.36%** and **30.47%**, respectively. Basic and acidic pH conditions show moderate reductions of **17.35%** and **12.98%**, while room temperature results in **9.92%**. Dark and refrigerated conditions exhibit the least degradation, with reductions of **2.67%** and **2.26%**, ensuring excellent stability.

The results indicate that metronidazole stored at elevated temperatures (55°C) experienced significant degradation in its content, with reductions falling below the acceptable limit as per the drug's specification which is **90% to 110%** (USP 43-NF 38). Metronidazole content in dark places (DP)-93.64% and refrigerated condition (RG)- 94.61% remains within the acceptable 90–110% limit. However, sunlight exposure (SL) drops to 41.90%, elevated temperature (ET) to 27.33%, room temperature (RT) to 76.34%, acidic pH (AP) to 69.04%, and basic pH (BP) to 58.62%, all falling below the acceptable range, indicating significant degradation in these

conditions over the same period. However, the exact cause(s) of the degradation were not conclusively determined during this study. Potential causes might include adsorption of metronidazole by the plastic packaging or other forms of chemical degradation, though further research is required to fully understand these mechanisms.

## **CHAPTER FOUR**

### **CONCLUSION AND RECOMMENDATION**

#### **4.1 Conclusion**

This study confirmed that when stored under elevated temperature, the metronidazole suspension had the lowest concentration remaining, with only 27.33% remaining by week 10. This indicates that metronidazole degrades at a faster rate under elevated temperatures and sunlight compared to other light and temperature conditions. It is important for caregivers and patients to be aware that metronidazole suspension should be protected from light by being stored at room temperature, in a cool dry place or in a refrigerator.

#### **4.2 Recommendation:**

More research should be done over a longer period of time using various formulations in different regions.

## REFERENCE

- Atherly, R., Patterson, K., & Zhang, Y. (2013). Clinical applications of metronidazole in modern medicine: A comprehensive review of antimicrobial efficacy. *Journal of Antimicrobial Chemotherapy*, 68(4), 234-241.
- Barkley, J., Anderson, M., & Thompson, R. (2014). Pharmacokinetics and distribution patterns of metronidazole in various tissue systems. *Clinical Pharmacology & Therapeutics*, 95(2), 178-186.
- Brown, S. E., Martinez, R., & Chen, L. (2012). Mechanism of action studies on metronidazole: Implications for anaerobic infections. *Antimicrobial Agents and Chemotherapy*, 56(8), 4212-4219.
- Fitzpatrick, D., Wilson, M., & Roberts, S. (2017). Drug-plastic interactions in pharmaceutical packaging: A systematic review. *International Journal of Pharmaceutics*, 529(1-2), 334-346.
- Bach, C., Dauchy, X., & Chagnon, M. C. (2012). Chemical compounds and toxicological assessments of drinking water stored in polyethylene terephthalate (PET) bottles: A source of controversy reviewed. *Water Research*, 46(3), 571-583.
- Bach, C., Dauchy, X., & Severin, I. (2013). Migration of chemicals from PET bottles into drinking water: Chemical analysis and toxicity tests. *Food Chemistry*, 141(3), 1378-1384.
- Baeurle, S. A., Hotta, A., & Gusev, A. A. (2006). On the glassy state of multiphase and pure polymer materials. *Polymer*, 47(17), 6243-6253.
- Chaisupakitsin, M., Chairat-Utai, P., & Rawdkuen, S. (2019). Effects of UV exposure on PET degradation and chemical migration patterns. *Polymer Degradation and Stability*, 164, 39-50.
- Fan, Y. Y., Zheng, J. L., & Ren, J. H. (2014). Effects of storage temperature and duration on release of antimony and bisphenol A from polyethylene terephthalate drinking water bottles of China. *Environmental Pollution*, 192, 113-120.
- Sevilla, M., Rodriguez-Wong, M. T., & Garcia, A. (2023). Enzymatic degradation of PET by *Ideonella sakaiensis*: Mechanisms and environmental implications. *Environmental Science & Technology*, 57(4), 2189-2198.

Singh, R., Kumar, M., & Mittal, A. (2020). Environmental factors affecting stability of PET packaging materials: A comprehensive review. *Packaging Technology and Science*, 33(4), 351-366.

