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**Photodegradation of Antibiotics from Wastewater
using TiO₂ Nanoparticles prepared by a Green
Synthesis Method using the Directing Agent PEG300**

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Dedication

To my beloved parents and sisters...

Sawsene

Dedication

To myself, who endured every hardship and never gave up throughout my academic journey in pursuit of my goal; to my parents, who went to great lengths for me, and whose constant support and encouragement have been a source of strength to me along the way; and to my brothers, for whom I remain a role model as they forge their own paths.

Djihane

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Praise be to God, Lord of the Worlds, and peace and blessings be upon the noblest of messengers, our Prophet Muhammad, and upon his family and companions.

By the grace and mercy of God, and with His guidance, we have been able to complete this research project. To Him be all praise and thanks, first and last, for granting us the strength, patience, and perseverance to overcome all obstacles.

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Abstract

This study aims to evaluate the photocatalytic efficiency of titanium dioxide nanoparticles (TiO₂ NPs) prepared using an environmentally friendly method for the degradation of pharmaceutical pollutants in wastewater, specifically antibiotics, into secondary compounds. TiO₂ NPs were prepared using bioactive compounds from Swiss chard (*Beta vulgaris* subsp. *cicla*) leaf extract as reducing and stabilizing agents instead of harmful chemicals, and using polyethylene glycol 300 (PEG300) as a growth promoter for the nanoparticles.

Biologically synthesized TiO₂ NPs were characterized, and their surface, structural, and optical properties were analyzed using various analytical techniques. X-ray diffraction (XRD) results revealed a pure, highly crystalline anatase phase. The crystal particle size was calculated to be approximately 50 nanometers. Furthermore, Fourier-transform infrared (FTIR) spectroscopy confirmed the presence of (Ti-O) bonds, and scanning electron microscopy (SEM) images revealed a porous nanostructure with slight agglomeration. On the other hand, photometric measurements using UV-Vis spectroscopy revealed a significant decrease in the photonic bandgap to 2.4 eV, confirming the success of the modification in shifting the catalyst's response slightly toward the visible range.

The catalytic efficiency of these particles was studied through the heterogeneous photocatalytic degradation of the antibiotics ciprofloxacin (CIP) and norfloxacin (NFL) at different pH levels over 120 minutes, during which they exhibited varying responses depending on the pH of the medium. The degradation efficiency of CIP reached its maximum value of 40.53% in an acidic medium (pH = 3), while NFL recorded the highest degradation efficiency of 33.08% in a basic medium (pH = 8). This average and variable performance in efficiency is attributed to the influence of the catalyst's surface charge and zero-charge point, in addition to the kinetic limitations on catalyst diffusion resulting from the absence of mechanical agitation.

These findings open up significant opportunities for establishing the appropriate hydrodynamic conditions and environment for photo-catalytic degradation processes, as well as for extending the duration of the process to improve wastewater treatment in the future.

Keywords: titanium dioxide (TiO₂) nanoparticles, Swiss chard (*Beta vulgaris* subsp. *cicula*), PEG-300, band gap, pharmaceutical contaminants, heterogeneous photocatalysis.

المخلص

تهدف هذه الدراسة إلى تقييم كفاءة التحفيز الضوئي لجسيمات ثاني أكسيد التيتانيوم النانوية (TiO₂ NPs) المحضرة بطريقة صديقة للبيئة، وذلك لتفكيك الملوثات الصيدلانية في مياه الصرف الصحي وتحديد المضادات الحيوية إلى مركبات ثانوية، حيث تم تحضير جسيمات ثاني أكسيد التيتانيوم النانوية باستخدام المركبات الحيوية لمستخلص أوراق نبات السلق (*Beta vulgaris subsp.cicla*) كعوامل اختزال وتثبيت بدلا من المواد الكيميائية الضارة، وباستخدام البوليمر بولي إيثيلين غليكول 300 (PEG300) كعامل موجه لنمو الجسيمات النانوية.

تم تشخيص جسيمات ثاني أكسيد التيتانيوم النانوية المصنعة حيويا وتحليل خصائصها السطحية والبنوية والضوئية باستخدام تقنيات تحليل مختلفة، حيث أظهرت نتائج تحليل حيود الأشعة السينية (XRD) تشكلا نقيًا لطور الأناتاز ذو التبلور العالي، كما تم حساب حجم الحبيبات البلورية والذي قدر بحوالي 50 نانومتر. كما أكدت نتائج مطيافية الأشعة تحت الحمراء (FTIR) ظهور روابط (Ti-O)، وبينت صور المجهر الإلكتروني الماسح (SEM) بنية نانوية مسامية بتكامل بسيط. من جهة أخرى، كشفت القياسات الضوئية بمطيافية الأشعة فوق البنفسجية والمرئية (UV-Vis) عن انخفاض ملحوظ في فجوة الطاقة الضوئية لتصل إلى 2.4 إلكترون فولط، مما يؤكد نجاح التعديل في إزاحة إستجابة المحفز نحو النطاق المرئي قليلا.

تمت دراسة الكفاءة التحفيزية لهذه الجسيمات من خلال عملية التحفيز الضوئي الغير متجانس للمضادين الحيويين السيبروفلوكساسين (CIP) والنورفلوكساسين (NFL) في درجات حموضة مختلفة مدة 120 دقيقة، حيث أظهرت إستجابة متباينة وفق درجة حموضة الوسط، فقد بلغت كفاءة تحلل السيبروفلوكساسين قيمتها القصوى بنسبة 40.53% عند الوسط الحمضي (pH = 3)، بينما سجل النورفلوكساسين أعلى كفاءة تحلل بنسبة 33.08% عند الوسط القاعدي (pH = 8). يعود هذا الأداء المتوسط والمتباين في الكفاءة إلى تأثير شحنة السطح ونقطة الشحنة الصفرية للمحفز، بالإضافة إلى القيود الحركية لانتشار المحفز الناتجة عن غياب التحريك الميكانيكي.

تفتح هذه النتائج آفاقا هامة لتبني الشروط الهيدروديناميكية والبيئة المناسبة لعمليات التحلل الضوئي المحفز وكذا إطالة وقت العملية لتحسين معالجة مياه الصرف مستقبلا.

الكلمات المفتاحية: جسيمات ثاني أكسيد التيتانيوم النانوية (TiO₂)، نبات السلق (*Beta vulgaris subsp.cicla*)، PEG300، فجوة الطاقة، الملوثات الصيدلانية، التحفيز الضوئي غير المتجانس.

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List of Abbreviations

Symbol	Significance
PEG300	Polyethylene Glycols
NP	Nanoparticle
PbS	Lead (II) sulfide
BC	This abbreviation stands for “Before Christ.”
AD	It is an abbreviation of the Latin phrase “Anno Domini,” which means “in the year of the Lord.”
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
NPs	Nanoparticales
PNPs	Polymeric Nanoparticles
0D	Zero-dimensional
1D	One-dimensional
2D	Two-dimensional
3D	Three-dimensional
Na-Y	Zeolite Na-Y ‘Aluminosilicate’
MCM	Mobil Composition of Matter
SBA	Santa Barbara Amorphous
CNTs	Carbon Nanotubes
SMART	Self-Monitoring, Analysis, and Reporting Technology
LEDs	Light-emitting diodes
LSPR	Local Surface Plasmon Resonance
MRI	Magnetic Resonance Imaging
PVC	Polyvinyl Chloride
VO	Vacant Oxygen
UV	Ultraviolet
MBE	Molecular Beam Epitaxy
DC	Direct Current
RF	Radio Frequency
TEM	Transmission Electron Microscope
CTEM	Conventional Transmission Electron Microscope
HRTEM	High-Resolution Transmission Electron Microscope

STEM	Scanning Transmission Electron Microscope
SEM	Scanning Electron Microscopy
XRD	X-ray Diffraction
FWHM	Full Width at Half Maximum
UV-Vis	Ultraviolet- Visible
FTIR	Fourier Transform Infrared
VB	Valence Band
CB	Conduction Band
ROS	Reactive Oxygen Species
mPEG	Methoxy-Polyethylene Glycol
CIP	Ciprofloxacin
NFL	Norfloxacin
JCPDS	Joint Committee on Powder Diffraction Standards
EDX	Energy Dispersive X-ray

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General Introduction

The discovery of antibiotics in the late 1920s is considered one of the most significant breakthroughs in the field of medicine. Antibiotics are drugs derived from natural sources produced by microorganisms, or from synthetic or semi-synthetic sources; they are capable of combating bacterial infections in humans, animals, and aquatic organisms by killing bacteria or inhibiting their growth and reproduction[1, 2]. With the continuous growth of the global population, the consumption of these drugs has increased significantly. Their role is no longer limited to treating and combating various infectious diseases; they are now also used for preventive purposes and to stimulate and enhance animal growth by adding them to feed as preventive agents and growth promoters[1].

The overuse of antibiotics has led to an increase in their release into the environment through various pathways, as they can enter sewers and water bodies via wastewater—particularly from pharmaceutical factories, hospitals, livestock farms, and aquaculture facilities— from which they are then transferred to soil and subsequently to agricultural ecosystems via the manure produced by livestock treated with antibiotics. They can reach humans through all these pathways (via animals, plants, and water). The danger of these antibiotics lies in their persistence and accumulation in these environments, particularly aquatic environments, leading to the emergence of what is known as antibiotic resistance in bacteria, which have been classified as emerging environmental contaminants threatening public health. The risk can be severe enough to cause death due to reduced antibiotic efficacy and the inability to combat resistant bacteria[1, 3]. These antibiotic-resistant bacteria are constantly evolving, having undergone genetic and mutational changes in their structure that allow them to recognize all types of antibiotics, rendering antibiotics incapable of fighting or eliminating them.

To address this growing threat, limit the spread of these pollutants, and attempt to eliminate them, many researchers and scientists have worked to propose various solutions, including traditional methods for removing these pollutants, such as filtration and reverse osmosis. However, due to their low removal efficiency, current studies have shifted toward developing modern methods such as biodegradation and advanced oxidation technologies. Among advanced oxidation methods, heterogeneous photocatalysis stands out as a promising and sustainable method for eliminating organic pollutants in aquatic environments. Among the most widely used photocatalysts in this process are oxygen-doped nanocrystals due to their low cost, high chemical stability, and significant ability to generate reactive oxygen species (such as hydroxyl radicals) through

electron transfer mechanisms between energy bands upon photostimulation[4]. The production of electron-hole pairs begins when the photocatalyst excites electrons, causing them to transition from the valence band to the conduction band and leaving behind an energy gap. Thus, after migrating to the surface of the catalyst, these electrons and holes generate highly reactive free radicals that break down organic pollutants, including antibiotics[4, 5].

Titanium dioxide is one of the most well-known photocatalysts that has been extensively studied for the treatment of wastewater contaminated with various organic and pharmaceutical pollutants, as it is environmentally safe, low-cost, and exhibits high photocatalytic activity. However, it faces several challenges, including a wide bandgap that limits its absorption to the deep ultraviolet range, rapid recombination of electron-hole pairs, and issues with surface stability and reactivity, particularly in aqueous media[4, 5].

With this in mind, and to overcome these limitations, researchers have focused on modifying the surface and structural properties of titanium dioxide nanoparticles (TiO_2 NPs) to enhance their photocatalytic efficiency and extend their activity beyond the ultraviolet spectrum into the visible spectrum. Among the modern and sustainable methods is the green synthesis method, which relies on natural sources instead of harmful chemicals as an alternative to reducing titanium salt ions and forming environmentally friendly TiO_2 NPs. The addition of directing and coating agents is also a pioneering step toward improving and controlling the morphology and size of these particles.

This study aims to develop and enhance the efficiency of TiO_2 NPs and apply them as a photocatalyst in the photodegradation of certain antibiotics commonly found in wastewater (such as ciprofloxacin (CIP) and norfloxacin (NFL)), in line with the principles of green chemistry and sustainable development.

Based on the foregoing, the central issue of this research revolves around the following questions: How can the surface and photophysical properties of titanium dioxide nanoparticles (TiO_2 NPs) be modified, and can this modification contribute to increasing the efficiency of the photodegradation of antibiotics? What are the other experimental factors and conditions that can influence the efficiency of the photocatalytic degradation of antibiotics?

To address these questions, this study aims to achieve the following objectives:

To prepare and modify titanium dioxide nanoparticles using an environmentally friendly method.

Characterize the structural, surface, and photophysical properties of the prepared particles using various analytical techniques (such as Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy coupled with X-ray energy-dispersive spectroscopy (SEM/EDX), and Ultraviolet and Visible Spectroscopy UV-Vis)).

Study the photocatalytic efficiency of the prepared particles in the degradation of the studied antibiotics and evaluate the effect of pH on degradation efficiency over time.

Accordingly, this thesis has been divided into four chapters to address this issue and achieve the stated objectives as follows:

Chapter One provides a literature review on nanotechnology and nanomaterials, including their properties, methods of preparation, and characterization.

Chapter Two discusses the problem of antibiotic contamination and its potential risks to the environment and human health. It also covers traditional and modern methods for their removal.

Chapter 3 outlines the experimental methodology, including the extraction and biosynthesis of TiO₂ NPs, their characterization, and finally, an evaluation of their performance in the photocatalytic degradation of the antibiotics “ciprofloxacin and norfloxacin”.

Chapter 4 presents and analyzes the experimental results, discusses the characteristics of the biosynthesized titanium dioxide nanoparticles and their efficiency in the photocatalytic degradation of the antibiotics, and draws conclusions based on this.

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Chapter One

Nanotechnology

The foundations of molecular and quantitative understanding laid the groundwork for modern chemistry, transforming it from an empirical science to a structure-focused science with improvements in molecular processing. Synthetic chemistry began with the synthesis of urea by Friedrich Wöhler. In 1828, this led to deliberate interventions in the nanoworld through the selection of reactions, relying on the statistical distribution of molecules. Recent decades have seen the diverse synthesis of molecular structures and solids, with a focus on topology, extending to nanophysical techniques[1].

Nanoscience has emerged in various fields of knowledge, including physics, organic chemistry, colloid chemistry, polymer chemistry, biology, and materials science. One of the driving factors behind this development is that comprehensive methods for developing materials science have been largely exhausted, paving the way for research into ways to improve and modify the properties of materials and reduce their structural elements[2].

I.1 What is Nano?

The word "Nano" means one billionth. One nanometer is one billionth of a meter, or approximately one billionth of a yard. To get an idea of the scale of nanometers, the diameter of a human hair is about 50,000 nanometers, while the diameter of a germ cell is a few hundred nanometers. In 2002, the width of the smallest lines etched into microchip integrated circuits was about 130 nanometers. The smallest objects visible to the naked eye are about 10,000 nanometers in size. Ten hydrogen atoms lined up in a straight-line measure one nanometer. Indeed, a nanometer is very small. Nanoscience, in its simplest definition, is the study of the fundamental principles of molecules and structures in which at least one dimension is between 1 and 100 nanometers. These structures are called nanostructures, and nanotechnology is the use of these structures in useful two-dimensional devices. It is important to understand that the nanoscale is not just small, but a special kind of small[3].

We will discuss various important scientific terms in nanotechnology, including:

Table I.1 The most important scientific terms relating to nanotechnology[4].

Scientific terms	Definitions
Nanoscale	It is a scale used to measure very small objects, with sizes ranging from approximately 1 to 1000 nanometers.
Nanoscience	It is the scientific field specializing in the study of matter at the nanoscale, aiming to uncover the unique properties that matter acquires when its size is reduced, and to observe the differences between the behavior of individual atoms and that of materials in their conventional form.
Nanotechnology	The science that focuses on the engineering of matter and the control of its properties at the atomic and molecular levels, with a view to applying these capabilities to cutting-edge innovations in industrial and medical fields.
Nanomaterial	Materials with internal or external structures on the nanoscale.
Nano-object	Material that has at least one of its end dimensions (length, width, height) in the nanoscale dimensions.
Nanoparticle	It is a Nano-object with three external dimensions at the nanoscale. The terms "nanorod "or "nanoplate" are used instead of 'nanoparticle' (NP) when there is a significant difference between the length of the longest axis and the shortest axis of the nanoparticle.
Nanofiber	If a nanomaterial has two external dimensions of similar size and a third dimension that is larger, it is referred to as a " nanofiber".

I.2 History of Nanoparticles

Humans have known about nanotechnology for a long time and have used it with natural skill; for more than 4,500 years, people have reinforced ceramic materials by adding natural 'asbestos' fibers to them. The ancient Egyptians, meanwhile, were pioneers in nanochemistry 4,000 years ago, having devised a chemical process to manufacture microscopic particles of lead (PbS) no larger than 5 nanometers in diameter for use in hair dye. Furthermore, 'Egyptian blue' is considered the first synthetic dye in history, developed by the Egyptians in the 3rd century BC by combining a sintered mixture of glass and quartz at the nanoscale, producing a complex compound consisting of $\text{CaCuSi}_4\text{O}_{10}$ and silicon dioxide (SiO_2)[4].

The Lycurgus Cup, dating from the 4th century AD, has become a unique historical artifact. It is the only remaining complete example of what is known as ‘bicolored glass’. This cup possesses remarkable optical properties; its color changes from a deep green to a glowing red and becomes transparent when illuminated from within (i.e., when light falls on the cup at a 90-degree angle to the line of sight). The stained-glass windows of León Cathedral in Spain (**Figure I.1**) are a living example of the application of nanomaterials in the Middle Ages. Although the scientific understanding of the concept was lacking at the time, craftsmen were able to utilize the optical properties of nanoparticles to color vast areas of glass, covering a total of 2,000 square meters, creating a unique spectacle, which has made it one of the most important monuments on the Way of St James[5].

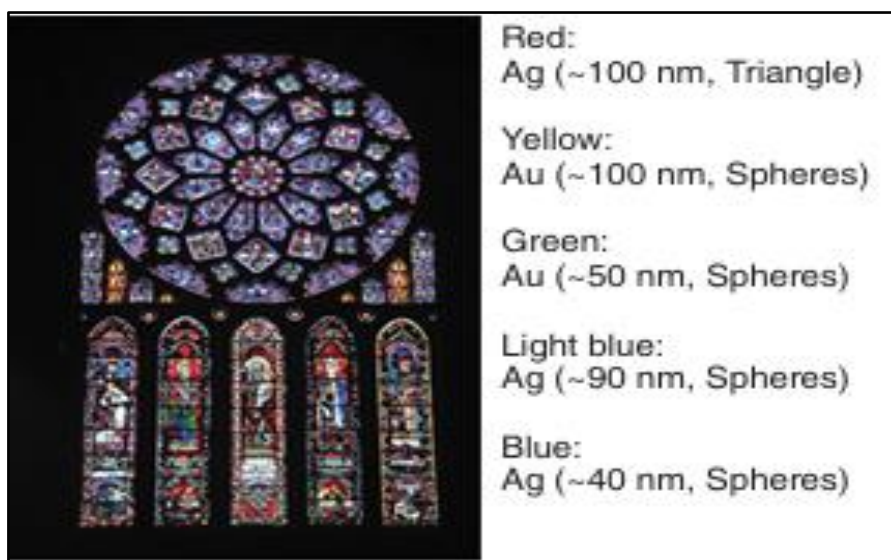


Figure I.1 Rosace Nord stained glass in the Cathédrale Notre-Dame de Chartres (France), color changes depend on the size and shape of gold and silver nanoparticles[5].

Between the 9th and 17th centuries, the Islamic world witnessed the invention of ceramic glazes characterized by their luster and vibrant sheen, a technique that was later introduced to Europe. This unique appearance was achieved by incorporating nanoparticles of metals such as silver (Ag) and copper (Cu) into the glaze layer, giving the ceramic vessels exceptional optical properties and an attractive metallic luster[6].

In 1857, the scientist Michael Faraday presented the first systematic description of nanoparticle preparation by synthesizing a gold colloidal solution, marking a fundamental turning point in the scientific history of nanomaterials. Faraday did not stop at preparation; he also observed that the optical properties of gold colloids differ radically

from those of gold in its solid (bulk) state; this is considered one of the earliest scientific reports describing what is known today as ‘size effects’. In 1908, the scientist Meek provided a precise physical explanation for the distinctive colors of metal colloids based on their interaction with light[4].

In 1990, using a transmission electron microscope, it was found that the presence of nanoparticles with a diameter of 50–100 nanometers was responsible for the dichroism (two colors) observed. These nanoparticles were identified by X-ray analysis as a silver-gold (Ag-Au) alloy with an Ag: Au ratio of approximately 7:3 and 10% copper (Cu) dispersed in a glass matrix. The gold nanoparticles absorb light at a wavelength of approximately 520 nm, giving them a red color. The green color results from light scattering by silver nanoparticles larger than 40 nm, whilst the reddish-purple color results from absorption by the larger particles[6]. In the 1940s, nanoparticles of SiO₂ were manufactured as alternatives to carbon black for reinforcing rubber[4].