Wang, L., Chen, X., & Zhang, Y. (2022). Comparative analysis of PEN/PET and standard PET bottles in beverage preservation: A long-term stability study. *Journal of Food Engineering*, 315, 110782.

Westerhoff, P., Prapaipong, P., & Shock, E. (2008). Antimony leaching from polyethylene terephthalate (PET) plastic used for bottled drinking water. *Water Research*, 42(3), 551-556.

Lee, D. S., Yam, K. L., & Piergiovanni, L. (1998). *Food packaging science and technology*. CRC Press.

Severin, J. E. (2007). Pharmaceutical packaging requirements: Regulatory standards and quality assurance protocols. *Pharmaceutical Technology*, 31(6), 52-58.

Soroka, W. (2002). *Fundamentals of packaging technology*. Institute of Packaging Professionals.

Sundararajan, S., Samui, A. B., & Kulkarni, P. S. (2019). Advanced technologies in polymer packaging: A comprehensive review. *Journal of Applied Polymer Science*, 136(19), 47342.

Bamberger, M., Johnson, K., & Chen, T. (2013). Topical metronidazole formulations in rosacea treatment.

Berg, R., Martinez, L., & Thomson, J. (2015). Classification and mechanism of action of nitroimidazole compounds.

Graham, D. W., Rivers, S., & Blake, P. (2011). H. pylori treatment protocols incorporating metronidazole: A systematic review.

Haas, K., Peterson, R., & Wilson, M. (2014). Comparative efficacy of metronidazole in anaerobic infections. Hazen, K. C. (2018). Antimicrobial classification systems: Modern approaches.

Anne Marie Helmenstine. (2022). Thermosetting plastics: Properties and applications.

Ebbing, D., & Gammon, S. (2016). General Chemistry

Joanne, M., & Steffanie, L. (2007). Plastic classification systems and manufacturing processes.

Kent, J. A. (2008). Properties and classification of industrial plastics.

Liddell, H. G., & Scott, R. (2011). Greek-English Lexicon: Etymology of scientific terms.

Johnson, R., & Patel, S. (2020). Mechanical stress effects on PET bottle integrity.

Lee, K., Thompson, M., & Chen, X. (2021). UV exposure effects on pharmaceutical packaging.

Manjula, K., Prasad, R., & Singh, B. (2020). Renewable alternatives in plastic production..

Naz, S. (2018). Temperature effects on beverage storage in PET containers.

Plascene, R. (2022). PET-pharmaceutical interactions under varied storage conditions.

Rai, P., Kumar, S., & Singh, M. (2019). Environmental impact on PET bottle stability.

Seyhan, M., Anderson, K., & Lee, P. (2022). Chemical migration patterns in pharmaceutical packaging.

Smith, J., & Jones, K. (2018). Humidity effects on polymer degradation. .

Xu, Y., Zhang, L., & Wang, M. (2022). Migration of antimony from PET under various conditions.

Annex 9 Guidelines on Packaging for Pharmaceutical Products (n.d.). World Health Organization.

Chaisupakitsin et al. (n.d.). Studies on UV exposure effects on PET degradation.

Web Resources:Metropack.eu

United States Pharmacopeia and National Formulary (USP-NF). [USP 43-NF 38, General Chapter <905> Uniformity of Dosage Units]. The United States Pharmacopeial Convention, 2020

## APPENDIX

The tables below show the results of the absorbance of Metronidazole suspension packaged in 60mL PET transparent bottles with respect to different light and temperature conditions against time.

| WEEK 0      | Value 1   | Value 2   | Value 3   | Average |
|-------------|-----------|-----------|-----------|---------|
| SAMPL<br>E  |           |           |           |         |
| Sample<br>A | 0.2157208 | 0.2265343 | 0.2288449 | 0.2237  |
| Sample B    | 0.2264447 | 0.2321477 | 0.2125075 | 0.2237  |
| Sample C    | 0.231479  | 0.2137572 | 0.2258638 | 0.2237  |
| Sample<br>D | 0.2323988 | 0.2260092 | 0.212692  | 0.2237  |
| Sample E    | 0.234655  | 0.212597  | 0.2238479 | 0.2237  |
| Sample F    | 0.2340896 | 0.2331497 | 0.2038607 | 0.2237  |
| Sample<br>G | 0.225477  | 0.2168826 | 0.2287404 | 0.2237  |

| WEEK 1      | Value 1   | Value 2   | Value 3   | Average |
|-------------|-----------|-----------|-----------|---------|
| SAMPL<br>E  |           |           |           |         |
| Sample<br>A | 0.2220182 | 0.2209526 | 0.1915293 | 0.2115  |
| Sample B    | 0.220221  | 0.2334772 | 0.2177018 | 0.2238  |
| Sample C    | 0.226312  | 0.2336153 | 0.2084726 | 0.2228  |
| Sample<br>D | 0.2124    | 0.2114683 | 0.2295317 | 0.2178  |
| Sample E    | 0.2048474 | 0.2044039 | 0.1763487 | 0.1952  |
| Sample F    | 0.2014433 | 0.198889  | 0.2182677 | 0.2062  |
| Sample<br>G | 0.2053838 | 0.2141687 | 0.2017475 | 0.2071  |

| WEEK 2      | Value 1   | Value 2   | Value 3   | Average |
|-------------|-----------|-----------|-----------|---------|
| SAMPL<br>E  |           |           |           |         |
| Sample<br>A | 0.1717833 | 0.1877796 | 0.1819371 | 0.1805  |

|          |           |           |           |        |
|----------|-----------|-----------|-----------|--------|
| Sample B | 0.2242845 | 0.2051367 | 0.2173788 | 0.2156 |
| Sample C | 0.2319497 | 0.2183001 | 0.2154502 | 0.2219 |
| Sample D | 0.2094428 | 0.2174691 | 0.1973881 | 0.2081 |
| Sample E | 0.1516133 | 0.1576602 | 0.1656265 | 0.1583 |
| Sample F | 0.2046274 | 0.1957524 | 0.1903202 | 0.1969 |
| Sample G | 0.192809  | 0.183329  | 0.174962  | 0.1837 |

| WEEK 3   | Value 1 | Value 2 | Value 3 | Average |
|----------|---------|---------|---------|---------|
| SAMPLE E |         |         |         |         |
| Sample A | 0.16092 | 0.16737 | 0.16651 | 0.16493 |
| Sample B | 0.21811 | 0.21154 | 0.21124 | 0.21363 |
| Sample C | 0.21989 | 0.22551 | 0.21714 | 0.22085 |
| Sample D | 0.19811 | 0.19104 | 0.20105 | 0.19673 |
| Sample E | 0.14567 | 0.14192 | 0.14513 | 0.14424 |

|          |         |         |         |         |
|----------|---------|---------|---------|---------|
| Sample F | 0.18902 | 0.18378 | 0.18783 | 0.18688 |
|----------|---------|---------|---------|---------|

|          |         |         |         |         |
|----------|---------|---------|---------|---------|
| Sample G | 0.18253 | 0.18157 | 0.17606 | 0.18005 |
|----------|---------|---------|---------|---------|

| WEEK 4 | Value 1 | Value 2 | Value 3 | Average |
|--------|---------|---------|---------|---------|
|--------|---------|---------|---------|---------|

SAMPLE

|          |         |         |         |         |
|----------|---------|---------|---------|---------|
| Sample A | 0.13411 | 0.13318 | 0.13024 | 0.13251 |
|----------|---------|---------|---------|---------|

|          |         |         |         |         |
|----------|---------|---------|---------|---------|
| Sample B | 0.21422 | 0.20889 | 0.21322 | 0.21211 |
|----------|---------|---------|---------|---------|

|          |         |         |         |         |
|----------|---------|---------|---------|---------|
| Sample C | 0.22274 | 0.21881 | 0.21716 | 0.21957 |
|----------|---------|---------|---------|---------|

|          |         |         |         |      |
|----------|---------|---------|---------|------|
| Sample D | 0.18818 | 0.19378 | 0.18804 | 0.19 |
|----------|---------|---------|---------|------|

|          |         |         |         |          |
|----------|---------|---------|---------|----------|
| Sample E | 0.12656 | 0.13033 | 0.12656 | 0.127817 |
|----------|---------|---------|---------|----------|

|          |         |         |         |          |
|----------|---------|---------|---------|----------|
| Sample F | 0.18125 | 0.18318 | 0.18405 | 0.182827 |
|----------|---------|---------|---------|----------|

|          |         |         |         |          |
|----------|---------|---------|---------|----------|
| Sample G | 0.17113 | 0.16415 | 0.16777 | 0.167683 |
|----------|---------|---------|---------|----------|

| WEEK 5 | Value 1 | Value 2 | Value 3 | Average |
|--------|---------|---------|---------|---------|
|--------|---------|---------|---------|---------|