In an article published on 22 February 2005, The New York Times summarized the relationship between the color of stained glass and the size and shape of nanoparticles (**FigureI.2**). Several decades later, the components present in stained glass in various churches were identified, thanks to the development of analytical instruments[5].

The development of nanotechnology in the field of computer science has transformed computers from huge, room-sized machines into portable devices that are easy to carry. At the beginning of the 21st century, interest in nanoscience and related technologies, as well as the progress made in this field, has increased. Recently, the significant potential of nanotechnology in biomedicine has become apparent, particularly in the detection and treatment of a range of diseases, as demonstrated by numerous studies[6].

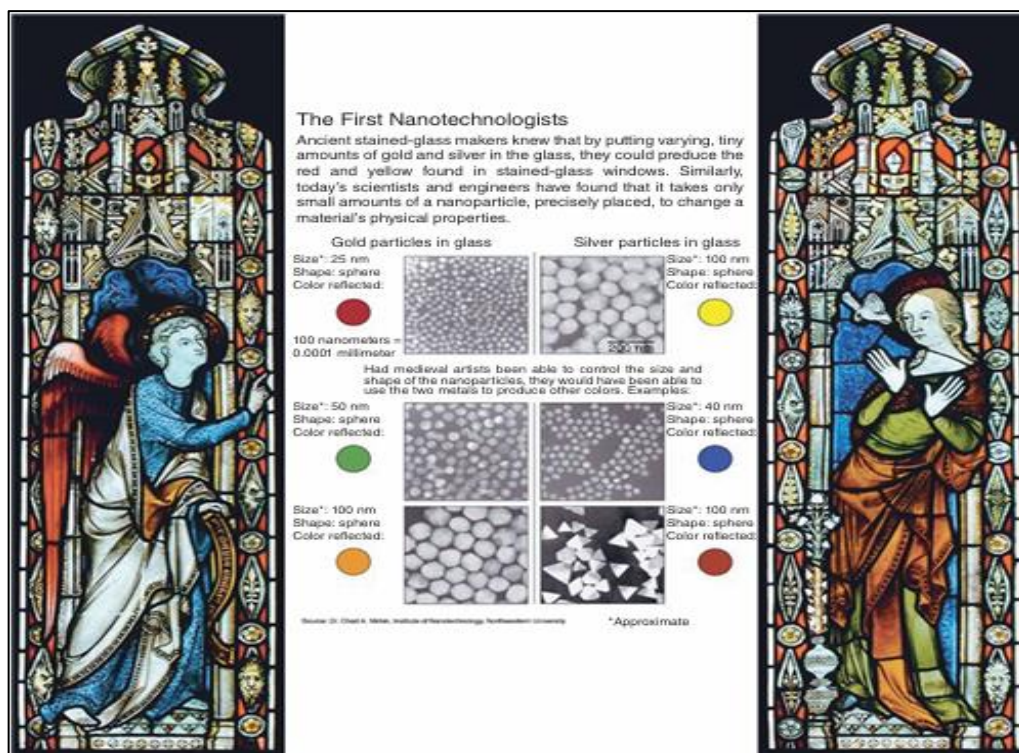


Figure 1.2 Comparison of the effect of size and shape of nanoparticles on the coloring of stained glass (Stained Glass Museum, Great Britain)[5].

1.3 Importance of NPs

Over the past few years, a small word with enormous potential has rapidly entered the public consciousness: 'nano'. The term has sparked speculation about a major transformation in virtually every aspect of science and engineering, along with the implications this holds for ethics, the economy, international relations, and everyday life. Optimists saw it as a panacea for all our problems, whilst pessimists regarded it as the next step towards chemical and bioweapons, perhaps one of the more extreme cases[3].

The considerable interest in nanoparticles has captured the attention of both the research and industrial sectors primarily due to their physical and chemical properties, which differ significantly from those of bulk (macroscopic) materials. At present, virtually all branches of applied science and technology are witnessing progress in one way or another with the advent of nanotechnology[7].

Hence, the importance of these nanocomposites in medicine (pharmaceuticals), genetic engineering (DNA, RNA), biotechnology (proteins, individual cells, and enzymes), and the food industry[2].

I.4 Classification of nanomaterials based on origin

I.4.1 Natural nanomaterials

Natural nanomaterials arise in the environment either through the biological processes of living organisms or through natural phenomena. Synthetic surfaces with distinctive micro- and nanoscale properties can also be derived from these natural sources for use in technical applications. These materials are distributed throughout the Earth's spheres (hydrosphere, atmosphere, lithosphere, and biosphere) independently of human intervention. They are an integral part of our planet's components, found in the troposphere, in all water bodies and underground sources, in rocks, soil, and lava, and even inside microorganisms and complex biological systems, including the human body[4].

I.4.2 Synthetic nanomaterials

Engineered nanomaterials are manufactured using various techniques, including mechanical grinding, combustion of engine exhaust and smoke, or physical, chemical, biological, or hybrid methods. With the increasing use of these materials in industry and consumer goods, there is a growing interest in developing risk assessment strategies to understand their behavior and fate in the environment. The real challenge lies in determining whether current scientific rules are sufficient to predict their effects, or whether they have unique environmental properties that differ from what we know about natural nanomaterials[4].

I.5 Classification of nanomaterials based on the structural configuration

I.5.1 Organic nanomaterials

At the nanoscale, organic compounds are converted into organic nanomaterials, which are the most prevalent of all types and are frequently used in the biomedical industry and various cancer treatments. Examples of organic nanoparticles or polymers include liposomes, dendrimers, micelles, and ferrites (**FigureI.3**). These particles are biodegradable and non-carcinogenic, but they are highly sensitive to heat and radiation and are unstable due to their non-covalent bonds. Their classification and effectiveness

depend on specific structural properties such as size, shape, morphology, stability, and ability to carry lipids and proteins[6, 8].

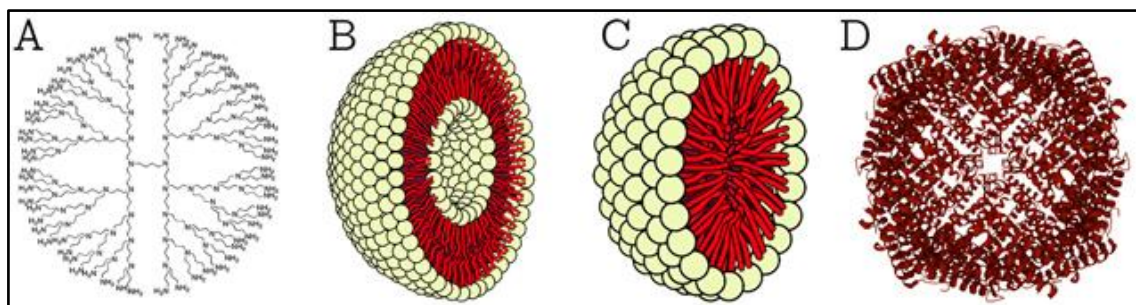


Figure I.3 Types of organic NPs. **A.** Dendrimers; **B.** liposomes; **C.** micelles; and **D** ferritin[9].

I.5.2 Inorganic nanomaterials

They are NPs whose basic composition does not contain carbon atoms. These materials are mainly classified into two categories: nanoparticles based on metals or metal oxides[6].

- **Metal NPs**

These particles are produced from various common metals through two manufacturing methods: construction and destruction. Due to their small size, increased surface area, and quantum confinement effects, these particles exhibit superior thermal and catalytic properties, as well as high antibacterial activity and sensitivity to ultraviolet radiation. They also have remarkable electrical conductivity due to the high density of atoms on their surface[10].

- **Metal Oxide NPs**

These particles are found in most metals (such as aluminum, copper, zinc, and silver) and are manufactured in laboratories using photochemical or electrochemical techniques, often by reducing metal ions in solutions. Due to their high surface energy, they have a great capacity to absorb small molecules, making them ideal for bioassays and environmental testing. Gold nanoparticles are also used to coat electron microscope (SEM) samples to improve image quality and resolution[10].

- **Ceramic NPs**

These are solid inorganic materials produced through thermal cycles (heating and cooling) of materials such as carbides, oxides, and phosphates. Their structural forms vary between porous, hollow, and dense, and their states between crystalline and non-crystalline. They are of great research interest for their use in chemical and photocatalysis, and are also considered a promising substrate in drug delivery systems for the treatment of cancer, eye diseases, and viral and bacterial infections[10].

- **Semiconductor NPs**

These particles combine the properties of metals and non-metals and consist of elements from groups (II-VI), (III-VI), and (IV-VI) in the periodic table (such as silicon and gallium nitride). They are characterized by wide band gaps that can be precisely tuned to create innovative applications. Their uses range from advanced electronics, photonic optics, photocatalysis, and water splitting for energy production[10].

- **Polymeric NPs**

Scientifically known as PNPs, these are organic materials that often take the form of capsules or nanospheres. These particles are easy to handle and provide effective protection for drug molecules, ensuring controlled release and targeted delivery of treatments. Due to their high biocompatibility and natural degradability, they are widely used in medical diagnostics and the manufacture of advanced drug delivery systems[10].

- **Lipid-based NPs**

These spherical platforms (10-100 nanometers in diameter) consist of a double lipid layer surrounding a solid core containing lipophilic molecules. They are easy to prepare, highly biocompatible, and capable of carrying large drug doses. These particles are a revolutionary tool in modern medicine, where they are mainly used in cancer treatment by effectively delivering drugs and releasing ribonucleic acid (RNA) technologies within the body[10].

1.5.3 Carbon-based nanomaterials

This category includes nanoparticles made exclusively from carbon atoms. These carbon nanoparticles combine the features of sp hybrid carbon bonds with unconventional physical and chemical properties at the nanoscale. Thanks to their outstanding electrical conductivity, exceptional strength, electron affinity, and optical, thermal, and absorptive

qualities, carbon nanoparticles are applied in various fields, including drug delivery, energy storage, biomedical imaging, photovoltaic devices, and environmental sensing systems for monitoring microbial environments or detecting microbial pathogens. Carbon nanodiamonds and carbon nanoballs are more complex carbon nanoparticles. Due to their low toxicity and outstanding biocompatibility, they are used in drug delivery and tissue engineering fields[9].

- **Fullerenes**

Fullerenes are spherical carbon nanoparticles (**FigureI.4**) bonded by sp^2 hybridization. Depending on the number of layers, fullerenes can range in size from 4 to 36 nanometers in diameter for multi-layered fullerenes, and up to 8.3 nanometers for single-layered fullerenes[10].

- **Graphene**

Graphene is a term that refers to a type of carbon characterized by a hexagonal structure and a flat, two-dimensional surface (**FigureI.4**). A single layer of graphene is only 1 nanometer thick[10].

- **Carbon nanotubes**

Single-layer carbon nanotubes have a diameter of less than 0.7 nanometers. However, there is variation in the length of multi-layer carbon nanotubes, which can range from micrometers to several centimeters, and their ends can be closed or hollow. Carbon nanotubes are produced by rolling graphene into hollow tubes, composed of carbon atoms(**FigureI.4**)[10].

- **Carbon nanofibers of carbon**

Most of the nano-foils are rolled into cup- or cone-shaped cylinders, rather than straight tubes, to produce carbon nanofibers[10].

- **Carbon black**

Carbon black has a diameter of 20-70 nanometers and is amorphous in structure. When agglomerates of about 500 nm in size are formed, the interactions between the particles become very strong, and the particles mix to form larger agglomerates (**FigureI.4**) [10].

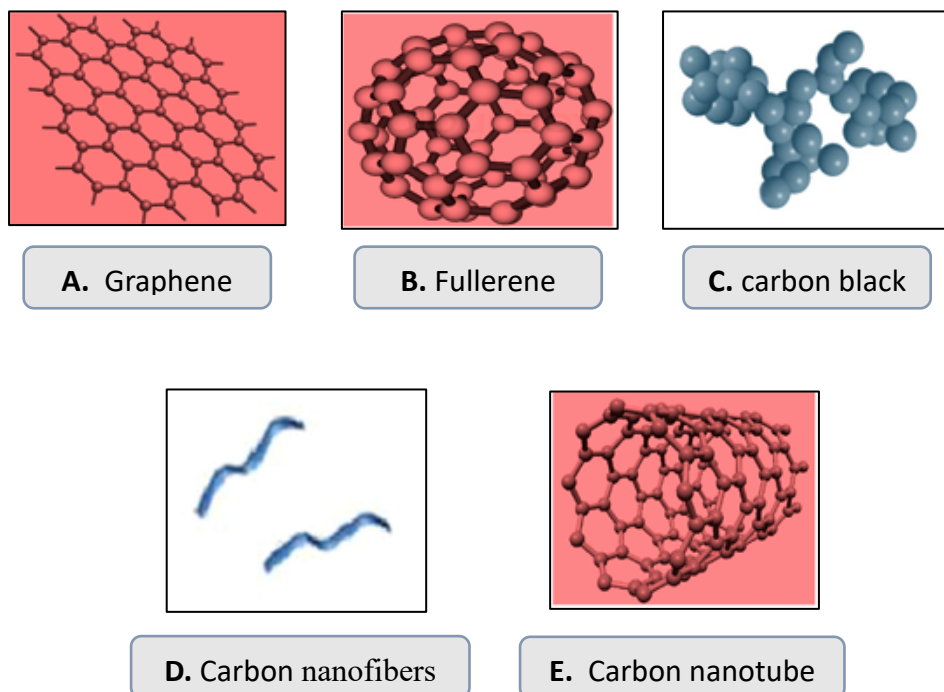


Figure I.4 Different types of carbon-based NPs. **A.** Graphene, **B.** Fullerene, **C.** Carbon black, **D.** Carbon nanofibers, **E.** Carbon nanotube[8, 9, 11].

I.5.4 Composites nanomaterials

Composite nanomaterials involve combining nanoparticles with each other, combining them with larger materials, or combining nanomaterials with bulk materials. These materials are currently used to enhance the mechanical, thermal, and fire-resistant properties of a wide range of products, from car parts to packaging materials[8].

I.6 Classification of nanomaterials according to the number of dimensions

Nanomaterials are characterized by their extremely small sizes, falling within the nanoscale range in one, two, or three dimensions. These materials can be divided into four main categories based on their origin, dimensions, and structural composition[12].

I.6.1 Zero-dimensional (0D) nanomaterials

These materials are characterized by the restriction of electron movement in all three directions. They often appear in specific geometric shapes such as spheres with an

average diameter between 1 and 50 nanometers, as well as cubic and polyhedral structures. Examples: quantum dots, gold balls, and silver clusters[12].

I.6.2 One-dimensional (1D) nanomaterials

In this category, the movement of free electrons is restricted to only one direction. These materials are characterized by diameters that remain within the nanoscale, while their length extends beyond this range, sometimes reaching several micrometers. Examples: nanotubes, nanorods, nanowires, and nanofibers[12].

I.6.3 Two-dimensional (2D) nanomaterials

These materials allow electrons to move freely in only two dimensions, while the third dimension (thickness) remains confined to the nanoscale. These materials often have large surface areas despite their thinness, which can extend several micrometers in length and width. Examples: thin films, nanotapers, and nanoballs[12].

I.6.4 Three-dimensional (3D) nanomaterials

The movement of free electrons in these materials is not restricted to specific dimensions, but rather moves in all three directions. These structures usually consist of clusters of nanoparticles or building blocks that together form a bulk material or hollow spheres. Examples: bulk materials, nanoparticles, and hollow spheres composed of nanoparticles[12].

I.7 Classification of nanomaterials based on pore dimensions

In this context, the pore diameter determines the size of the particles and provides data on diffusion and interaction properties. When the guest particles are smaller than the pore size, the interaction between the particles and the wall decreases, while the interaction between the particles themselves increases during the diffusion process. These materials are useful in applications related to absorption and diffusion that rely on these properties. They are divided into three sections[8].

I.7.1 Micro porous materials

Microporous materials are characterized by very narrow pores with a diameter of less than 2 nanometers, which makes them capable of absorbing only small molecules such as gases or linear molecules, with slow diffusion kinetics and high reaction properties. Examples of these materials include Na-Y and natural clay materials, and they are used in applications such as gas purification, filter membranes, and gas storage[8].

I.7.2 Mesoporous materials

Medium-pore materials have pores with diameters ranging from 2 to 50 nanometers, allowing them to absorb relatively larger molecules. Examples include MCM-41, MCM-48, SBA-15, and carbon-based mesoporous materials, which can be used as nanoreactors for polymerization or as absorption systems for liquids and vapors[8].

I.7.3 Macro porous materials

Microporous materials are characterized by large pores with diameters greater than 50 nanometers, allowing them to accommodate large molecules such as polyaromatic systems or small biological molecules. Examples include carbon nanotubes, porous gels, and porous glass, which are mainly used as matrices for storing functional molecules, structures for implanting functional groups such as catalytic sites, and sensing materials[8].

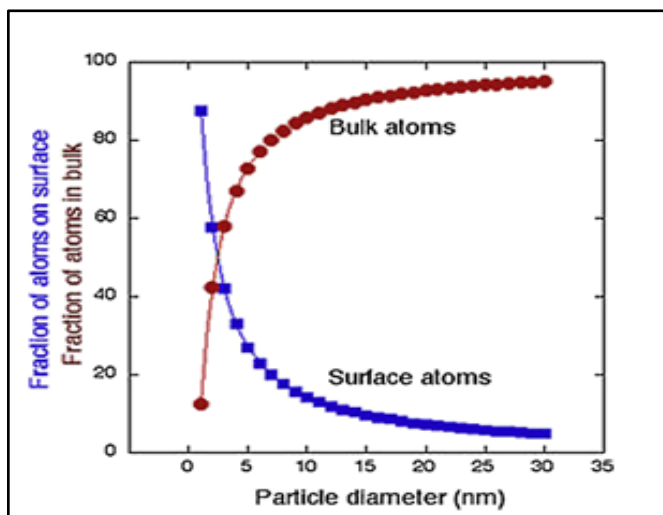
I.8 Properties of NPs

I.8.1 Physical properties

The change observed when the size of a material is reduced to the nanoscale is a change in the surface-to-volume relationship, where the surface area increases rapidly as the size decreases, in accordance with the law (1-1):

$$\frac{A}{V} = \frac{4\pi r^2}{\frac{4}{3}\pi r^3} \quad (1-1)$$

Here, **A** represents the surface area ratio and **V** the volume of the material; that is, the ratio of surface area to volume is inversely proportional to the particle diameter. Therefore, as the particle diameter decreases, the surface area-to-volume ratio increases, as illustrated by the curve in **(FigureI.5)**.

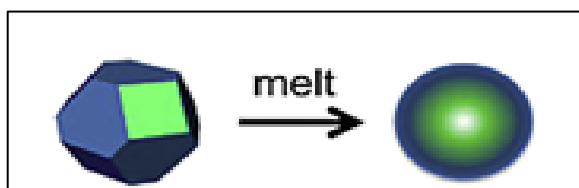


FigureI.5 Curves representing the fraction of available atoms on the surface of bulk material and nanomaterial[13].

This is where the importance of the surface area-to-volume ratio lies in determining surface activity; an increase in a material's surface area leads to an improvement in the catalytic sites on the material's surface and, consequently, an improvement in the catalytic activity of these sites. Furthermore, an increase in the surface area-to-volume ratio affects both physical and chemical properties. Among the physical properties, we shall mention the following[13].

- **Melting point**

The temperature at which a solid substance changes into a liquid. We observe that the temperature decreases; the polyhedral solid molecules take on a spherical shape after melting to achieve the lowest possible energy, as shown in (FigureI.6).



FigureI.6 An illustration showing how the shape of a particle changes as its size decreases[13].

The melting point of nanomaterials is lower than that of their bulk counterparts, as the particle surface interacts with the surrounding environment, leading to a change in surface composition and consequently a reduction in the melting point. An example of this is the melting point of gold in its bulk form, which is 1337 K; this decreases rapidly at 5 nm,

and this decrease is observed in metallic particles such as copper, tin, indium, and lead. There is also a phase transition temperature, which decreases as the size of the material decreases. For example, the Curie temperature for the transition of barium titanite from the photoconductive state to the semiconductive state in its bulk form is 130 °C, which decreases to 75 °C at a size of 120 nm[13].

- **Mechanical properties**

The mechanical properties of nanomaterials generally comprise ten components, namely: strength, brittleness, hardness, toughness, stress resistance, ductility, elasticity, malleability, stiffness, and yield stress. Most inorganic and non-metallic materials are brittle and lack important properties such as hardness, ductility, elasticity, or malleability; in contrast, organic materials. Due to surface and quantum effects, nanoparticles exhibit different properties compared to bulk materials. For example, conventional FeAl powder, consisting of fine particles (larger than 4 micrometers), is brittle, in contrast to ultrafine FeAl powder, which exhibits a good combination of strength and ductility. These properties arise due to various interaction forces between the nanoparticles or between them and the surface[9].