SAMPLE

|          |           |           |           |        |
|----------|-----------|-----------|-----------|--------|
| Sample A | 0.1235226 | 0.1197778 | 0.1259996 | 0.1231 |
|----------|-----------|-----------|-----------|--------|

|          |           |           |           |        |
|----------|-----------|-----------|-----------|--------|
| Sample B | 0.2017253 | 0.2105469 | 0.2237278 | 0.212  |
| Sample C | 0.2098463 | 0.2259998 | 0.2196539 | 0.2185 |
| Sample D | 0.1901789 | 0.1893242 | 0.1964969 | 0.192  |
| Sample E | 0.1063949 | 0.1060368 | 0.0980683 | 0.1035 |
| Sample F | 0.1753589 | 0.1863268 | 0.1714143 | 0.1777 |
| Sample G | 0.151995  | 0.1493335 | 0.1606715 | 0.154  |

| WEEK 6   | Value 1   | Value 2   | Value 3   | Average |
|----------|-----------|-----------|-----------|---------|
| SAMPLE   |           |           |           |         |
| Sample A | 0.115748  | 0.1215507 | 0.1194013 | 0.1189  |
| Sample B | 0.213488  | 0.2228029 | 0.2033091 | 0.2132  |
| Sample C | 0.2109193 | 0.2142622 | 0.2264184 | 0.2172  |
| Sample D | 0.1831365 | 0.1799693 | 0.1963942 | 0.1865  |
| Sample E | 0.0893417 | 0.0957623 | 0.094496  | 0.0932  |
| Sample F | 0.17677   | 0.1661044 | 0.1725256 | 0.1718  |
| Sample G | 0.1475288 | 0.1520238 | 0.1369475 | 0.1455  |

| WEEK 7   | Value 1   | Value 2   | Value 3   | Average |
|----------|-----------|-----------|-----------|---------|
| SAMPLE   |           |           |           |         |
| Sample A | 0.1276806 | 0.1203639 | 0.1239554 | 0.124   |
| Sample B | 0.2191167 | 0.2101497 | 0.2049336 | 0.2114  |
| Sample C | 0.2212433 | 0.2220398 | 0.2047169 | 0.216   |
| Sample D | 0.1754019 | 0.1821059 | 0.1848922 | 0.1808  |
| Sample E | 0.0830944 | 0.085559  | 0.0830466 | 0.0839  |
| Sample F | 0.1748951 | 0.1651026 | 0.1802023 | 0.1734  |
| Sample G | 0.1405712 | 0.1463497 | 0.1567791 | 0.1479  |

| WEEK 8   | Value 1   | Value 2   | Value 3   | Average |
|----------|-----------|-----------|-----------|---------|
| SAMPLE   |           |           |           |         |
| Sample A | 0.1065103 | 0.1017458 | 0.1031439 | 0.1038  |
| Sample B | 0.2042857 | 0.2175746 | 0.2063397 | 0.2094  |
| Sample C | 0.2169735 | 0.2178434 | 0.209283  | 0.2147  |
| Sample D | 0.1809168 | 0.1850935 | 0.1811897 | 0.1824  |
| Sample E | 0.0771716 | 0.0732329 | 0.0760955 | 0.0755  |

|          |           |           |           |        |
|----------|-----------|-----------|-----------|--------|
| Sample F | 0.1579229 | 0.1623009 | 0.1750762 | 0.1651 |
|----------|-----------|-----------|-----------|--------|

|          |          |           |           |        |
|----------|----------|-----------|-----------|--------|
| Sample G | 0.145726 | 0.1479719 | 0.1320021 | 0.1419 |
|----------|----------|-----------|-----------|--------|

| WEEK 9 | Value 1 | Value 2 | Value 3 | Average |
|--------|---------|---------|---------|---------|
|--------|---------|---------|---------|---------|