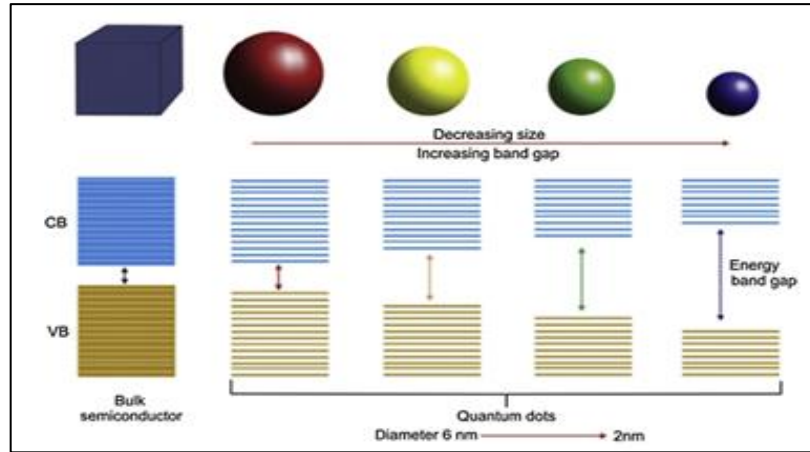
- **Hardness, ductility, and Young's modulus/strength**

Hardness is a material's resistance to deformation; Hall-Petch's law states that a reduction in grain size increases a material's strength, making nanomaterials significantly stronger than bulk materials. When irreversible deformation occurs under the influence of external or internal stress, this is referred to as the material's ductility. Their strength can be understood through Young's modulus and ductility. For example, carbon nanotubes have a high Young's modulus of 1 terapascal along their axial axis; however, they are compressible and deformable along the transverse direction due to the weakness of the π bond. Graphene is also considered one of the strongest materials, as it has been found that its intrinsic strength and stiffness remain unchanged at high defect densities[13].

- **Optical properties**

When the size of a material is reduced to the nanoscale, its optical properties change through two different mechanisms: quantum confinement and surface plasmon resonance. When particles are reduced to a size smaller than the de Broglie wavelength, quantum confinement of charges occurs, causing energy levels to appear separately, as shown in **Figure I.7**, where the distinctness of the energy levels gradually increases as the bulk

material is transformed into a two-dimensional, then one-dimensional, and finally zero-dimensional state. The confinement of energy levels causes a change in the appearance of the material, particularly through a change in its color[13].



FigureI.7 Quantum confinement with decreasing volume[13].

- **Magnetic properties**

All magnetic materials contain a ‘magnetic component’ in their composition, including metals such as Fe, Co, or Ni (at ambient temperatures), except certain metals such as Pd, Au, or Ag, which are diamagnetic; but all this change at the nanoscale, where anti-magnetic metals in their bulk state, when in the form of nanoparticles, acquire the ability to be magnetized. This is a result of the uneven distribution of electrons. The magnetic properties are determined by key parameters, namely: composition, crystal structure, magnetic anisotropy, and vacancy defects. At the nanoscale, there are two further important parameters that play a significant role: size and shape.

The importance of size has become apparent in determining the transition of a material from the superparamagnetic state, in which it loses its magnetization immediately, to the single-domain state, where, as the particle size increases, the magnetic coercivity (resistance to changes in magnetization) increases until it reaches its maximum strength, at which point all electrons align in a single direction. It then transitions to a multi-domain state; as the particle size increases further, it divides into distinct magnetic domains, which facilitates the magnetization process and consequently reduces the coercivity (**FigureI.8**). The magnetic properties of a nanoparticle are influenced by its shape (cubic or spherical); for example, cubic nanoparticles such as CoFe_2O_4 exhibit lower magnetic coercivity than spherical ones. The composition also affects the magnetism of

nanoparticles, as a change in crystal phase or the creation of alloys (such as combining cobalt with platinum) can transform the material from a strong magnet into a superparamagnetic material with zero coercivity at room temperature. Furthermore, structural modification of nanoparticles through the use of magnetic impurities significantly alters their magnetic properties[9].

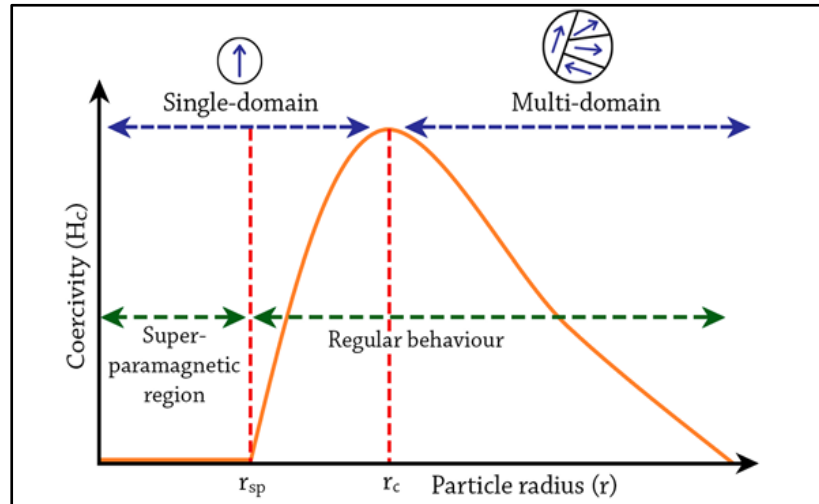


Figure 1.8 The change in magnetic coercivity of NPs as a function of particle radius[9].

- **Thermal properties**

Thermal properties such as energy transfer, thermal conductivity, thermal stability, electrothermal energy, and heat capacity are key factors in the process of heat transfer in nanoparticles. The thermal and electrical conductivity of these particles is significantly influenced by their size, as reducing the size of nanoparticles increases the ratio of the particle's surface area to its volume. This increase in the ratio provides a larger surface area, which enhances electron supply and supports heat transfer, which relies on one of the two main mechanisms of thermal conduction. Furthermore, convection enhances the thermal efficiency of nanoparticles, but its effect is particularly evident when crystalline nanoparticles are dispersed in liquids, forming a nanoliquid[14].

1.8.2 Chemical properties

The decrease in particle size affects their behavior in chemical processes; as the particle size is reduced, the surface area increases dramatically, meaning that a greater number of free bonds become available to interact with the surrounding substances, which causes the reaction rate to increase exponentially. Another advantage of nanoparticles is the lower reaction temperature. Furthermore, some reactions occur only at the nanoscale

and cannot take place outside this scale[13]. The chemical properties of nanoparticles are influenced in terms of:

- **Surface energy**

The reduction in the size of a substance leads to an increase in the energy of its surface atoms; this additional energy is known as surface energy, surface free energy, or surface tension[13].

- **Chemical potential**

The energy absorbed or released as a result of a change in the number of particles in a given state, where the chemical potential depends on the curvature: an atom on a convex surface has a high chemical potential, whilst an atom on a concave surface has a low chemical potential. The curvature of the surface also affects the solubility of particles; solubility decreases as the curvature of the surface increases. This means that a smaller particle has greater solubility than a larger particle[13].

- **Oxidation process at the nanoscale**

In the case of metallic nanoparticles, it is observed that oxidation does not occur at low temperatures. However, at high temperatures, oxidation does occur; furthermore, the rate of oxidation decreases at high temperatures. The reason oxidation does not occur at low temperatures is that when nanoparticles are exposed to air, a thin layer of adsorbed gases forms on their surface. Until this gas is removed, the nanoparticles will not undergo oxidation[13].

- **Catalysis**

Nanoscale materials exhibit increased catalytic activity; as the size of the nanomaterial decreases, catalytic activity increases. Noble metals, which are inert in their bulk form, are considered weak catalysts, yet at the nanoscale they act as highly effective catalysts. For example, gold in its bulk form is a weak catalyst. However, when reduced to the nanoscale, it exhibits enhanced catalytic activity in the oxidation of carbon dioxide[13].

- **Functionalization of nanostructures**

Carbon nanotubes (CNTs) are extremely important, but the fact that their ends are capped by fullerene hemispheres renders them inactive in chemical reactions. However, carbon nanotubes (CNTs) can be activated to make them effective in chemical reactions

using various activation methods, including surface activation, end-wall activation, and inner-wall activation[13].

I.9 Nanotechnology applications

I.9.1 Automobile

Given the strength and lightness of nanomaterials, they can be used in the manufacture of equipment and components for high-performance sports cars and motorcycles, as well as in dirt-repellent coatings, car windscreens, and anti-fog mirrors coated with a water-repellent nano-layer[9].

I.9.2 Aviation and Aerospace

Carbon fiber composites are used in the manufacture of modern aircraft and helicopters, where they play a key role in reducing the overall weight of the vehicles, which has a positive impact on fuel efficiency, in addition to their mechanical advantages, as these composites play a vital role in reducing the radar signature of fighter aircraft (stealth technology). Current research efforts are also focused on developing smart wings and blades capable of changing shape in future aircraft and space shuttles, intending to achieve superior maneuverability and aerodynamic control[9].

I.9.3 Construction and Building

The construction sector is undergoing a technological transformation through the integration of biotechnology and nanotechnology; ‘self-healing’ concretes have been developed that utilize nanocomposite applications to automatically repair cracks. In a related development, smart glass for skyscrapers has been developed, coated with a layer of nanorods, giving it ‘easy-clean’ properties; this technology allows surfaces to be cleaned using water alone, without the need for chemical detergents or surfactants[9].

I.9.4 Applications in the field of defense and security

Research and development in the defense sector focuses on enhancing personal safety by using nanomaterials to produce lighter, more robust helmets and bulletproof vests. There is also significant interest in 'SMART' technology (Self-Monitoring, Analysis and Reporting Technology), which is being integrated into the uniforms of tactical and special forces to enable self-monitoring systems and enhance the efficiency of field responses through the real-time exchange of data and information[9].

I.9.5 Applications in electronics

The electronic and optical properties of nanoparticles can be utilized and applied in imaging and electronics technologies. In the field of lithium-ion batteries, separators play a vital role in preventing direct contact between the cathode and anode whilst providing pathways for ion transport. Given the limitations of commercial polyolefin separators in terms of thermal stability and electrolyte absorption, nanoparticles (such as zinc oxide, ZnO) are emerging as an ideal alternative due to their porous structure, which enhances the batteries' ability to store greater amounts of energy. In a related context, Pt-Au bimetallic nanoparticles help improve the efficiency of oxygen reactions in lithium-air batteries, whilst the use of nickel nanoparticles in metal hydride batteries provides a longer operational life and reduces the need for recharging. In display technology, particles such as CdS and ZnSe are used in LEDs to produce larger, brighter, and more energy-efficient screens, whilst ensuring a longer lifespan for these devices[7].

I.9.6 Applications in medicine and pharmacy

Nanoparticles, whether metallic or semiconducting, possess tremendous potential for the detection and treatment of cancer. This is due to their enhanced light scattering and absorption properties, which result from the local surface plasmon resonance (LSPR). For example, light is efficiently absorbed by gold nanoparticles, where it is converted into localized heat, which can be utilized in selective photothermal therapy for cancer. This works by killing cancer cells through the heat generated in tumor tissue. Due to the unique optical properties of gold nanoparticles, they are an excellent candidate for photodynamic therapy of cancer, using a light-activated drug to kill cancer cells. It also found that gadolinium-based nanoparticles have a significant ability to inhibit tumor growth and metastasis, as well as to enhance contrast in MRI imaging of tumors. Zinc and iron oxide nanoparticles are effective tools in the field of pharmacy, having demonstrated high efficacy in targeted drug delivery and the selective destruction of cancer cells[7].

I.9.7 Applications in agriculture

Nanoparticles can be utilized in agriculture to provide solutions to current agricultural and environmental problems, namely in the form of nano-fertilisers and nano-pesticides. Nano-fertilizers are used in smaller quantities than traditional chemical fertilizers, as they are highly efficient. This is due to their ability to release nutrients exactly when and where plants need them, thereby preventing excess fertilizer from turning into gaseous forms or leaching into water. Many nanoparticles have proven effective in combating microbes,

insects, and worms, making them a promising alternative to chemical pesticides and a cheaper alternative to biological pesticides[7].

I.9.8 Applications in the food industry

Nanoparticles can be applied in the food industry, including food storage and packaging. For example, silver nanoparticles show great promise in food packaging due to their antimicrobial activity; they play a key role in reducing the risk of pathogens and extending the shelf life of food. The effectiveness of incorporating nanoparticles, including silver (Ag) and zinc oxide (ZnO), into the formulations of both biodegradable and conventional non-biodegradable packaging materials has been verified to provide comprehensive protection for food products such as meat, baked goods, fruit, and milk. These nanomaterials have demonstrated a superior ability to inhibit the activity of spoilage agents, including yeasts, bacteria, and both aerobic and anaerobic bacteria. A notable practical example is the fortification of polyvinyl chloride (PVC) film with silver nanoparticles, used to wrap minced meat at a temperature of 4°C, which significantly contributed to inhibiting bacterial growth; this had a positive effect on extending the product's shelf life from two to seven days, confirming the high efficacy of this technique in food preservation[7].

I.10 Synthesizing nanomaterials methods

Various types of nanomaterials can be obtained using several methods and manufacturing techniques that reduce material dimensions to the nanoscale. In recent years, much effort has been devoted to researching and developing these methods, and even to discovering new, practical ones. These developments aim to improve the efficiency and behavior of nanomaterials in various applications by precisely controlling their physical and chemical properties through adjusting and modifying several variables, such as size, morphology, crystal structure, and purity[15].

Synthesis methods range from physical and mechanical to chemical, leading to modern trends in sustainable green synthesis as shown in **FigureI.10**.

I.11 Nanomaterials synthesizing approaches

Generally, there are two main approaches to synthesizing different forms of nanomaterials (powders, tubes, wires, rods, spheres, or thin films) as shown in **Figure I.9**: the top-down approach (descending way) and the bottom-up approach (ascending way)[12].

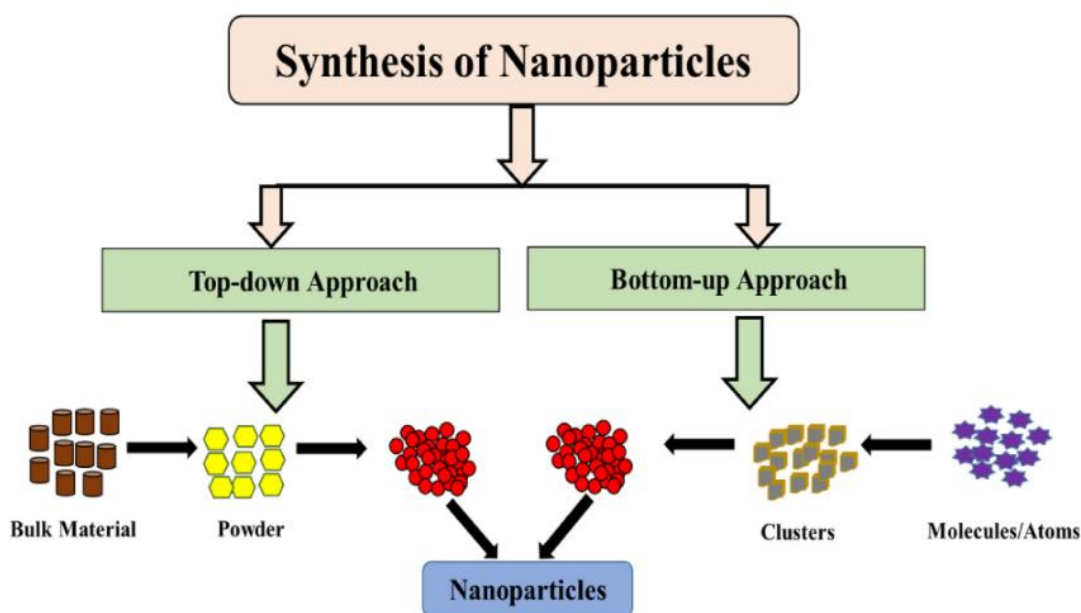
- **Top-down approach**

In the production of nanomaterials, this approach relies on reducing the size of bulk materials from the macroscale to the nanoscale through milling, fracturing, or crushing by physical, mechanical, and chemical processes. The methods used in this approach are easy to implement but are not ideal for producing nanomaterials with uniform and controllable sizes and shapes[12, 15, 16].

- **Bottom-up approach**

Bottom-up approaches produce nanomaterials from smaller particles such as atoms or molecules through a process of self-assembly, where atoms are stacked on top of each other to form layers, which in turn stack to form nanostructures[15, 16].

The bottom-up approach gives a more homogeneous and orderly chemical composition[12].



FigureI.9 A diagram illustrating the two main approaches used in the fabrication of nanoparticles: the top-down approach and the bottom-up approach[15].

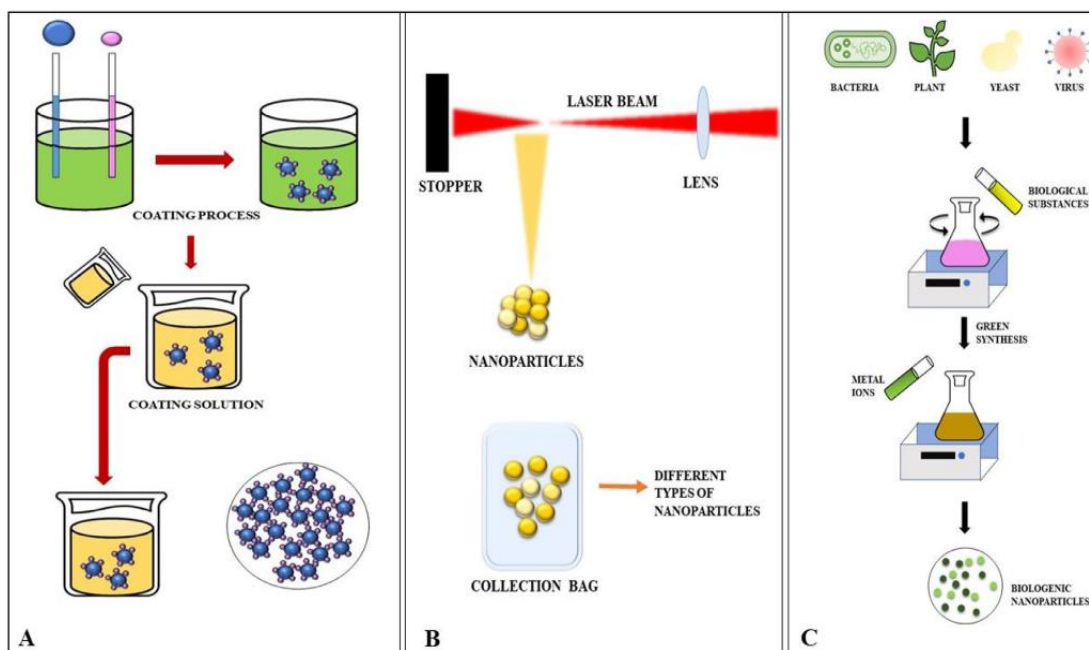


Figure I.10 Methods of NPs Synthesis: **A. Chemical method** **B. Physical method**
C. Biological method[15].

I.12 Physical synthesis methods

I.12.1 Ball milling (mechanical milling)

Ball milling is one of the oldest methods used in the synthesis of nanomaterials and nanoparticles. It is simple and low-cost. This method reduces particle size using ball milling, so it falls under the top-down approach[12].

The ball mill consists of a hollow cylindrical shell coated on the inside with a corrosion-resistant layer, rotating around a horizontal axis. The interior space is filled with rubber, steel, or carbide balls. These balls produce high-energy impacts that enable the production of fine powder from bulk materials[16]. The process is illustrated in **Figure I.11**.

In order to obtain nanoparticles, high-energy ball milling must be used with high-energy mills such as planetary ball mills, attrition ball mills, vibrating ball mills, etc. Where high-energy effects are applied to powder particles, chemical reactions begin, leading to the deformation of the powder particles, which then harden and break[7, 16].

This process has several drawbacks, including the formation of agglomerated NPs, the lack of uniform distribution, and the inability to control the morphology of NPs[16].

Ball milling can be used to synthesize many types of NPs, such as zinc oxide NPs ranging in size from 5 to 110 nm, copper oxide NPs with a size of 11 to 20 nm, and titanium dioxide NPs ranging in size from 10 to 37 nm[12].

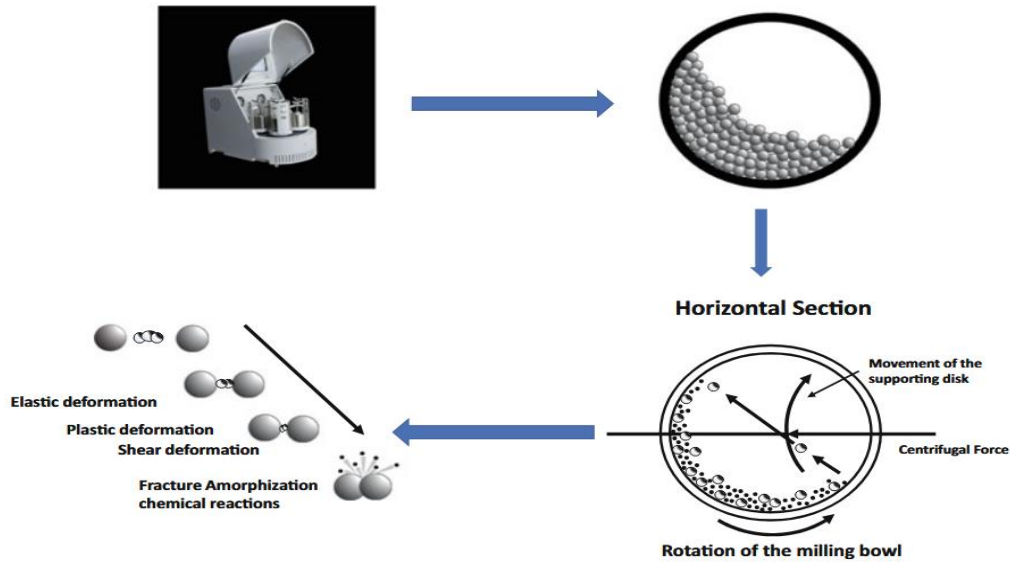


Figure I.11 Mechanical grinding process[12].

I.12.2 Physical vapor deposition

A method of depositing the precursor on a substrate by vaporizing the material to be deposited and then condensing this vapor on the substrate in the form of thin films or NPs. In this method, no chemical reactions occur; only physical methods are used, so it has a low environmental impact[2, 12, 15, 16].

The most important vapor deposition techniques used to synthesize thin films and NPs are[7]:

- **Evaporation technique:** The principle is to thermally evaporate the precursor in a low-pressure vacuum chamber and then condense it on the substrate to form a thin film.

Some techniques based on this principle have been developed, such as Laser ablation and Molecular Beam Epitaxy (MBE).

- **Sputtering technique:** The precursor is bombarded with energetic particles, causing its atoms to fly toward the substrate and settle on it. This technique is divided into two types according to the energy source: Direct Current (DC) sputtering and Radio Frequency (RF) sputtering.

I.12.3 Lithography

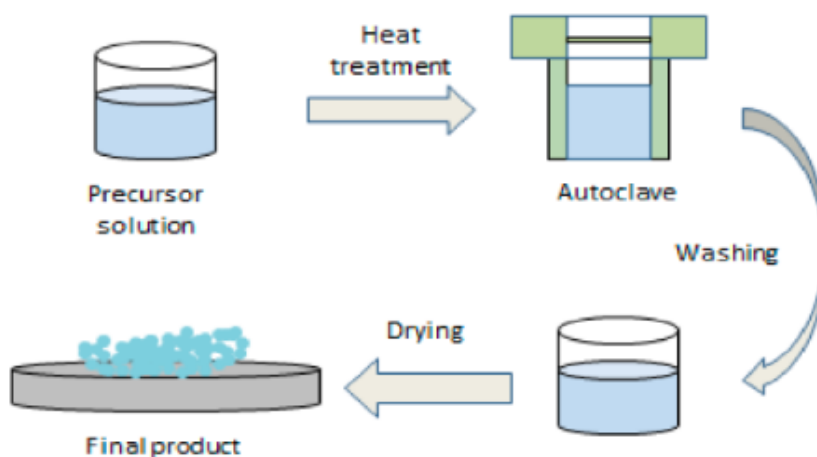
The word lithography is derived from two Greek words: litho, meaning stone, and graphine, meaning to engrave a pattern on stone[7].

It is printing on a flat surface where the area to be shaped is the receptive part of the ink, while the rest of the surface is the ink-repellent part, according to Merriam-Webster Online Dictionary. This method is expensive because it requires costly equipment and high energy consumption. It was used in the past to create micron-sized particles and aided in the production of integrated electronic devices, but today it has evolved to be capable of creating NPs[17].

I.13 Chemical synthesis methods

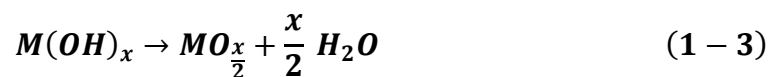
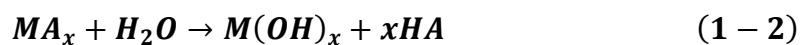
I.13.1 Hydrothermal synthesis

This method relies on the reaction between the solid material and the aqueous solution at high temperatures and pressures. The size of the resulting NPs can be controlled by increasing or decreasing the pressure, as can the rate of hydrolysis and the solubility of the metallic compounds. The process involves heating the initial solutions to very high temperatures, up to 300°C, after placing them in an autoclave. The reaction is then completed by cooling with cold water[12]. The process is illustrated in **FigureI.12**.



FigureI.12 Schematic diagram of the hydrothermal synthesis process[17].

Among the metallic NPs that can be synthesized using this method are metal oxides, metal phosphates, and metal sulfides. The hydrolysis and drying processes in this method are carried out according to reactions (1-2) and (1-3)[12]:



The nanomaterials produced by this method are characterized by low stability at high temperatures. On the other hand, this process takes a long time compared to other methods, and it is also difficult to provide the very high temperatures and pressures required by this method[16].

I.13.2 Sonochemical method

This method is one of the easiest, fastest, and simplest ways to manufacture nanomaterials. It relies on the energy generated by ultrasound waves, which are responsible for the phenomenon of acoustic cavitation. This cavitation creates bubbles that burst, generating intense heat and high pressure, which in turn leads to high-energy chemical reactions.

This technique is used to manufacture various nanomaterials, including metallic NPs and metal oxide NPs.

One of the most significant drawbacks of this technology is the difficulty in controlling the reactions that occur, as the reaction to form NPs is not the only reaction that can take place, since a reaction also occurs between the reagents[7, 12].

I.13.3 Sol-Gel method

The advantages of this method lie in its simplicity, low cost, and the ability to produce nanoparticles with precisely controlled surface area, pore size, and grain size by controlling the process chemistry. It is used to manufacture thin films and powders (nanoparticles)[7, 12].

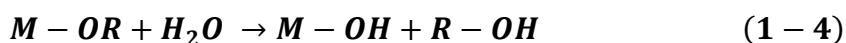
This method is a wet process combining chemical and physical processes for the synthesis of inorganic oxides[12, 15].

A sol can be defined as the stable dispersion of colloidal particles in a solvent. Colloidal particles are solid particles suspended in a solution in a dispersed manner without clumping or settling. A gel, on the other hand, is defined as a continuous three-

dimensional network surrounding a liquid. Both alkoxide and non-alkoxide materials can be used as precursor materials in this process, with alkoxides being the most important and widely used. The process involves the gradual transformation of a liquid sol into a solid gel through hydrolysis and condensation reactions[7]. The main mechanism of this method is polymerization (formation of metal-oxide-metal bonds) to form colloidal particles. Then comes the step of linking these particles in the form of a three-dimensional network in the solvent[7], so the liquid colloidal solution turns into a solid gel phase, and then into a powder through the calcination process[18].

The steps of the sol-gel process can be arranged as follows and as shown in **FigureI.13**:

1. **Hydrolysis:** At a high temperature, water or alcohol and the metal alkoxide are mixed (with an adjusted pH) to form a hydroxide solution according to the reaction (1-4):

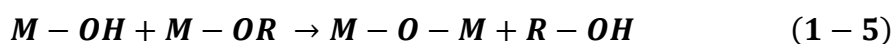


Where:

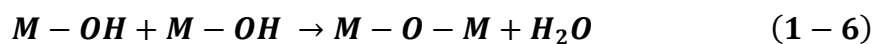
M: is a metal (Si, Ti, Al...).

R: is an alkyl (methyl, ethyl, butyl...).

2. **Polycondensation:** The formation of metal oxide bonds increases the viscosity of the solvent, which leads to the formation of a porous structure, according to the reaction (1-5):

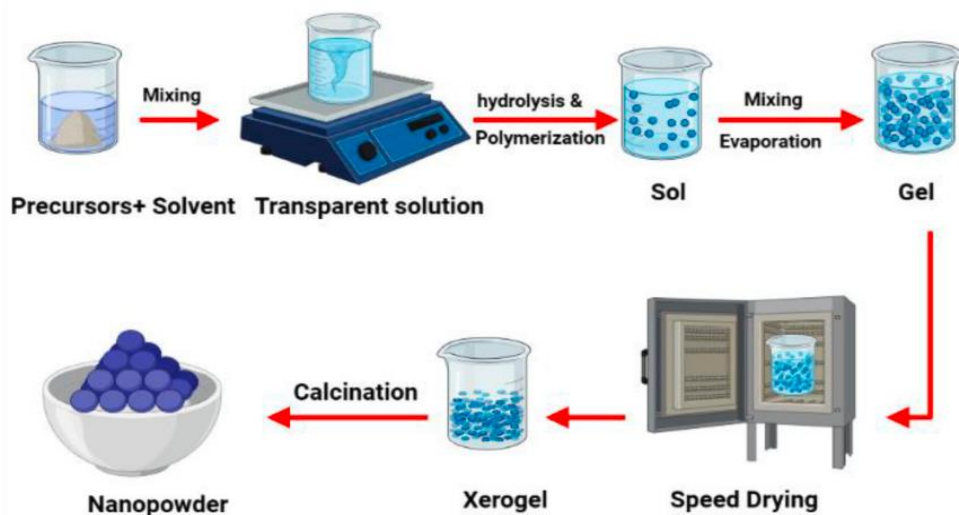


3. **Aging:** The porosity of colloidal particles decreases as a result of the redeposition of the three-dimensional gel network, according to the reaction (1-6):



4. **Drying:** At moderate temperatures, water and alcohol are removed.
5. **Calcination:** This step is carried out at controlled temperatures to remove the rest of the water and organic residues, resulting in a powder of nanoparticles with a specific shape and size.

This method can be divided into three different techniques: auto-ignition, auto-combustion, and Pechini synthesis[12, 17].



FigureI.13 Flowchart of the Sol Gel Process[19].

I.14 Biological synthesis methods

Careful consideration must be given to selecting the appropriate method for synthesizing NPs, as the stability of their shape and size, as well as the diversity of their properties, are directly influenced by the synthesis technique used and the control of the variables[16]. Although both physical and chemical methods achieve this, they are not without drawbacks: physical methods are costly because they require high pressure, temperature, and energy for operation, as well as large and sophisticated equipment; On the other hand, although chemical synthesis is simple and relatively low-cost, it has harmful effects on the environment due to the toxic byproducts it generates[20, 21].

The biological synthesis of NPs using plants, microorganisms, and various biological sources is considered a promising new approach, as it is both green and effective[12].

Among the advantages of this method are that it is non-toxic and environmentally friendly, simple, and low-cost, making it an economical and sustainable approach that allows for easy scaling up to large-scale production[12, 21, 22].

Nanoparticles manufactured using the green method have many promising applications in various fields such as agriculture and animal feed, cosmetics, biomedical sciences, technical applications, and environmental health[12].

Green synthesis for the production of NPs utilizes biological systems such as bacteria, viruses, biomolecules, and various plant extracts, among others, thereby linking nanotechnology and biotechnology[15, 21, 22].

In general, the biological methods used in the synthesis of NPs can be divided into three categories[15]:

- Biomolecule-based synthesis.
- Microorganism-based synthesis.
- Plant-based synthesis.

I.14.1 Biological synthesis of nanoparticles using biomolecules

Biomolecules such as proteins, DNA, and RNA act as nanotemplates thanks to their sophisticated structure and information sequences, serving as guiding tools in the synthesis of NPs[15].

DNA is an excellent biological framework for the precise formation and assembly of NPs due to its high capacity to bind transition metal ions. Amino acids found in proteins and enzymes can act as metal-reducing agents, while the remaining peptide chain can help stabilize the resulting NPs and prevent their aggregation[15].

I.14.2 Biological synthesis of nanoparticles using microorganisms

Microorganisms (bacteria, viruses, yeasts, fungi, etc.) are tiny living organisms that can adapt to and survive in environments rich in metal ions, as they feed on them.

Many researchers have successfully synthesized NPs using this method, which combines nanotechnology with microbial biotechnology, which is a field known as bio-nanotechnology. This process relies primarily on the reductive components within the microbial cell through two main mechanisms[15]: extracellular synthesis and intracellular synthesis.

Various reduction enzymes found in microorganisms also store heavy metals and remove their toxicity[23].

I.14.3 Biological synthesis of nanoparticles using plants

Plants are characterized by their diverse chemical compounds, which make them widely used in the pharmaceutical, dietary supplement, and other industries; consequently, they have become a promising avenue for the synthesis of NPs with various applications[16].

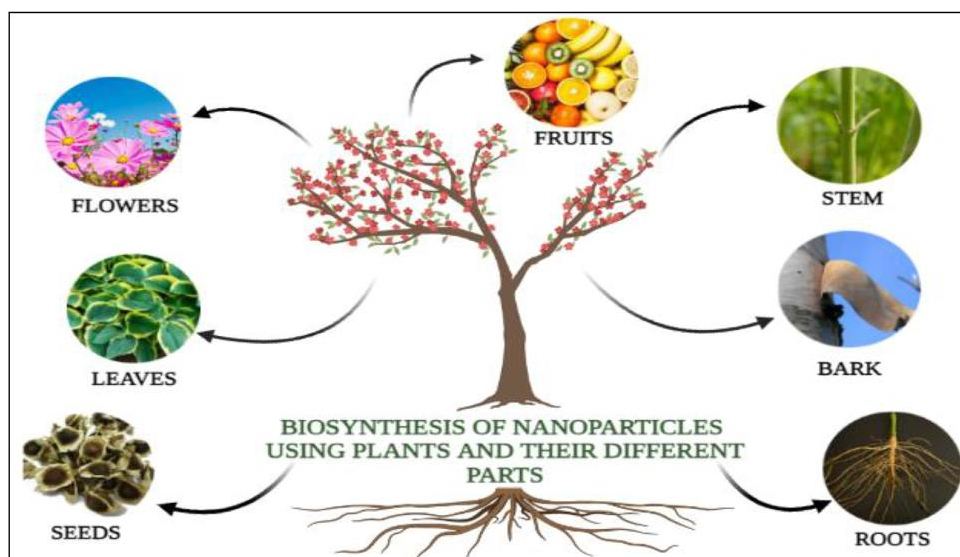
Plants, particularly medicinal herbs, are safe and inexpensive raw materials, making them the ideal choice for synthesizing nanoparticles. Furthermore, synthesis using plant extracts rich in natural biomolecules is much easier and faster than microbial synthesis[12, 15].

Extracts from various parts of plants, such as leaves, stems, roots, shoots, flowers, seeds, fruits, and bark (as shown in **FigureI.14**), have been successfully used in the synthesis of NPs[15, 22].

Examples of such plants used in the synthesis of NPs:

- *Pongamia pinnata*[24].
- *Cannabis sativa*[25, 26].
- Red cabbage[27].
- *Moringa oleifera* seeds[28].

Secondary metabolites found in various parts of plants, such as alkaloids, flavonoids, phenols, and terpenoids, act as reducing, coating, and stabilizing agents through the biological reduction, encapsulation, and stabilization of metal ions by plant extracts, leading to the formation of metal NPs with specific and uniform sizes and shapes[15, 16].



FigureI.14 Different parts of plants used in the biomanufacturing of nanoparticles[15].

- ***Beta vulgaris* subsp. *cicla***

Chard leaves belong to the Amaranthaceae family[29]. This family belongs to the dicotyledonous flowering plants, specifically to the order Caryophyllales, which is a sibling group of the Amaranthaceae family. This family is closely related to the Amaranthaceae family, as they are always classified as closely related groups[30]. Swiss chard belongs to the genus *Beta* (*Beta vulgaris*)[30] and falls within the group of cultivated varieties[31], specifically the ‘*cicla*’ group[32], so its full scientific name is ‘***Beta vulgaris* subsp. *cicla***’. It is also known as leaf beet, Swiss chard, or spinach beet and is characterized by its large, glossy, dark green leaves borne on fleshy white stems[29]. Chard leaves are a rich source of nutrients, containing high levels of fiber, sodium, magnesium, flavonoids, and vitamin C, while the stems contain high levels of potassium[30]. Many parts of the chard plant are used in alternative medicine, owing to its various biological properties, such as its use as an antioxidant, antidepressant, antimicrobial, antifungal, and anti-inflammatory agent. It also contains secondary metabolites called betalains, which are used as natural colorings in the food industry and have demonstrated anti-cancer activity[33].

I.15 Factors that influence the morphology and size of NPs

The green synthesis of nanoparticles can be influenced by various conditions and factors, which in turn affect the size, shape, and stability of these particles. Among the main factors that can affect the size, shape, and formation rate of nanoparticles are pH, the temperature of the reaction mixture, the ratio of metal ions to plant extract, and reaction time[34, 35].

- pH: The pH can affect the size, shape, and even the concentration of nanoparticles due to the potential change in the electrical charges of the bioactive compounds present in the plant extract, which may affect their ability to stabilize and coat the nanoparticles[35].
- Temperature of the reaction mixture: The temperature of the reaction mixture can significantly affect the rate of nanoparticle formation, as well as their size and shape[35].
- Ratio of plant extract concentration to metal ion concentration: The ratio of plant extract concentration to metal ion concentration can affect the size, shape, stability, and distribution of nanoparticles, as it has generally been found that at

high concentrations of plant extracts (i.e., high concentrations of reducing and stabilizing agents) relative to the concentration of metal ions, smaller, spherical nanoparticles are obtained[35].

- Reaction time: Reaction time plays an important role in determining the size, shape, stability, and distribution of nanoparticles[35].

I.16 The Role and Types of Coating and Directing Agents

Polymers, metal ligands, and surfactants are coating agents that aid in the synthesis of metal nanoparticles with precise size and shape. These agents consist of long-chain hydrocarbons modified with heteroatoms and can be classified as “green” coating agents as follows: biomolecules and polysaccharides. (440)

The final shape of the nanoparticles depends on the kinetics of nucleation during synthesis; they can grow isotropically, resulting in spherical particles, when the reaction is subject to thermodynamic control. Alternatively, they may grow asymmetrically, resulting in nanoparticles with various geometric shapes when the reaction is under kinetic control. Nanoparticle synthesis occurs under conditions far from thermodynamic equilibrium; therefore, any change in reaction conditions leads to significant differences in surface free energy between different crystal faces, which stimulates asymmetric growth. This is where coating agents come into play: when adsorbed onto the crystal faces, they modify the surface free energy differences, thereby promoting particle growth in specific directions and thus determining their geometric shape.(440)

• Polyethylene glycol (PEG)

Polyethylene glycol (PEG), also known as macrogol, is a polyether consisting of ethoxy units derived from the ring-opening polymerization of ethylene oxide. The general formula for PEG is $\text{HO}-(\text{CH}_2\text{CH}_2\text{O})_n\text{-H}$, where n represents the number of ethylene oxide units. It is a linear polymer with chemically active hydroxyl groups at both ends, facilitating binding to functional groups. PEG is synthesized through the ring-opening polymerization of ethylene oxide in methanol or water, as a reaction catalyst, to produce methoxy-PEG (mPEG) and diol PEG, respectively. The resulting PEG polymers differ in chain length and, consequently, in molecular weight[36, 37]. (PEG) Polymers are an important class of excipients due to their widespread use in various pharmaceutical formulations. PEG is commercially available in a wide range of molecular weights, ranging from 200 to 10,000,000 g/mol, with their physical states ranging from a clear,

non-volatile liquid at room temperature (low molecular weight PEG: 200–700), through a semi-solid ‘putty-like’ state (medium molecular weight PEG: 800–2,000), to a solid state, appearing either as a white waxy solid, in the form of flakes, or as a powder (PEG with a molecular weight exceeding 3,000)[38]. NPs are coated with a dense layer of (PEG) to modify their surface properties[39, 40]; this is a crucial factor in improving the biophysical and chemical properties of the NPs[37]. There are three typical strategies for coating the surface of nanoparticles, and they are:

- By physical adsorption: PEG adheres to the NPs via electrostatic or hydrophobic interactions.
- By the formation of a stable chemical bond: Polyethylene glycol is firmly grafted onto the surface of the NPs.
- By linking PEG with hydrophobic molecules to form large molecules that can self-assemble with themselves or with other compounds to form PEG-coated NPs in solution[37].

The addition of Polyethylene glycol after the titanium dioxide synthesis process improves the photocatalytic properties of the prepared titanium dioxide NPs[40].

I.17 The mechanism of the biological synthesis of TiO₂ NPs

Although there is a wide range of studies on the characterization of bioactive compounds found in plants and on understanding the mechanisms by which they are used to synthesize NPs, the exact mechanism remains unclear; Nevertheless, numerous studies have demonstrated that bioactive compounds (such as alkaloids, alcohols, phenolics, and polysaccharides) found in various plants play a fundamental role in the biosynthesis and stabilization of nanoparticles[41]. Research has also shown that the synthesis mechanism may vary depending on the plant species used[42]. **Figure I.15** shows the proposed mechanism for the bioreduction of titanium ions and the formation of TiO₂ NPs.