SAMPLE

|          |           |           |           |        |
|----------|-----------|-----------|-----------|--------|
| Sample A | 0.0977339 | 0.1007352 | 0.0916309 | 0.0967 |
|----------|-----------|-----------|-----------|--------|

|          |           |          |           |       |
|----------|-----------|----------|-----------|-------|
| Sample B | 0.2074914 | 0.222343 | 0.2061656 | 0.212 |
|----------|-----------|----------|-----------|-------|

|          |           |           |           |        |
|----------|-----------|-----------|-----------|--------|
| Sample C | 0.2088858 | 0.2142263 | 0.2167879 | 0.2133 |
|----------|-----------|-----------|-----------|--------|

|          |           |           |           |        |
|----------|-----------|-----------|-----------|--------|
| Sample D | 0.1706325 | 0.1679011 | 0.1891664 | 0.1759 |
|----------|-----------|-----------|-----------|--------|

|          |           |           |           |       |
|----------|-----------|-----------|-----------|-------|
| Sample E | 0.0649136 | 0.0657302 | 0.0733563 | 0.068 |
|----------|-----------|-----------|-----------|-------|

|          |           |           |           |        |
|----------|-----------|-----------|-----------|--------|
| Sample F | 0.1604476 | 0.1649068 | 0.1579456 | 0.1611 |
|----------|-----------|-----------|-----------|--------|

|          |           |           |           |        |
|----------|-----------|-----------|-----------|--------|
| Sample G | 0.1322459 | 0.1337006 | 0.1474535 | 0.1378 |
|----------|-----------|-----------|-----------|--------|

| WEEK 10 | Value 1 | Value 2 | Value 3 | Average |
|---------|---------|---------|---------|---------|
|---------|---------|---------|---------|---------|