The following table summarizes some applied studies on the synthesis of titanium dioxide NPs using different plant species:

TableI.2 Some applied studies on the synthesis of titanium dioxide nanoparticles using extracts from various plant species.

The plant used	The essential components found in plants	The main results and observations	Reference
E.heteradena Jaub (Roots)	Hydroxyl groups in Phenols	Phenols are responsible for the reduction of $Ti(OH)_2$ and act as coating agents on the surface of NPs.	[43]
Aloe vera	Carboxylic Acids, Terpenoids, Flavonoids, and Proteins	Hydroxyl groups contribute to the formation and synthesis of particles by acting as reducing and masking agents.	[44]
L.Jatropha curcas	Phenols, Hydroxyl groups, and Tannins	The phenols coat the NPs, and a distinct absorption peak at 336 nm confirms the success of the process.	[45]
A.squarrosa	Hydroxyl groups in water-soluble compounds	Hydroxyl groups act as a catalyst at 60°C to convert titanate hydroxide into stable NPs.	[46]

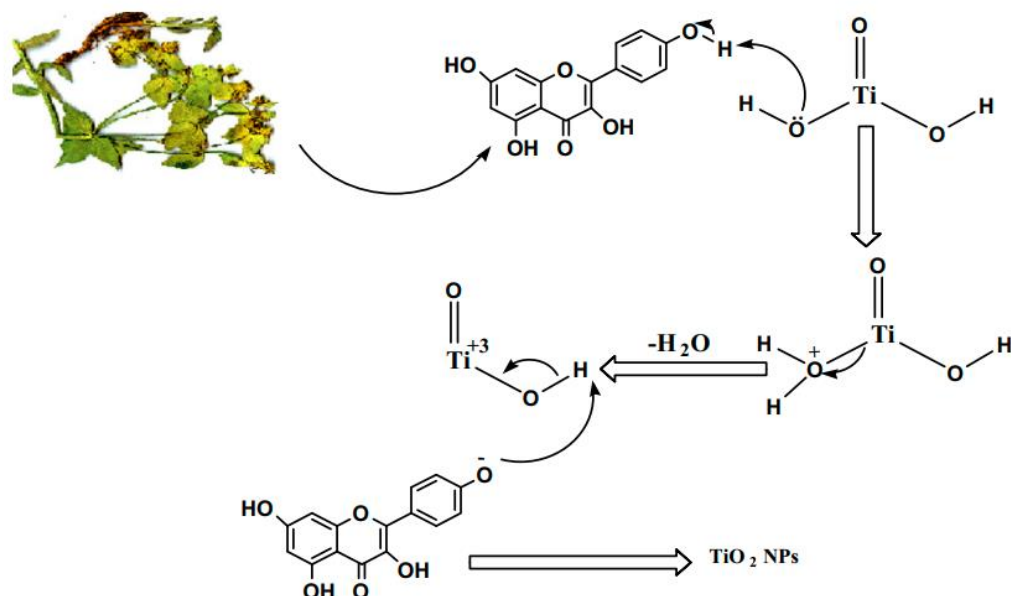


Figure I.15 The proposed mechanism for the bioreduction of titanium ions and the formation of TiO₂ NPs[43].

I.18 Techniques for characterizing nanoparticles

After successfully synthesizing NPs using various methods, it is essential to verify their quality, understand their interaction mechanisms, and identify their potential applications. This is achieved through various analytical techniques specific to NP characterization, which allow for the determination of the particles' size, shape, and crystallinity, as well as their surface area.

Among the most important techniques used in the characterization of NPs are:

Ultraviolet and visible spectroscopy, Fourier transform infrared spectroscopy, transmission electron microscopy, scanning electron microscopy, and X-ray diffraction[15].

The following is a detailed description of these techniques:

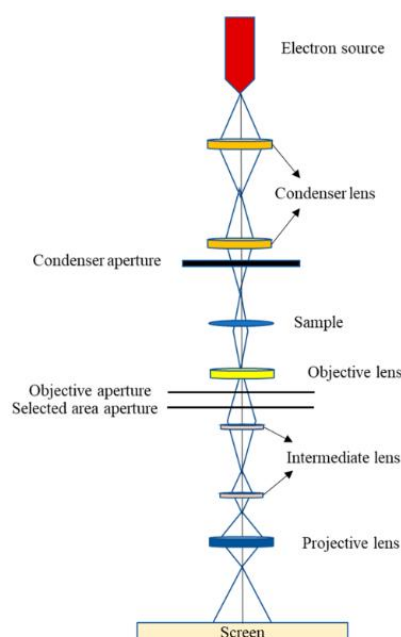
I.18.1 Transmission electron microscope (TEM)

The transmission electron microscope (TEM) is one of the most advanced and valuable technologies used to study the internal structure of a sample (crystal lattice structure and crystal size). It also provides information on grain boundaries, the microstructure of interfaces, and crystal defects in NPs at magnifications ranging from low to high[12, 15].

There are several types of transmission electron microscopes depending on the application, including the conventional transmission electron microscope (CTEM), the high-resolution transmission electron microscope (HRTEM), and the scanning transmission electron microscope (STEM)[47].

The electron beam is emitted from a tungsten filament and accelerated at high voltage toward a very thin sample placed inside a vacuum chamber, where it penetrates the sample. As a result, the electrons scatter, providing detailed images of the crystal structure and the spacing between crystal planes[7, 48].

The **FigureI.16** shows the Internal structure of a TEM device.



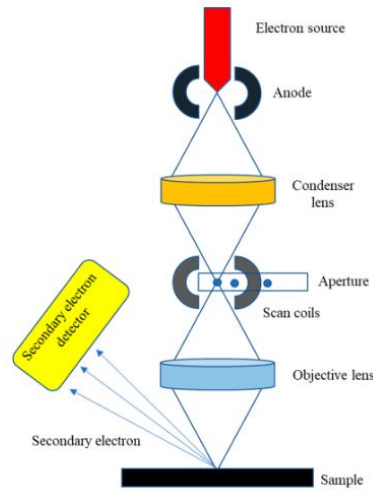
FigureI.16 Internal structure of a TEM device[47].

I.18.2 Scanning electron microscope (SEM)

Scanning electron microscopy is a widely used technique primarily focused on studying the surface morphology of NPs; it also provides information on the size, shape, and chemical composition of nanomaterials.

This technique involves scanning the sample surface with a focused beam of high-energy electrons, detecting the backscattered electrons, and generating detailed three-dimensional images[7, 12, 47].

The **FigureI.17** shows the Internal structure of a SEM device.



FigureI.17 Internal structure of a SEM device[47].

I.18.3 X-ray diffraction (XRD)

X-ray diffraction is an important technique for analyzing the composition, structure, and crystal phases of NPs; it also accurately determines the lattice constants and crystal grain size[15].

The **FigureI.18** shows the X-ray diffraction apparatus.

This technique relies on the interaction between a beam of X-rays incident on the sample and the periodic arrangement of atoms within the crystal lattice. This results in scattered waves that interfere with one another, producing constructive or destructive interference depending on how the waves interact. According to Bragg's law, constructive interference (diffraction) occurs when X-rays are simultaneously scattered by the crystal planes, resulting in a series of peaks at specific angles[48].

Bragg's law (1-7) expresses the relationship between the angle of incidence, the wavelength of the X-rays, and the interplanar spacing of the crystal planes[49]:

$$2d \sin \theta = n\lambda \quad (1 - 7)$$

Where:

d: the distance between two parallel atomic planes.

θ: diffraction angle (Bragg angle).

n: integer (diffraction order).

λ: wavelength of the X-rays used.

Obtaining various important pieces of information (crystal size, lattice constants, and phase composition) from this analysis on the analysis of the full width at half maximum (FWHM) of the Bragg diffraction peaks. Each peak is associated with a specific crystal orientation, and its width is influenced by the number of atoms contributing to the scattering process, which is directly related to the size of the crystal planes responsible for the reflection. This size can be calculated using the Scherrer equation (1-8)[50]:

$$D_{hkl} = \frac{K\lambda}{B_{hkl} \cos \theta} \quad (1 - 8)$$

Where:

K: crystal aspect ratio (from approximately 0.89 to 0.94).

λ : wavelength of the X-rays used.

B_{hkl} : full width at half maximum (FWHM), measured in radians.

θ : Bragg angle.

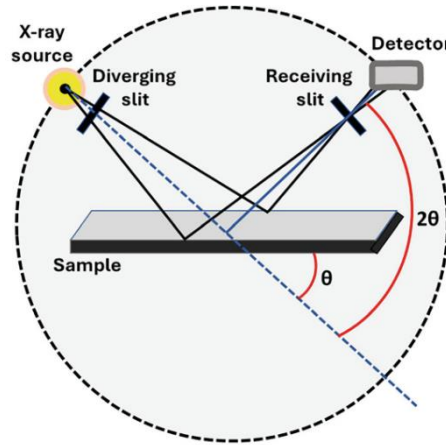


Figure I.18 X-ray diffraction apparatus[48].

I.18.4 Ultraviolet and visible spectroscopy

Ultraviolet and visible spectroscopy is a low-cost, simple, fast, and sensitive characterization technique used to characterize various materials, including nanomaterials, as it provides information on transport and absorption properties. It also helps determine the size and calculate the bandgap of nano-metal oxide particles based on Tauc's equation (1-9):

$$(\alpha h\nu)^n = A(h\nu - E_g) \quad (1 - 9)$$

α : absorption coefficient.

$\hbar$: Planck's constant.

ν : frequency of incident light.

n : a coefficient that determines the nature of electronic transitions.

$n = 2$ (direct allowed transition)

$n = 1/2$ (indirect allowed transition)

A : constant related to matter.

E_g : energy gap.

This technique relies on measuring the sample's light absorption in the UV-Vis region compared to the absorption of a reference material. The sample's absorbance can be calculated based on the Beer-Lambert law (1-10)[7, 15]:

$$A = \epsilon lc \quad (1 - 10)$$

Where:

A : absorbance.

ϵ : molar attenuation coefficient.

l : path length.

c : concentration.

The **Figure I.19** shows the Structural composition of ultraviolet and visible spectroscopy.

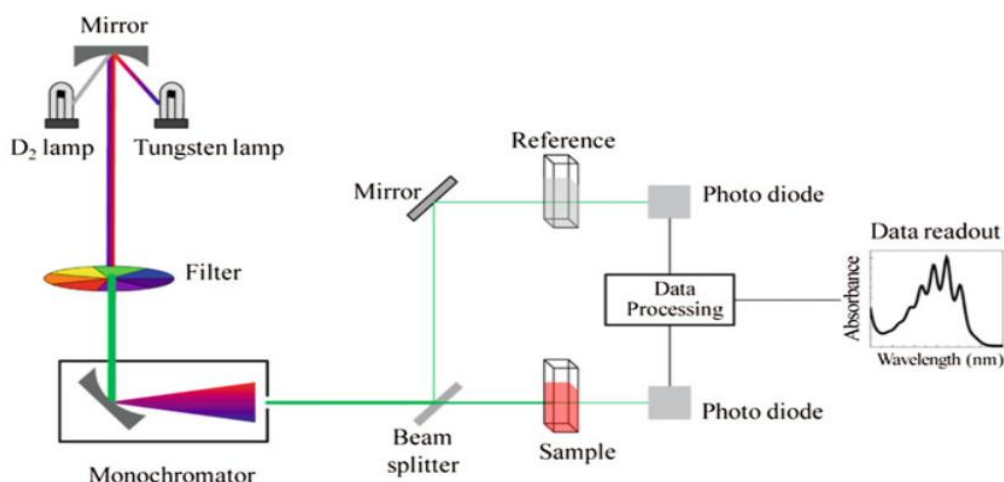


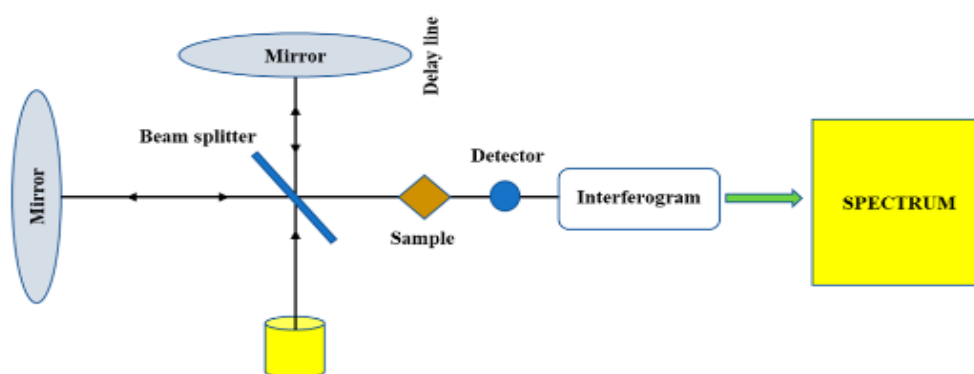
Figure I.19 Structural composition of ultraviolet and visible spectroscopy[7].

I.18.5 Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy is a common and widely used technique for analyzing various types of materials[47]. It is characterized by its speed and accuracy, as each material has its own unique spectral fingerprint that distinguishes it from other materials[48].

The **Figure I.20** shows the Structural composition of infrared spectroscopy by Fourier transform.

This technique relies on the sample's absorption of electromagnetic radiation in the mid-infrared range ($4000\text{--}400\text{ cm}^{-1}$). This analysis produces a spectrum showing the bands of functional groups and bonds, along with their types and strengths, due to the vibrational motion of atoms and molecules in the infrared region, thereby providing details about the molecular structure of the sample[15]. Regarding the analysis of nanoparticles, this technique provides information about the functional groups present on the surface of these particles (such as amine groups, carboxyl groups, and phenolic groups...)[51].



FigureI.20 Structural composition of infrared spectroscopy by Fourier transform[47].

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Chapter Two

*Antibiotics in the Environment and
Methods for Removing them*

II.1 Antibiotics as pollutants

II.1.1 Definition and classification of antibiotics

The term “antibiotics” is a compound word derived from the Greek “anti,” meaning “against,” and “biotikos,” meaning “related to life.” They are chemical compounds of natural, synthetic, or semi-synthetic origin that specifically affect microorganisms, particularly bacteria and parasites (protozoa), by reducing or halting their reproduction and even killing them. They are used in many fields, including human and veterinary medicine, aquaculture, and agriculture[1-3].

Antibiotics have been used as medications to prevent bacterial growth and treat various infectious diseases (wounds, abscesses, and infections). They have also been used to treat microbial infections in animals and livestock. Their use is not limited to treating and preventing diseases; they are also used to enhance and improve animal growth[1-3].

The first antibiotic to be isolated was penicillin (a natural substance produced by a type of fungus) by Alexander Fleming in 1928[1, 2].

Antibiotics can be classified in three ways based on their chemical structure, their spectrum of activity, and their mechanism of action, as summarized in the following table[3, 4]:

TableII.1 Classification of Antibiotics.

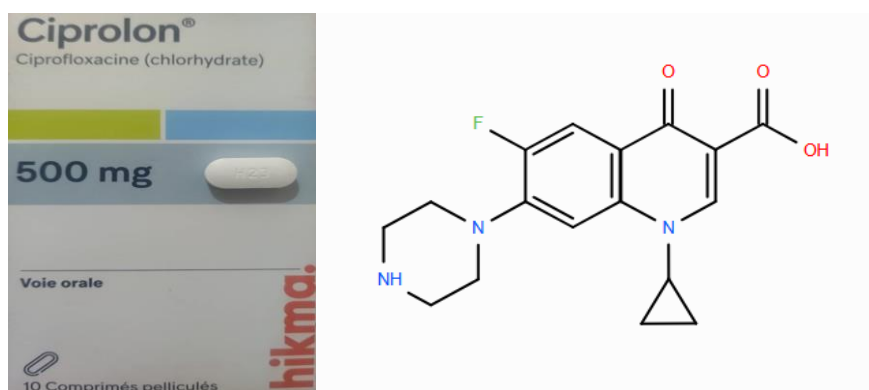
Based on	Classification of antibiotics
their chemical composition	β -lactams (penicillins, carbapenems...) Macrolides Fluroquinolones Tetracyclines Sulfonamides Lincosamides Vancoplanins Teicoplanins Ansamycins Aminoglycosides

their range of effectiveness	Narrow-spectrum antibiotics Broad-spectrum antibiotics Extended-spectrum antibiotics
their mechanisms of action	Bactericidal antibiotics Bacteriostatic antibiotics

- **Ciprofloxacin**

Ciprofloxacin, with the chemical formula $C_{17}H_{18}FN_3O_3$ and the chemical structure shown in **FigureII.1**, is one of the most prominent antibiotics belonging to the fluoroquinolone family, which is one of the main families of antibiotics. It is used as a medication to kill or inhibit bacterial growth in both humans and animals[5-7], to treat various conditions such as pneumonia and gastrointestinal disorders[8]. This type of antibiotic is often not completely metabolized, so it is excreted in large amounts through urine and feces[6]. Studies have also shown that very high levels of it have been detected in hospital wastewater, in particular, at concentrations reaching 150 mg/l[9], which can pose a significant risk if not properly disposed of, given its high stability in aquatic environments and poor biodegradability[5]. Consequently, its persistence in aquatic environments may lead to the emergence of antibiotic-resistant bacteria, as well as its toxicity toward many microorganisms[10]. Ciprofloxacin has been removed using several conventional methods, such as adsorption, biodegradation, and photodegradation, but photodegradation is one of the most recent methods. Being low-cost, environmentally friendly, and effective as it eliminates organic pollutants by converting them into harmless compounds such as water and carbon dioxide, it has become the focus of numerous studies[5]. In this process, free radicals formed with the aid of a photosensitizer attack and break down specific active sites in the ciprofloxacin structure (particularly aromatic rings and hydroxyl groups)[7]. Various studies have confirmed the success of the photodynamic process using different types of photosensitizers under visible light, ultraviolet radiation, and even sunlight.

The antibiotic used in this study and shown in **FigureII.1** was purchased from a local pharmacy.

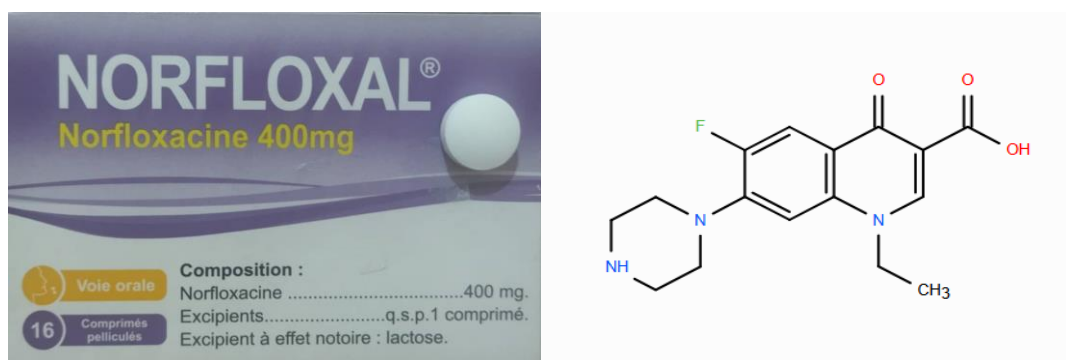


FigureII.1 The commercial CIP used and its chemical structure.

- **Norfloxacin**

Norfloxacin is an antibiotic with the chemical formula $C_{16}H_{18}FN_3O_3$ and the chemical structure shown in **FigureII.2** that belongs to the fluoroquinolone class. It is used to treat bacterial infections in both humans and animals [11], such as urinary tract infections, prostatitis, and cystitis[12]. Norfloxacin is also one of the antibiotics excreted in significant amounts in human and animal waste due to poor metabolic clearance[13, 14], and consequently, it is found in relatively high concentrations in wastewater, posing a threat to microorganisms as well as the risk of antibiotic-resistant bacteria emerging[12-14]. Several conventional methods are used to remove this antibiotic from aquatic environments, such as adsorption, membrane filtration, reverse osmosis, biological treatment, and photodegradation. However, these methods suffer from several drawbacks, including incomplete degradation, high cost, and the formation of toxic byproducts in some cases[11, 12]. Therefore, the use of advanced oxidation methods is considered a promising option for the degradation of organic pollutants in general.

The antibiotic used in this study and shown in **FigureII.2** was purchased from a local pharmacy.