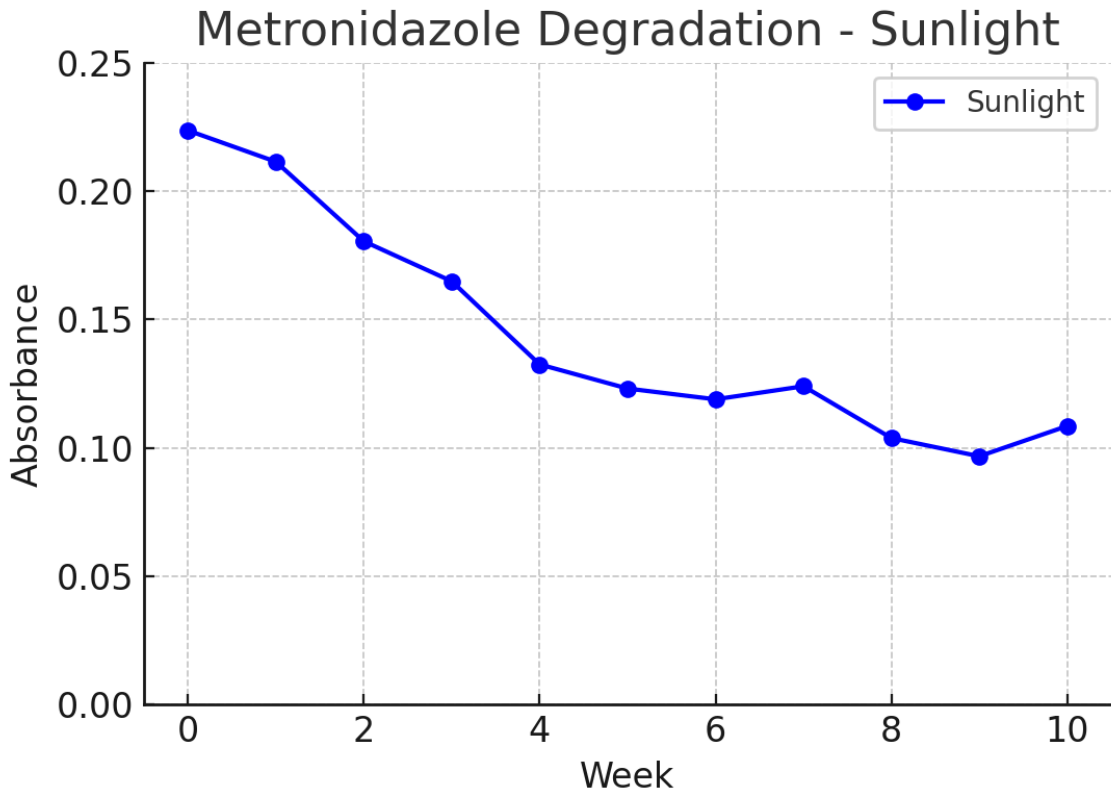
SAMPLE

|          |           |           |           |        |
|----------|-----------|-----------|-----------|--------|
| Sample A | 0.0906866 | 0.0917539 | 0.0989595 | 0.0938 |
|----------|-----------|-----------|-----------|--------|

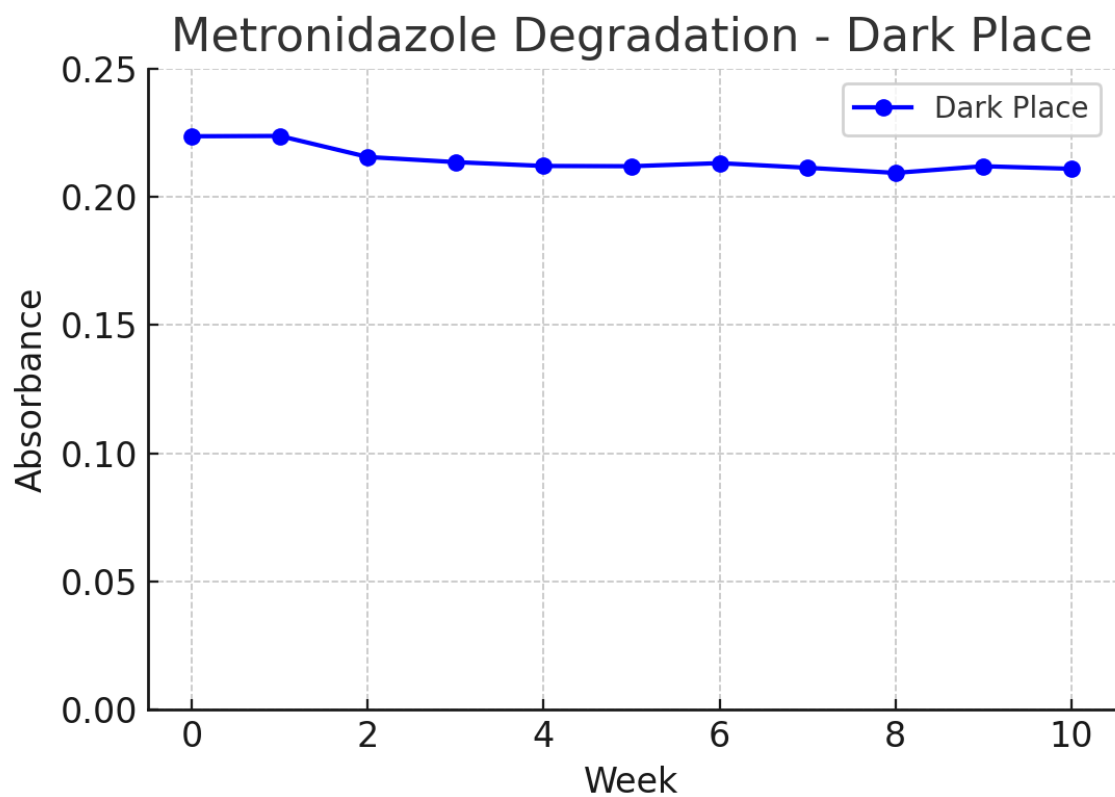
|          |           |           |           |        |
|----------|-----------|-----------|-----------|--------|
| Sample B | 0.210617  | 0.2038063 | 0.2185767 | 0.211  |
| Sample C | 0.2148867 | 0.20923   | 0.2112833 | 0.2118 |
| Sample D | 0.1663862 | 0.1627008 | 0.1830131 | 0.1707 |
| Sample E | 0.0633385 | 0.0641268 | 0.0561347 | 0.0612 |
| Sample F | 0.1547954 | 0.151781  | 0.1671237 | 0.1579 |
| Sample G | 0.1381671 | 0.1375509 | 0.125382  | 0.1337 |