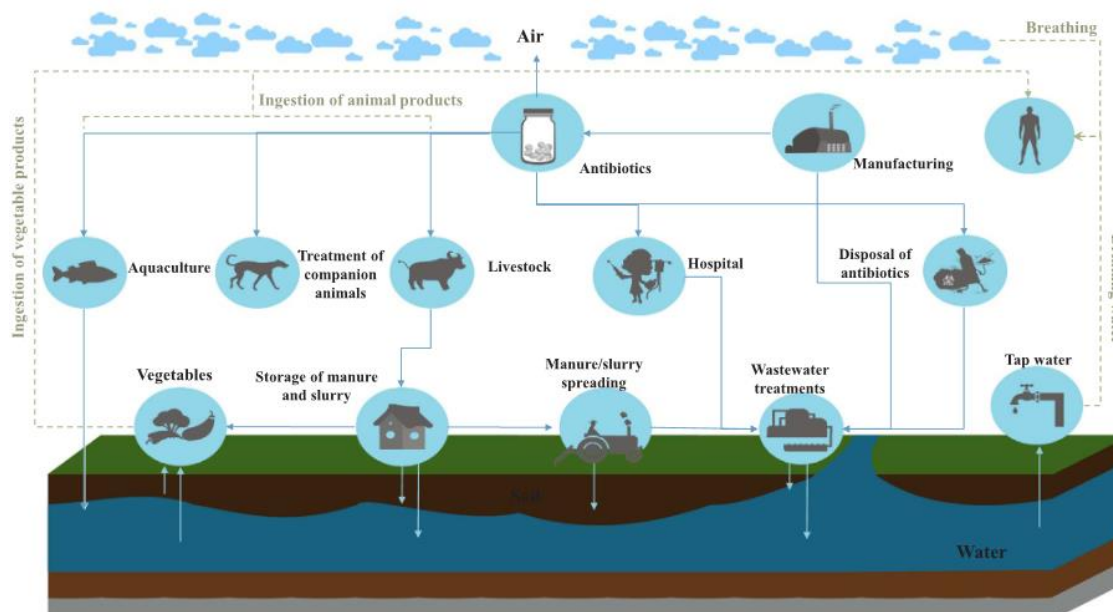


FigureII.2 The commercial NFL used and its chemical structure.

II.1.2 Environmental and health risks associated with the release of antibiotics into various natural environments

The consumption of antibiotics has increased significantly in recent years, particularly with population growth. Their use is no longer limited to treating infectious diseases and various microorganisms in humans and animals; they are now also used to improve livestock growth and increase productivity. This has led to these antibiotics entering aquatic and terrestrial ecosystems through various pathways, such as pharmaceutical industry waste, hospital waste, waste from livestock and aquaculture farms, medical waste landfills, and wastewater. These pathways are illustrated in **FigureII.3**[15].

It is worth noting that irrigating crops with treated wastewater and fertilizing them with organic manure (livestock manure) containing non-degradable antibiotic residues are among the primary pathways through which antibiotics enter the agricultural sector intended for human consumption. Although the concentrations of antibiotics measured in various environments (such as surface water, groundwater, wastewater, soil, organic fertilizer, and various crops) may appear low to be classified as contaminants (nanograms per liter (ng/L) or nanograms per gram (ng/g) and micrograms per liter ($\mu\text{g/L}$) or micrograms per gram ($\mu\text{g/g}$), they can still affect certain aquatic organisms and terrestrial plants, and their persistence in these environments has caused microorganisms to undergo genetic mutations, producing antibiotic-resistant genes. These have been classified as emerging contaminants in various environmental components, particularly wastewater and surface water, which means that microorganisms can resist the effects of antibiotics. Consequently, they can cause diseases resistant to all known, newly discovered, and even unknown natural antibiotics, causing growing concern, especially since this can lead to death due to the lack of a drug that can eliminate these antibiotic-resistant bacteria[1, 2]. Therefore, it has become necessary to explore effective and cost-effective methods for analyzing and disposing of these antibiotics.



FigureII.3 The Environmental Cycle of Antibiotics: From Industrial and Agricultural Sources to Ecosystems[15].

II.2 Methods for removing antibiotics from water

Methods and techniques for treating organic pollutants in general, and antibiotics in particular, range from biological techniques, known as biodegradation, to physicochemical techniques (such as filtration, adsorption, reverse osmosis, and photodegradation).

- **Biodegradation:** This technique relies primarily on the use of microorganisms, such as bacteria and fungi, or their enzymes, to break down antibiotics. This technique has shown excellent results in treating many types of antibiotics, but it is ineffective against others and is considered relatively dangerous due to the direct use of microorganisms[7].
- **Filtration:** Filtration is one of the oldest methods of water treatment, using porous membranes with pore sizes ranging from 10 to 1,000 Å to remove various contaminants; however, it is not effective in completely removing antibiotics[7].
- **Adsorption:** One of the most promising methods for removing pollutants, particularly organic ones, involves the use of various adsorbents, the most well-known of which are activated carbon, biochar, and carbon nanotubes. Although these methods are effective at removing most antibiotics from water, they do not completely break them down[3].

- Reverse osmosis: This process involves pumping water through a semi-permeable membrane, which allows clean water to pass through while trapping contaminants. This technique is also effective at removing antibiotics, but it does not break them down and is costly[7].
- Photodegradation: This technique includes direct photodegradation and indirect photodegradation (catalyzed photodegradation), with the latter having seen continuous improvements due to its effectiveness and low cost. This technique relies on the use of both light and a catalyst to accelerate the chemical degradation of organic pollutants. Semiconductors have been used as effective catalysts due to their chemical stability and unique properties. This technique relies primarily on the generation of free radicals, which in turn react with organic pollutants, leading to their degradation[3, 7].

II.3 Photocatalytic mechanisms

Improving the efficiency of photocatalysts necessitates a comprehensive understanding of fundamental scientific principles, such as photocatalyst design and the mechanisms of photoreactions. The photosynthetic process includes multiple important steps[16]. **(Figure II.4).**

II.3.1 Light Absorption (Electron Excitation)

The photocatalytic process begins when a photosensitizer, which is typically a semiconducting substance, absorbs light. For the reaction to begin, the energy of the incident photons must be equal to or greater than the bandgap energy of the semiconductor ($h\nu \geq E_g$), which represents the energy difference between the valence band (VB) and the conduction band (CB). This requires UV light to excite electrons and generate electron-hole pairs (e^- , h^+). When the semiconductor absorbs the appropriate wavelength and intensity of light, electrons are excited from the low-energy valence band (VB) and promoted to the high-energy conduction band (CB), leaving behind positively charged holes in the valence band (VB). These excited electrons and holes are now ready to initiate a cascade of chemical reactions on the surface of the photosensitizer[16, 17].

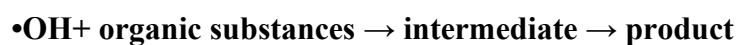
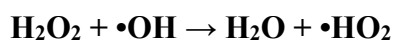
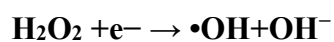
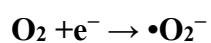
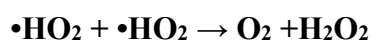
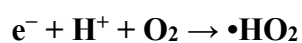
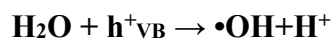
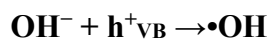
II.3.2 Charge separation and transfer

After electron-hole pairs are formed, they must remain separated for a sufficient period of time to initiate chemical reactions or be directly utilized in the primary reaction on the surface of the photocatalysts. If they recombine too quickly, all the energy they absorb from light is dissipated as heat. One way to achieve separation is by adding specific impurities (dopants) to the material or by fabricating structures composed of two different types of semiconductors, known as heterojunctions or nanocomposites. These methods help direct electrons and holes in opposite directions and generally enhance the efficiency of the photocatalysts[16].

II.3.3 Reduction-Oxidation (Red/Ox) Reactions

When charge carriers (e^- , h^+) reach the surface of semiconductors, they trigger redox reactions. Reduction reactions occur when electrons in the CB interact with neighboring molecules, such as oxygen (O_2), to reduce them and form reactive oxygen species (ROS) such as superoxide radicals ($\bullet O_2^-$). These reactive oxygen species (ROS) play a crucial role in the process of photocatalysis. These radicals are highly reactive and have the ability to decompose or oxidize pollutants; furthermore, they can facilitate the conversion of carbon dioxide (CO_2) into valuable chemicals, contributing to environmental and energy applications. Conversely, oxidation reactions occur when positively charged holes in the valence band interact with molecules, such as water, leading to the formation of highly reactive hydroxyl ($\bullet OH$) radicals. These hydroxyl radicals are important for many photocatalytic processes, as they can oxidize and degrade organic pollutants or kill harmful microorganisms such as bacteria[16].

The mechanism of an ideal photocatalytic process is based on the equations[18]:



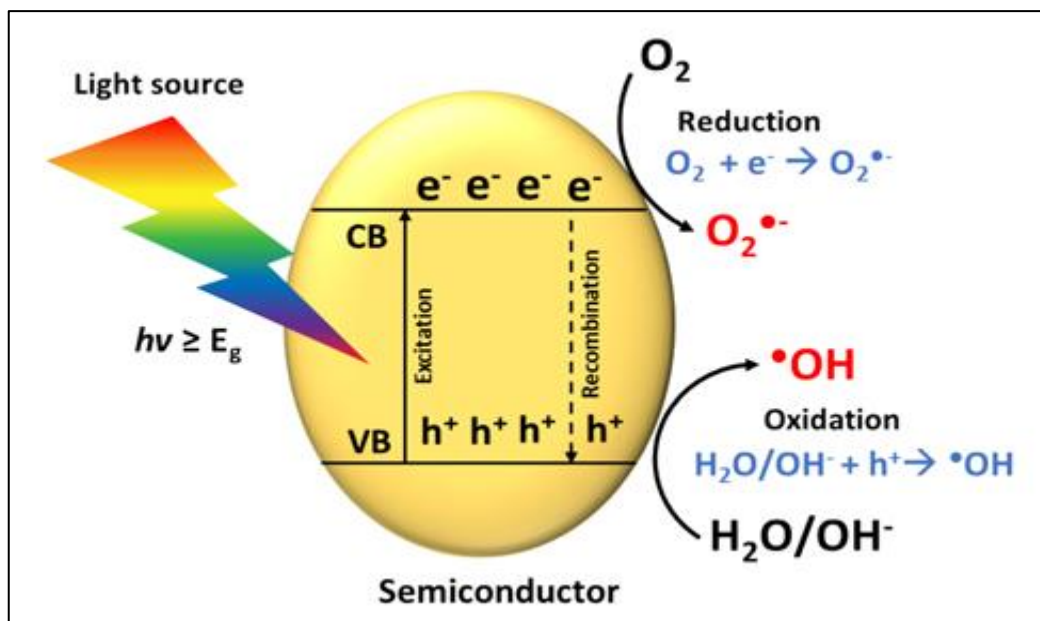


Figure II.4 A diagram of the photocatalytic mechanism in semiconductors[16].

II.4 TiO₂ as Photocatalyst

II.4.1 History of TiO₂

TiO₂ was discovered in 1791 from ilmenite (FeTiO₃). Titanium (IV) oxide, TiO₂ or Titania, has been used in numerous applications due to its physical and chemical properties. First commercially produced in 1916, this compound gained widespread popularity in the paint industry, where it is known as 'White Pigment No. 6', and later in the food industry as the coloring agent E171[19].

There are three different phases of this oxide found in nature: anatase, discovered in 1801; rutile, discovered in 1803; and brookite, discovered in 1825. The brookite form is relatively rare and difficult to synthesize compared to the anatase and rutile forms, which possess photocatalytic activity[19].

Environmental photocatalysis has attracted considerable attention since the discovery of the photocatalytic activity of titanium dioxide (TiO₂) in 1972, and the oil crisis was one of the factors that spurred research into non-fossil fuels in the early 1970s. Interest in TiO₂ was greatly boosted by research in the field of electrochemistry, particularly by the aforementioned achievement of Fujishima and Honda in 1972 in the field of the photoelectrochemical decomposition of water[18].

II.4.2 Crystal Structure TiO₂

Titanium dioxide has a diverse crystal structure and occurs naturally in three crystalline phases: anatase, rutile, and brookite. The different morphologies exhibit distinct properties and, consequently, varying performance in photocatalysis[20].

- **Anatase**

The crystal structure of the Anatase phase is tetragonal (**FigureII.5**); it is also considered the most efficient phase for photocatalysis and is a metastable phase; this is because it has a larger indirect band gap than rutile and brookite, amounting to approximately 3.2 eV, which increases its oxidizing capacity and facilitates electron transfer. The conduction band in anatase is narrower than that in rutile. Anatase has a smaller indirect band gap than a direct band gap, and therefore, the indirect band gap is utilized in the process of photocatalysis, where the indirect band gap in anatase leads to a longer lifetime of charge carriers, making it more efficient and photoreactive than rutile and brookite. Anatase has a large specific surface area, providing more active sites, as well as a high concentration of oxygen vacancies that enhance charge separation. The adsorption affinity of anatase for organic compounds is higher than that of rutile; adsorption affinity can be defined as the equilibrium ratio between the concentration in the solid phase and the concentration of the solute in the liquid phase, also known as the adsorption equilibrium constant. Anatase exhibits a lower regeneration rate than rutile due to its higher pore retention rate. Anatase is characterized by lower thermodynamic stability compared to the rutile phase; the morphological changes in the anatase surface help to increase its photocatalytic activity. Porous anatase shows long-term electron generation and allows for efficient electron transport; at the same time, porous rutile is significantly less efficient and more reflective than anatase[20].

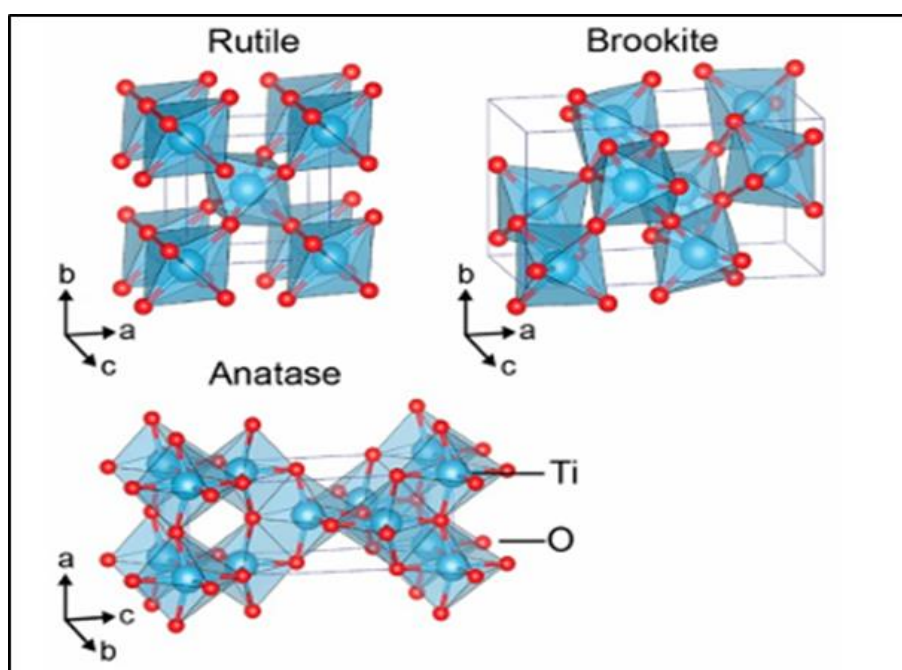
- **Rutile**

The crystal structure of the Rutile phase is tetragonal (**FigureII.5**); it is the most chemically stable phase; it is readily produced at high temperatures and has a narrow bandgap of about 3.0 eV, which allows it to absorb light close to the visible spectrum. Due to its lower photocatalytic activity compared to anatase, its low specific surface area, rapid reorganization, and the positive position of its lowest conduction band make it less capable of reduction than anatase. Rutile is rarely considered as a photocatalyst for the oxidation of dyes/organic compounds due to its poor oxygen absorption caused by the

lower position of the valence band compared to anatase; Rutile performs better than anatase in specific photocatalytic applications, including water oxidation and nitrogen fixation, due to its large specific surface area and abundant oxygen vacancies. Rutile is also used for the selective oxidation of aromatic alcohols to aldehydes in water oxidation. The vacant oxygen (VO) defect in rutile can increase the catalytic activity significantly, as VO is known to inhibit charge formation. This enables rutile to rival anatase in terms of photocatalytic efficiency[20].

- **Brookite**

Brookite is a metastable phase characterized by an orthorhombic crystal structure (**FigureII.5**); it has a smaller specific surface area compared to anatase. Brookite transforms into rutile at high temperatures, and this transformation depends on several factors, including the size of the crystals, their size distribution, and the contact area between them. Brookite is more stable than anatase at crystal sizes above 11 nanometers, whilst for sizes above 35 nanometers, rutile is the more stable phase. The catalytic activity of brookite depends heavily on the degree of crystal distortion. Electrons in brookite are trapped in shallow traps rather than through photogeneration, which reduces the number of free electrons; on the other hand, this prolongs the hole lifetime[20].



FigureII.5 Illustration of different crystal forms of TiO_2 [21].

II.4.3 General Properties of TiO₂

- **Physical Properties**

Table II.2 The physical properties of the crystal phases of TiO₂[21].

Property	Anatase	Rutile	Brookite
Crystal structure	Tetragonal	Tetragonal	Orthorhombic
Density (kg/m ³)	3830	4240	4170
Molecular volume (Å ³)	34.06	31.21	32.17
Refractive index	2.52	2.72	2.63
Hardness coefficient (Hs)	5.5–6	7–7.5	5.5–6
Electrical permeability	48	114	78

- **Optical and electronic properties**

The crystal phases of titanium dioxide, particularly rutile and anatase, have high refractive indices of 2.7 and 2.5, respectively. These values serve as a key measure of the mineral's ability to scatter photons. Rutile is considered an ideal pigment because it is more efficient at reflecting light than brookite and anatase. The large bandgap of titanium dioxide (TiO₂) improves the efficiency of coatings used in solar cells; it allows high optical transmittance for both visible light and near-infrared radiation whilst decreasing their absorption[21]. Titanium dioxide is classified as an n-type semiconductor with a wide energy bandgap of 3.2 eV for the anatase phase and 3 eV for the rutile phase. The electronic structure of TiO₂ is described such that the energy levels near the valence band maximum (VBM) are formed primarily and dominantly by the 2p atomic orbitals of oxygen, while the levels near the conduction band minimum (CBM) are formed primarily from the 3d orbitals of titanium. Stoichiometric TiO₂ is characterized by poor conductivity (less than $10^{-7} \Omega^{-1} \text{ cm}^{-1}$ at room temperature), which is due to a large band gap and the absence of intrinsic free electrons. In contrast, off-stoichiometric TiO₂ exhibits high conductivity of approximately 10^{-1} to $10^{-2} \Omega^{-1} \text{ cm}^{-1}$ at room temperature; this is attributed to the formation of oxygen vacancies during synthesis, in the material's post-processing, or through the addition of external additives, as each oxygen vacancy provides two free electrons that enhance the n-type conductivity of titanium dioxide.

Titanium dioxide (TiO₂) is characterized by a high capacity to absorb ultraviolet (UV) radiation, thanks to its photons possessing sufficient energy to overcome the band gap and excite electrons, thereby generating electron-hole pairs. Although this process reduces light transmittance in the UV region, UV light activates the photochemical processes that give TiO₂ its self-cleaning properties[22].

- **Properties of the phase transition**

The anatase phase can transform into the rutile phase at high temperatures; this is an irreversible process in terms of the transformation itself. It is also characterized by a restructuring process involving the breaking and reforming of bonds, which means it is also time-dependent. There is a wide temperature range for the transition from anatase to rutile, with temperatures ranging between 400 and 1200 °C. However, in air, the phase transition from anatase to rutile is considered to begin at temperatures of around 600 °C, and often at higher temperatures[22].

- **Thermodynamic properties**

Surface energy is an important factor in defining the thermodynamic properties of nanoparticles, as these particles have a larger surface area and therefore higher surface energy. The rutile phase is more stable than the anatase phase, followed by the brookite phase, in terms of the enthalpy of transformation from brookite to rutile and from anatase to rutile; the slower transformation of anatase to rutile is due to the higher surface energy of rutile compared to anatase. The relative stability of the phase depends on particle size, with the most stable phase being anatase at sizes below 4.9 nm, brookite at sizes between 4.9 and 30 nm, and rutile at sizes greater than 30 nm. **(FigureII.6)**[23].

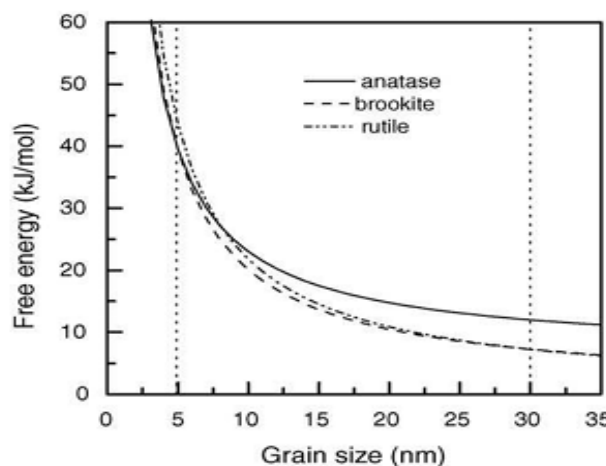


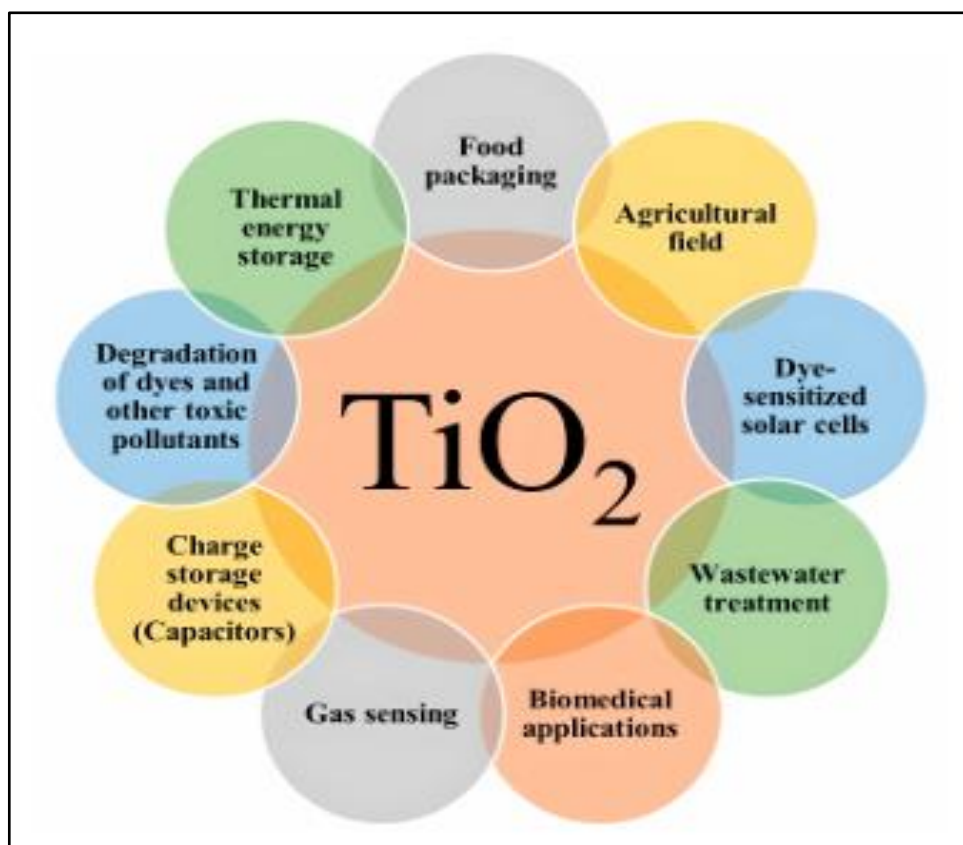
Figure II.6 A thermodynamic stability study of the anatase, brookite, and rutile phases as a function of particle size [23].

- **Photocatalytic degradation properties of TiO_2**

When TiO_2 is exposed to photonic radiation from an ultraviolet source, where the energy exceeds the bandgap energy of TiO_2 , electrons in the valence band move to the conduction band, thereby creating holes (+h) in the valence band. The photoexcited electrons (e^-) and holes (+h) then participate in oxidation and reduction reactions with species, forming peroxide radicals ($\text{O}_2^{\bullet-}$) and hydroxyl radicals ($\text{OH}^\bullet$). However, other species may also form as a result of further oxidation, such as $\text{OOH}^\bullet$ and H_2O_2 [20].

II.4.4 Applications of TiO_2

Titanium dioxide (TiO_2) is one of the most important metal oxides, due to its many functional properties; it displays great stability in both chemical and photochemical processes, and is also an environmentally safe and inexpensive substance. It also exhibits high photocatalytic activity, making it an essential component in a wide range of advanced technological applications. The following **Figure II.7** illustrates its exciting uses[21].



FigureII.7 Illustration of potential applications of TiO_2 [21].

References of Chapter Two

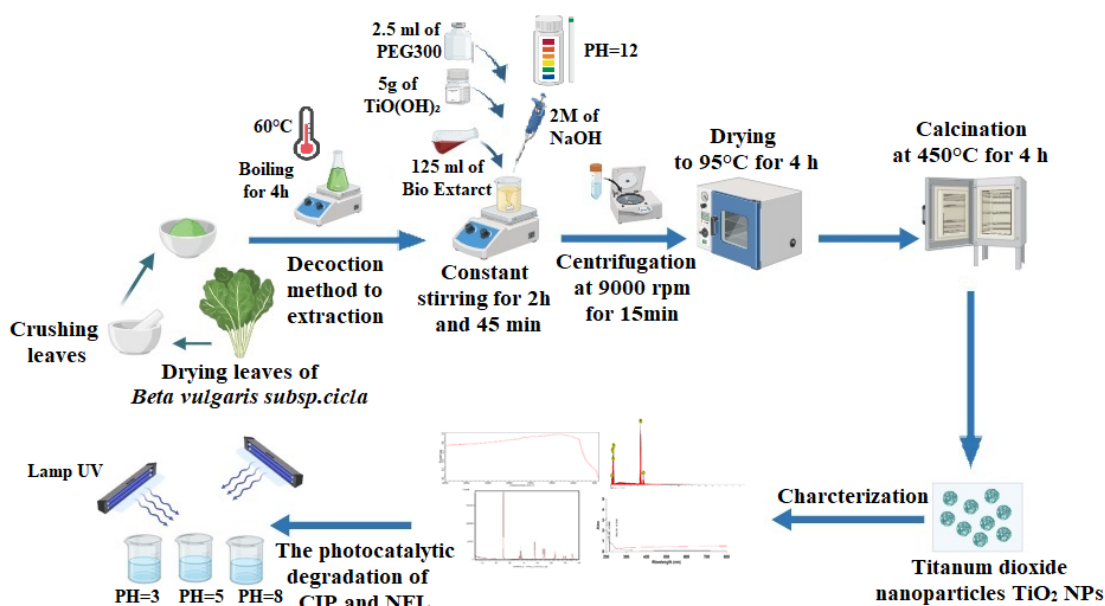
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Chapter Three

*Experimental Materials and
Methods*

This chapter focuses on presenting the experimental methodology used in the green synthesis of titanium dioxide nanoparticles using *Beta vulgaris subsp. cicla* leaf extract and the directing agent PEG300, and then using these particles as a photosensitizer in the photodegradation of antibiotics. The experimental work includes the extraction process, the synthesis of nanoparticles, their characterization, and finally the photosensitized photodegradation of two types of antibiotics.



FigureIII.1 Schematic diagram of the experimental part.

III.1 Equipment

Most of the equipment, glassware, and instruments are available in the pedagogical laboratories of the Chemistry Department, such as beakers of various sizes, graduated pipettes, volumetric flasks, graduated cylinders, spatulas, magnetic stir bars, Petri dishes, a precision balance, a magnetic shaker and heater, a centrifuge, an oven, and a muffle furnace. All vessels and glassware are thoroughly cleaned before use.

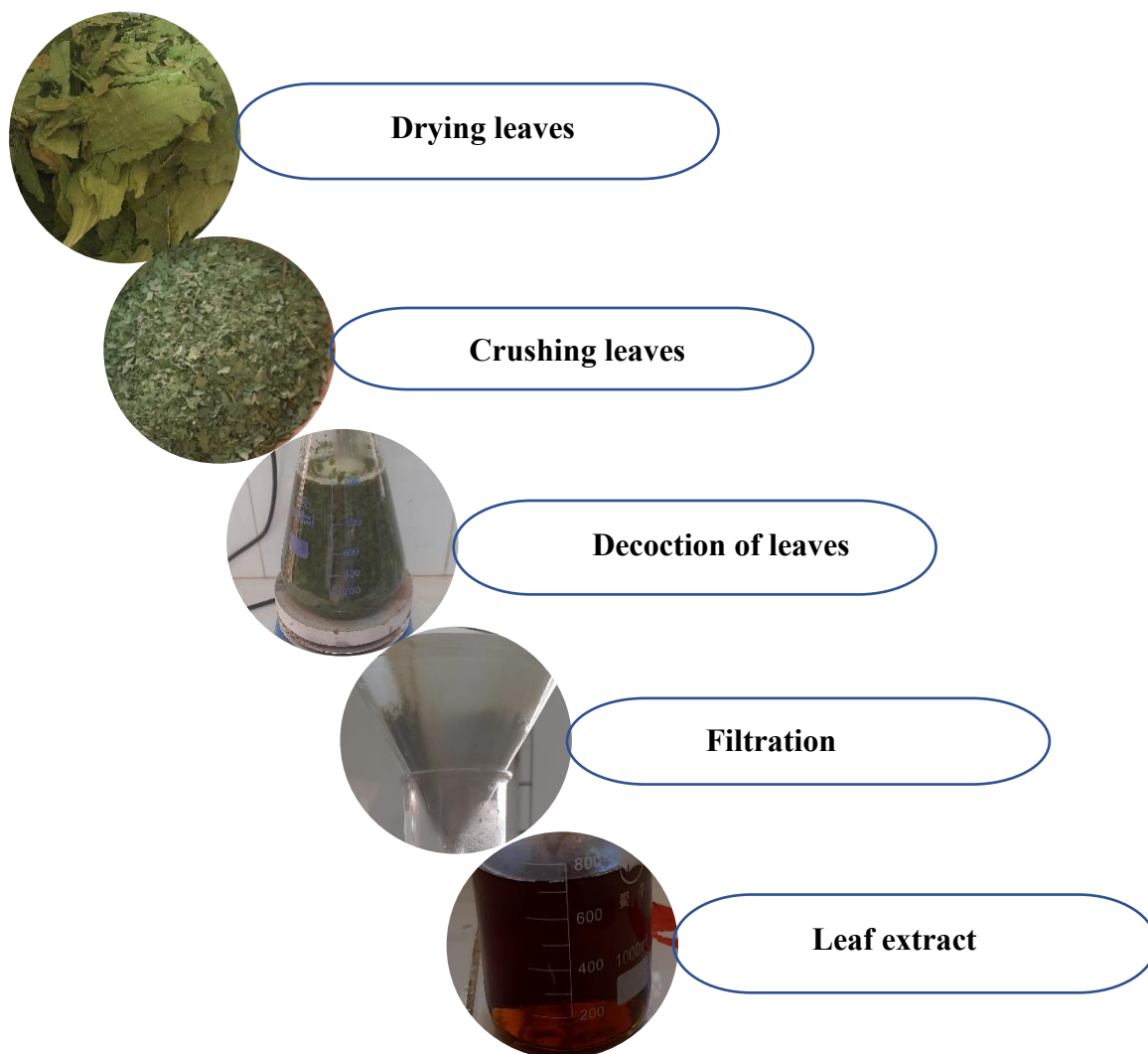
III.2 Chemical Products

TableIII.1 Properties of the chemical products used.

Materials	Formula	Molar mass (g/mol)	Purity (%)
Titanyl Hydroxide	$\text{TiO}(\text{OH})_2$	97.8	98-99
Polyethylene glycol (PEG300)	$\text{OH}(\text{CH}_2\text{CH}_2\text{O})_n\text{H}$	300	99.99
Sodium Hydroxide	NaOH	40	97-98
Hydrochloric acid	HCl	35.5	35-38

III.3 Chard Leaves Extraction

We bought some chard leaves from a local market in Touggourt, washed them thoroughly with distilled water, removed the white stalks, and left the leaves to dry in the open air for 12 days. Once the leaves had dried, we obtained a total weight of approximately 32 g. We ground the leaves thoroughly using a mortar and pestle until we had a fine powder. We used the ‘decoction’ method[1-3] to extract the bioactive compounds. The volume of distilled water used in the extraction process was approximately 800 ml. Once the extraction was complete, we filtered the extract thoroughly to remove any plant residue and obtained an extract volume of approximately 750 ml (**FigureIII.1**) (**FigureIII.2**).



FigureIII.2 Steps of Extracting Chard Leaves.

III.4 Titanium dioxide NPs synthesis

In a beaker, 125 mL of the prepared plant extract was placed, and then 5 g of titanium hydroxide ($\text{TiO}(\text{OH})_2$)[3-5] was added to achieve a solution concentration of 0.5 M. The mixture was left to stand with continuous stirring using a magnetic stirrer, and then 2.5 mL of the PEG300 directing agent was added[6]. After 45 minutes, a 2 M sodium hydroxide solution was added until the pH of the mixture reached 12[7], with continuous stirring for two hours. The resulting mixture was then separated by centrifugation at 9000 rpm for 15 minutes and washed with distilled water. The precipitate was placed in a Petri dish and dried in a laboratory drying oven set to 95°C for 4 hours. After drying, a dry mass weighing 8.21 g was obtained, which was ground before calcination in an electric

furnace at 450 °C for 4 hours[8]. Finally, a fine white powder of TiO₂ nanoparticles with a mass of 8.12 g was obtained (**FigureIII.1**) (**FigureIII.3**).



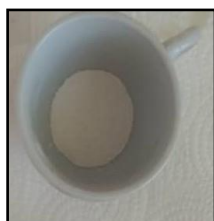
Constant stirring of the mixture of TiO₂ and Chard leaf extract.



Separated TiO₂.



Drying



Calcination

FigureIII.3 Steps of the preparation of titanium dioxide (TiO₂) nanoparticles.

III.5 Photocatalytic degradation of the antibiotics CIP and NFL

To investigate the catalytic efficiency of green-synthesized TiO₂ NPs, photocatalytic experiments were conducted on solutions prepared from the antibiotics CIP and NFL. This methodology relied on exploiting the unique properties of the nanoparticle catalyst, whose growth was directed and size stabilized using the PEG300 directing agent, giving it a high surface area that enhances its ability to adsorb pollutants and generate active free radicals upon exposure to light. This study aims to track the decrease in pollutant concentration over time and in different pH media using UV-Vis spectroscopy, in order to understand the effect of pH on the degradation of antibiotics and to assess the catalyst's effectiveness in breaking down the complex carbon structures of these antibiotics and converting them into simple, environmentally harmless compounds.

The protocol of photocatalytic degradation of CIP and NFL shown in **Figure III.4**.

III.5.1 Preparation of Stock Solutions

Stock solutions of the antibiotics (ciprofloxacin and norfloxacin) were prepared by dissolving 0.1 g of each antibiotic powder in 100 mL of distilled water. To ensure complete dissolution, an ultrasonic bath was used for 20 minutes at a temperature of 40°C. Subsequently, to obtain solutions with a concentration of 20 mg/L of the antibiotics, the diluted solutions were prepared by withdrawing 2 mL of the stock solution and making up the volume to 100 mL with distilled water[9].

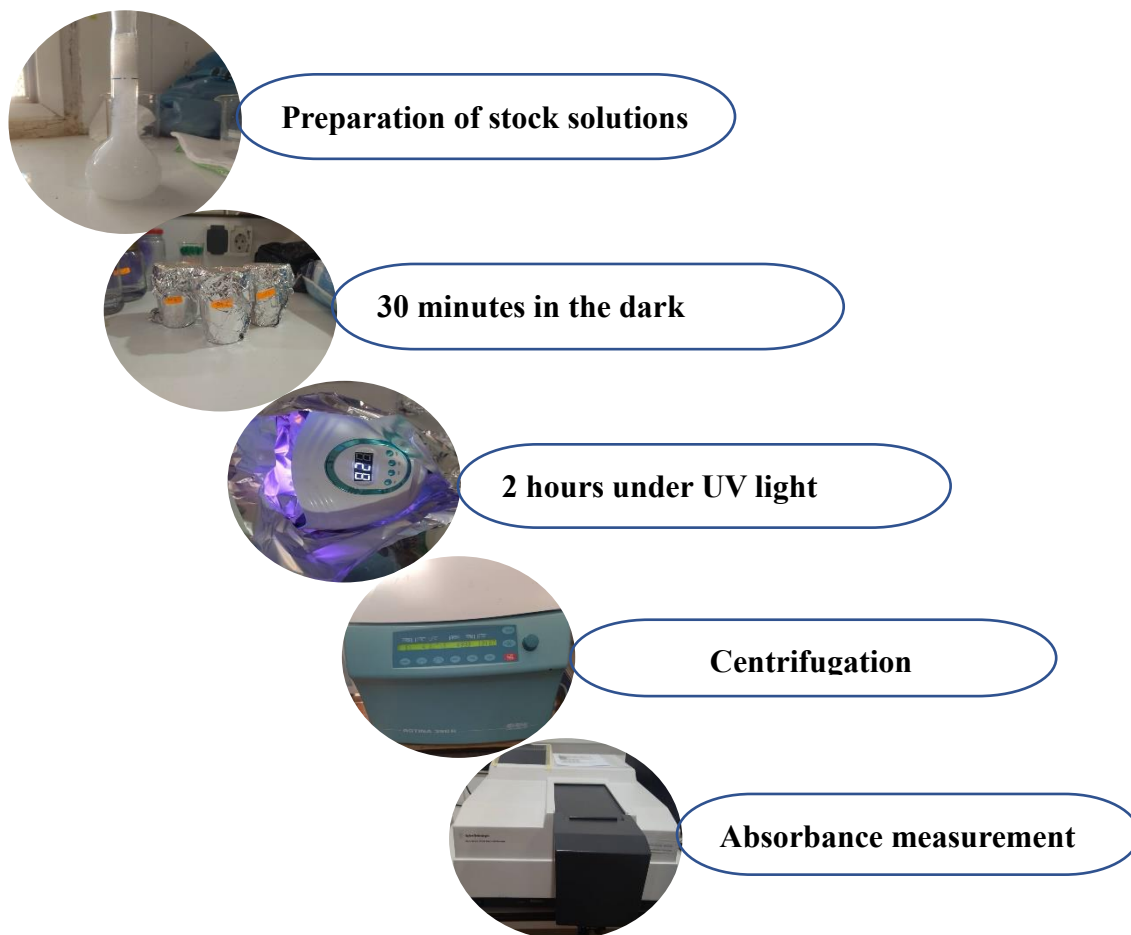
III.5.2 Preparation of Calibration Curves

To estimate the unknown concentrations of antibiotics during the degradation process, calibration curves were prepared for each antibiotic by preparing five known standard concentrations. The absorbance of each concentration was measured at a maximum wavelength of 277 nm using a UV-Vis spectrophotometer, and the linear relationship between concentration and absorbance was plotted according to the Beer-Lambert law.

III.5.3 Protocol for the Photocatalytic Degradation of each Antibiotic

- Three Becher flasks containing an antibiotic solution at a concentration of 20 mg/L were prepared, and the pH of these flasks was adjusted to 3, 5, and 8, respectively[10, 11].
- 50 mg of synthesized TiO₂ NPs were added to each beaker, which were then placed in the dark for 30 minutes while being stirred manually to achieve adsorption/desorption equilibrium[9].
- Before turning on the UV light source, 5 mL was withdrawn from each beaker (denoted as t₀), and the nanoparticle catalyst was separated using a centrifuge to measure the initial absorbance using a UV-visible spectrophotometer at the specific maximum wavelengths for each antibiotic in each sample.
- The solutions were then exposed to UV light (which is a commercial dual-source UV-LED lamp (48W/54W) emitting in the wavelength range of 365-405 nm) while being stirred manually from time to time, and samples (5 mL) were periodically withdrawn at specific time intervals (15, 30, 60, 90, 120) minutes, labeled as (t₁, t₂, t₃, t₄, t₅). After each sampling, the catalyst was

separated by centrifugation, and absorbance was measured to track the decrease in antibiotic concentration over time[10, 11].



FigureIII.4 Steps of photocatalytic degradation of CIP and NFL.

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Chapter Four

Results and Discussion

This chapter presents the results and discussion of structural, morphological, and optical measurements of TiO₂ NPs synthesized via a green method using '*Beta vulgaris* subsp. *cicla*' leaf extract. This chapter also discusses the effectiveness of these particles as a catalyst for the photodegradation of antibiotics in water at different pH levels.

IV.1 Ultraviolet and visible (UV-Vis) spectroscopy analysis

The ultraviolet and visible spectrum can help explain the absorption of radiant energy by atomic electrons and their transition to higher energy levels. Ultraviolet and visible analysis was performed on TiO₂ NPs prepared using plant leaf extract (chard). The UV-Vis absorption spectrum is shown in **FigureIV.1** exhibits a prominent absorption peak at a wavelength of 374 nm, which preliminarily indicates the formation of TiO₂ NPs[1]. To determine the photostimulation activity of these particles, we calculated the bandgap energy using Tauc's equation (4-1)[2]:

$$(\alpha h\nu)^n = A(h\nu - E_g) \quad (4 - 1)$$

Where:

α : absorption coefficient.

h : Planck's constant.

ν : frequency of incident light.

n : a coefficient that determines the nature of electronic transitions.