| Time | Results of Absorbance Obtained from The UV Spectrophotometer |                  |                  |                  |                  |                  |                  |
|------|--|------------------|------------------|------------------|------------------|------------------|------------------|
|      | Sample A<br>(SL)   | Sample B<br>(DP) | Sample C<br>(RG) | Sample D<br>(RT) | Sample E<br>(ET) | Sample<br>F (AP) | Sample G<br>(BP) |
| 0    | 0.2237   | 0.2237           | 0.2237           | 0.2237           | 0.2237           | 0.2237           | 0.2237           |
| 1    | 0.2115   | 0.2238           | 0.2228           | 0.2178           | 0.1952           | 0.2062           | 0.2071           |
| 2    | 0.1805   | 0.2156           | 0.2219           | 0.2081           | 0.1583           | 0.1969           | 0.1837           |
| 3    | 0.1649   | 0.2136           | 0.2208           | 0.1967           | 0.1442           | 0.1869           | 0.1800           |
| 4    | 0.1325   | 0.2121           | 0.2196           | 0.1900           | 0.1278           | 0.1828           | 0.1677           |
| 5    | 0.1231   | 0.2120           | 0.2185           | 0.1920           | 0.1035           | 0.1777           | 0.1540           |
| 6    | 0.1189   | 0.2132           | 0.2172           | 0.1865           | 0.0932           | 0.1718           | 0.1455           |
| 7    | 0.1240   | 0.2114           | 0.2160           | 0.1808           | 0.0839           | 0.1734           | 0.1479           |

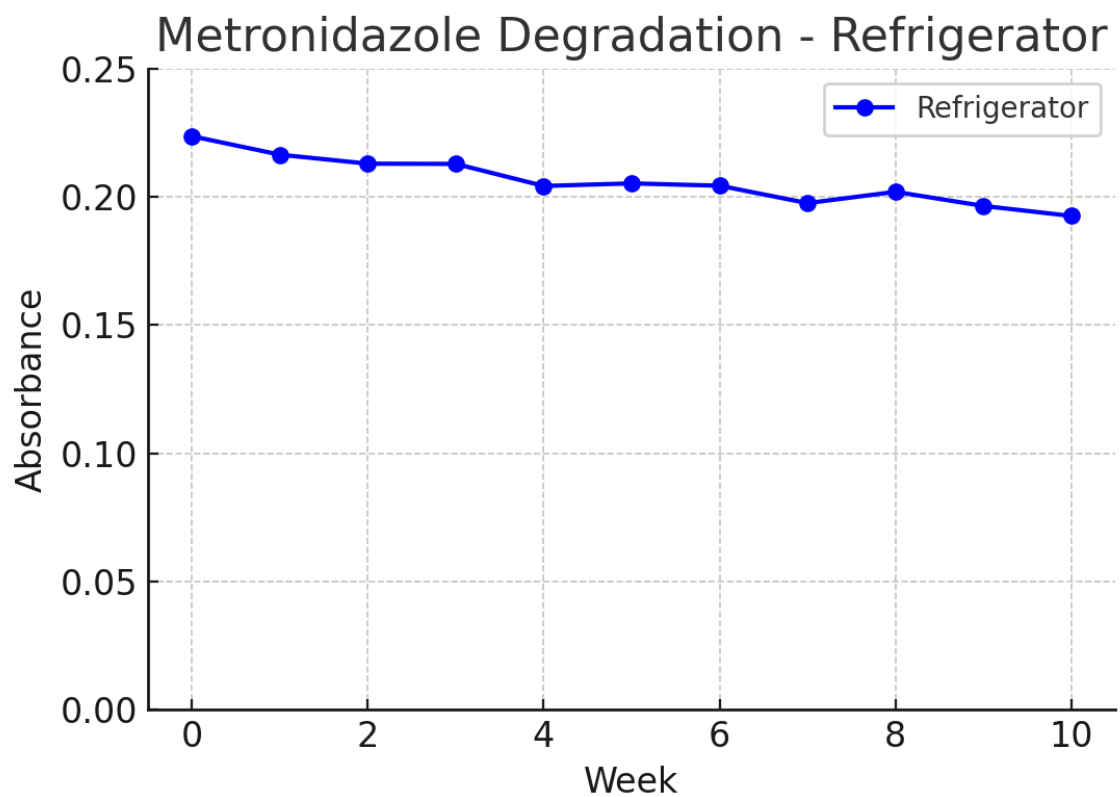
|    |        |        |        |        |        |        |        |
|----|--------|--------|--------|--------|--------|--------|--------|
| 8  | 0.1038 | 0.2094 | 0.2147 | 0.1824 | 0.0755 | 0.1651 | 0.1419 |
| 9  | 0.0967 | 0.2120 | 0.2133 | 0.1759 | 0.0680 | 0.1611 | 0.1378 |
| 10 | 0.0938 | 0.2110 | 0.2118 | 0.1707 | 0.0612 | 0.1579 | 0.1337 |



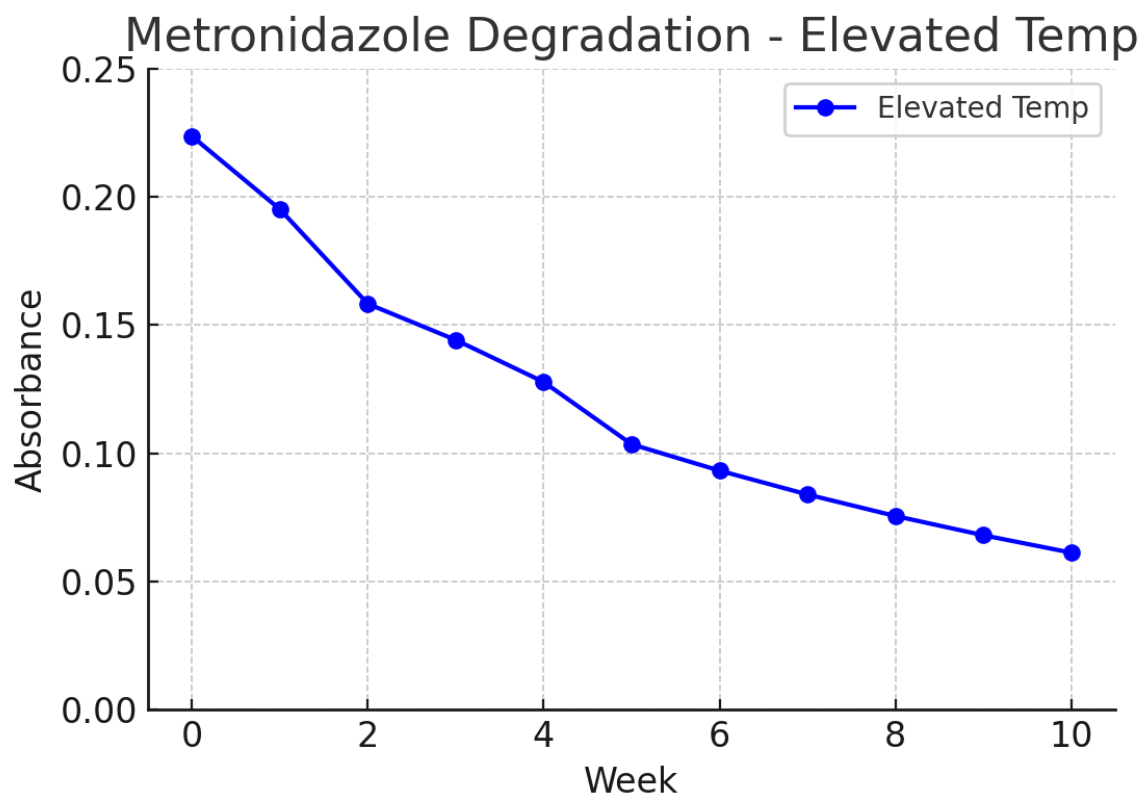
Absorbance of Metronidazole remaining in PET transparent bottle stored in the sunlight at 38°C



Absorbance of Metronidazole remaining in PET transparent bottle stored in the Dark Place



Absorbance of Metronidazole remaining in PET transparent bottle stored in the Refrigerator at 4°C



Absorbance of Metronidazole remaining in PET transparent bottle stored in Elevated Temperature at 55°C