$n = 2$ (direct allowed transition)

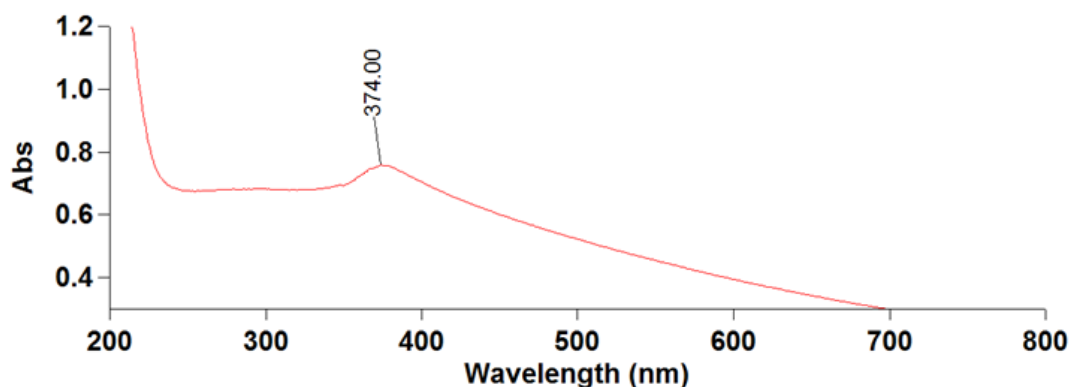
$n = 1/2$ (indirect allowed transition)

A : constant related to matter.

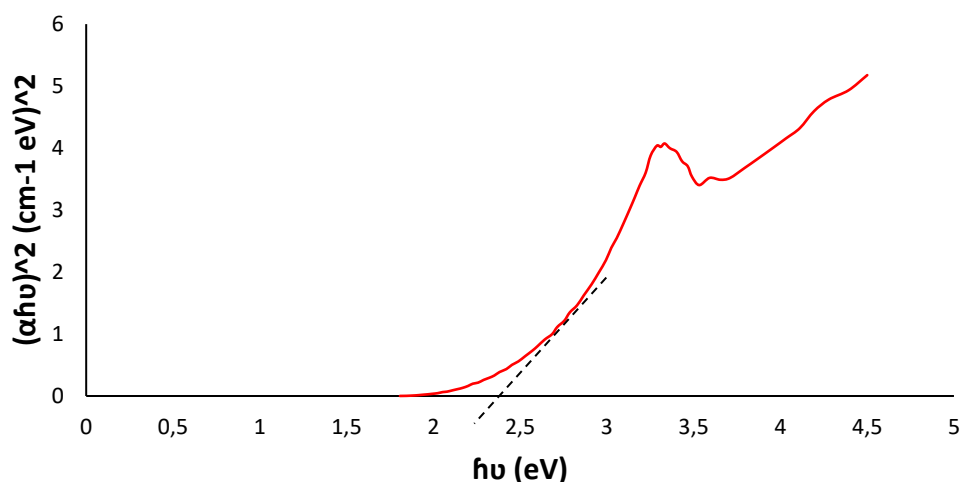
E_g : energy gap.

and the Tauc's plot shown in **FigureIV.2**, which indicates that the bandgap energy of sample A is 2.4 eV, which is low compared to the bandgap energy of pure titanium dioxide, which is 3.2 eV[2]. This is due to the combined role of the plant extract and the cation chains in creating surface and structural modifications in the crystal lattice of the TiO₂ NPs, where plant chemical compounds can contribute to a natural self-doping process with elements such as carbon or nitrogen within the TiO₂ NPs crystal lattice

during the calcination process, which may lead to the formation of mid-gap states located just below the conduction band. This structural modification can help reduce the energy required to excite electrons[3]. The directing agent PEG300 can also control particle morphology and prevent random agglomeration during the synthesis process, ensuring the maintenance of a precise nanocrystalline size, a uniform shape, and a high specific surface area.



FigureIV.1 The UV-Vis spectrum of TiO₂ NPs.

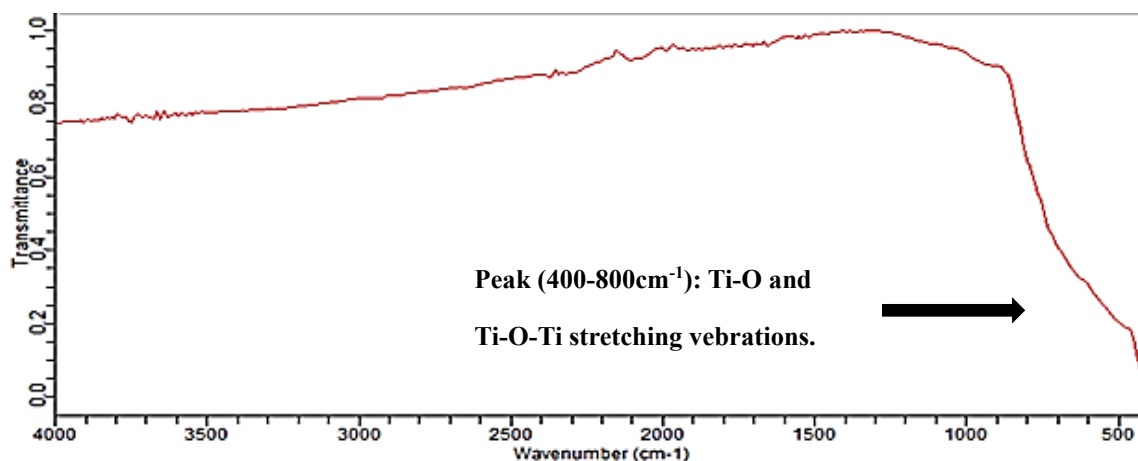


FigureIV.2 Tauc's plot of TiO₂ NPs.

IV.2 Fourier transform infrared (FTIR) spectroscopy analysis

The spectrum shows in(**FigureIV.3**) a broad absorption band and a sharp drop between 800 cm⁻¹ and 400 cm⁻¹, which is attributed to the presence of a Ti-O bond, confirming the formation of a metal-oxygen band[4-6]. There is a near-complete absence of absorption bands associated with the plant extract and PEG, specifically the aliphatic

C-H absorption band between 2850 cm^{-1} and 2950 cm^{-1} , and the very strong etheric C-O-C bond absorption band at 1100 cm^{-1} . The absence of the hydroxyl (OH) group absorption band at 3400 cm^{-1} is also noted. This explains the disappearance of the plant extract and polyethylene glycol (PEG) through several possibilities, including their complete decomposition and combustion during the calcination process, or perhaps during the repeated washing steps of TiO_2 .



FigureIV.3 The FTIR spectrum of TiO_2 NPs.

IV.3 X-ray diffraction (XRD) analysis

X-ray diffraction (XRD) was used to characterize the crystal structure, purity, and crystal size of the synthesized titanium dioxide nanoparticles. The XRD patterns are clearly shown in **FigureIV.4** in the 2θ range from 5° to 80° . The results confirmed the formation of the anatase phase. By comparing the positions of the diffraction peaks in the spectrum with standard reference charts (such as JCPDS card No. 21-1272)[7], we observe a high degree of crystallinity, with sharp and distinct diffraction peaks appearing at the following 2θ angles: 25.27° (which is the main and most intense peak), followed by peaks at 37.76° , 48.02° , 53.85° , 55.03° , 62.65° , 68.73° , 70.25° , and 75.03° . These peaks correspond exactly to the standard crystal planes of the anatase phase of titanium dioxide, which correspond to the following crystal planes in order: (101), (004), (200), (105), (211), (204), (116), (220), and (215).

The absence of any strange or unidentified peaks in the spectrum indicates the high purity of the prepared particles and their freedom from impurities, and that the starting material has been almost completely converted into nanoparticles; it also confirms the

efficiency of the washing and drying processes in removing unreacted organic residues from the plant extract.

The average size of the resulting nanoparticles was estimated to be approximately 50 nm using the Debye-Scherrer equation (4-2) on the most intense main peak of the (101) crystal plane[8]:

$$D_{hkl} = \frac{K\lambda}{B_{hkl} \cos \theta} \quad (4 - 2)$$

Where:

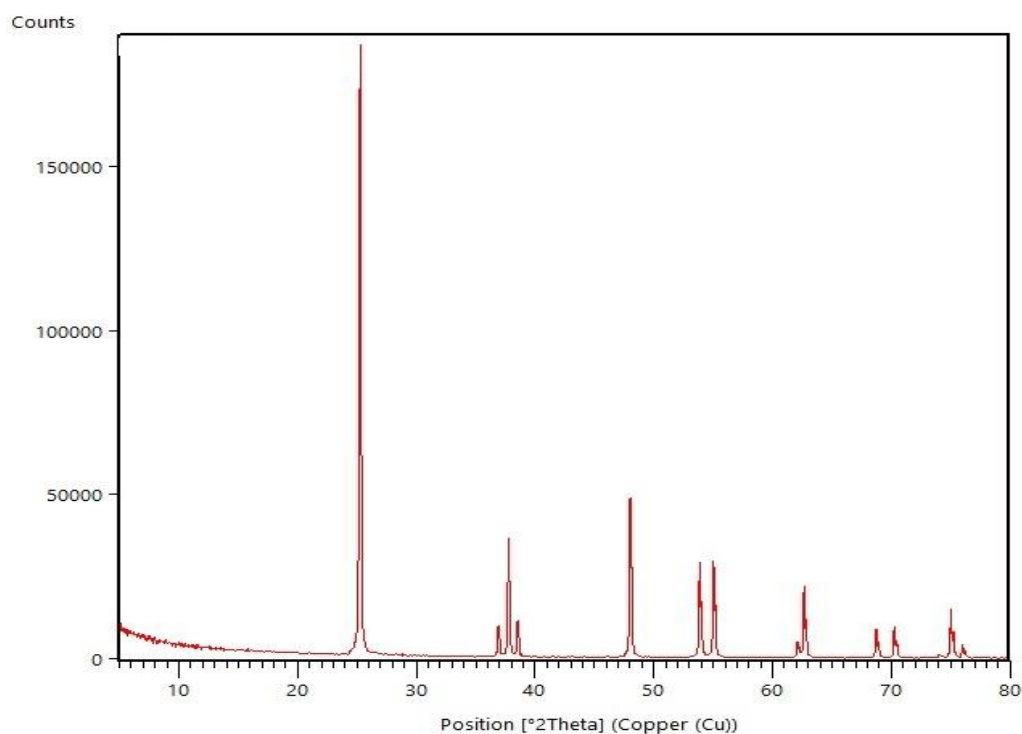
K: crystal aspect ratio (here 0.9).

λ : wavelength of the X-rays used (here 0.154 nm).

B_{hkl} : full width at half maximum (FWHM), measured in radians.

θ : Bragg angle (here 12.64°).

This result confirms the success of the strategy employed in combining the chard extract with PEG300, as it led to the production of nanoparticles with a homogeneous and geometrically stable size, where the biomolecules of the chard extract acted as excellent reducing, encapsulating, and stabilizing agents, and the PEG300 polymer chains limited the random growth of the particles and prevented their agglomeration by wrapping around them. From an applied perspective, a size of approximately 50 nanometers is considered ideal for photostimulation processes, as it ensures an excellent balance between high specific surface area (which increases the sites for antibiotic adsorption on the surface) and the physical stability of the sample, facilitating the separation and recovery of nanoparticles after water treatment is complete.



FigureIV.4 X-ray diffraction spectrum of TiO₂ NPs.

TableIV.1 XRD parameters and calculated crystallite size of the prepared samples.

2θ (°)	θ (°)	FWHM (°)	β (rad)	Crystallite size D (nm)
25,27	12,64	0,16	0,00279	49,81

IV.4 Diagnosis of TiO₂-NPs by Emission Scanning Electron Microscope (SEM) and Energy Dispersive X-ray (EDX)

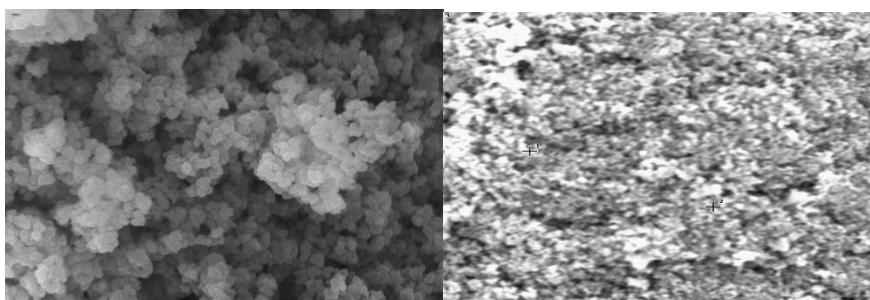
To study the morphological characteristics of (TiO₂ NPs), scanning electron microscopy (SEM) was employed. The topographical images obtained (**Figure IV.5**) allow the structure, morphology, and particle size of the titanium dioxide nanoparticles to be determined.

The images show that the TiO₂ nanoparticles are spherical in shape [9-11], and appear as simple aggregates. The resulting agglomerates were found to be approximately 50 nanometers in size. The sample contains numerous voids and pores, indicating that the particles have a porous network structure. Furthermore, the particles appear to have an agglomerated structure[10], suggesting that they possess high surface energy[12, 13].

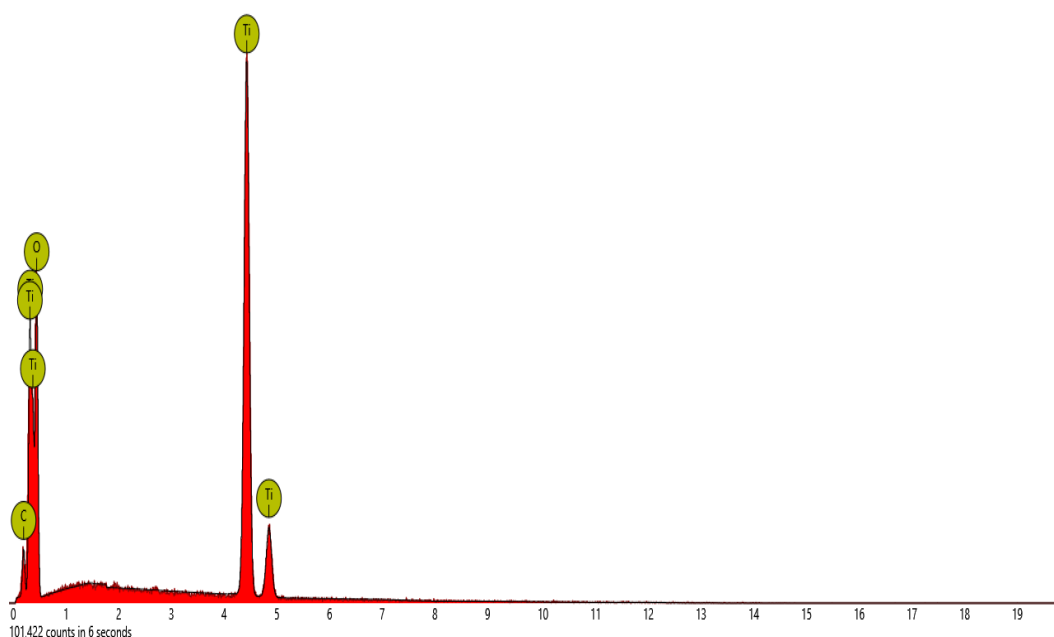
The spectrum shown in **Figure IV.6** reveals sharp, high-intensity peaks at the characteristic energy levels of titanium (Ti), with these peaks dominating the overall

spectrum; this is consistent with titanium being the base metal in the prepared sample. The spectrum shows a characteristic peak for oxygen (O), an expected signal that chemically confirms its bond with titanium to form the oxide compound TiO_2 [13]. A secondary peak of very low intensity was recorded, attributable to carbon (C); its presence is due to residual functional groups from the plant extract and the PEG polymer used as a stabilizing and capping agent for the nanoparticles, preventing their aggregation during the preparation of the TiO_2 NPs.

Table IV.2 shows the composition of the (TiO_2 NPs), where the elemental weight of titanium (Ti) was approximately 48.76% with an atomic percentage of 23.67%. Oxygen (O) had an elemental weight of 47.21% and an atomic percentage of 68.66%, whilst carbon (C) had a very low weight percentage, not exceeding 3.97%[10]. These results underscore the effectiveness of the preparation method employed, despite the presence of polyethylene glycol, in producing nanoparticles of high chemical purity, free of impurities or secondary metal contaminants[9, 10].



FigureIV.5 SEM Morphologies Analysis of the Synthesized TiO_2 NPs.



FigureIV.6 EDX Spectrum analysis of the synthesized TiO₂ NPs.

TableIV.2 EDX Morphologies analysis of the synthesized TiO₂ NPs.

Element Symbol	Atomic Conc.	Weight Conc.	Oxide Symbol
Ti	23,67	48,76	TiO ₂
O	68,66	47,27	/
C	7,67	3,97	/

IV.5 Photocatalytic Degradation of CIP and NFL

The rate of photodegradation of the antibiotics was estimated using the equation (4-3)[6, 12, 14]:

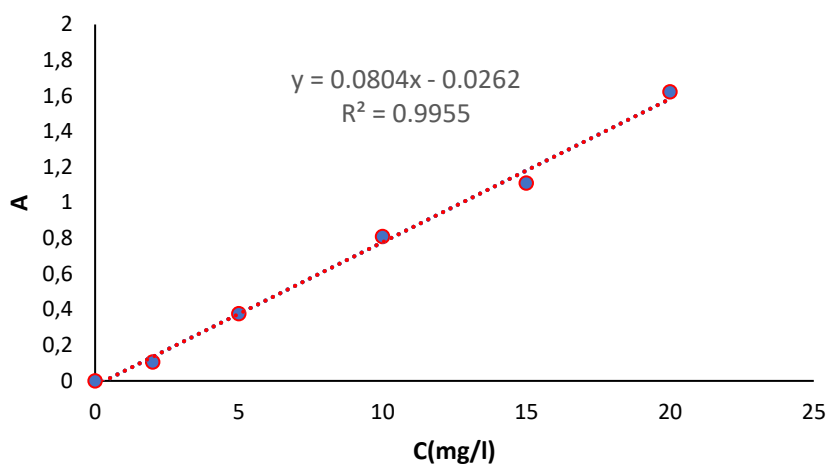
$$\text{Degradation \%} = \frac{C_0 - C_t}{C_0} \times 100 \quad (4 - 3)$$

Where C_0 is the initial concentration of the antibiotic, which was 20 mg/L, and C_t is the concentration of the antibiotic when investigating the various effects of degradation over different time periods.

IV.5.1 Photocatalytic Degradation of CIP

The effect of medium pH and irradiation duration on the photocatalytic degradation of the antibiotic ciprofloxacin using bio-synthesized TiO₂ NPs was studied over various time intervals, namely: 15 min, 30 min, 60 min, 90 min, and 120 min under UV light in three reaction media with different pH levels, namely 3, 5, and 8. This was done after achieving

adsorption-desorption equilibrium in the dark for 30 min. The results are shown in **TableIV.3** and **TableIV.4**, and the graph in **FigureIV.8** indicate a steady decrease in the amount of CIP in the solution with an increase in the rate of photodegradation over time, which peaked at 120 min at 40.53% for pH 3 and 35.41% at pH 8 and 31.4% at pH 5. The slow rate of photodegradation can be explained by the lack of continuous agitation of the reaction solution, which is a key factor in determining the efficiency of photocatalyzed degradation[6]. pH is also one of the most important factors influencing the photocatalytic degradation of CIP[15] catalyzed by TiO₂ NPs. As shown in **TableIV.3** and **TableIV.4** and the graph in **FigureIV.8**, the highest rate of photodegradation occurred at pH 3, with a value of 40.53% after two hours of radiation exposure, while the lowest photodegradation efficiency was observed at pH 5, with a value of 31.4%. These results clearly indicate the extent of the effect of pH on the degradation of CIP. The higher rate of degradation in the acidic medium can be explained by the abundance of hydrogen ions (H⁺) in the medium, which contributes to the acceleration of secondary reactions and the production of free hydroxyl radicals (•OH) in the solution, in addition to accelerating the degradation of intermediate compounds. The change in the chemical nature of the intermediates formed during the reaction facilitates their adsorption and attack by photons (h⁺)[14, 16]. At a pH of 5, a slow increase in the degradation of the antibiotic was observed in this medium, reaching 31.40%. After two hours, this may be due to a decrease in the concentration of hydrogen ions (H⁺) in the medium, which contributes to a reduction in the production of hydroxyl radicals (•OH) in the solution, and consequently reducing their binding to the antibiotic, which reduces the attack by photons (h⁺), making the photodegradation rate slow compared to other acidic media[14, 16]. While this antibiotic exists as a doubly charged ion at pH 8, which reduces the attractive forces between it and the surface of positively charged TiO₂ NPS (since the zeta potential of TiO₂ lies at approximately pH 6 [17]), the abundance of hydroxyl ions (OH⁻) in this medium significantly enhanced the production of highly reactive hydroxyl radicals (•OH), which aided the photodegradation process, reaching a maximum value of 35.31% after two hours.



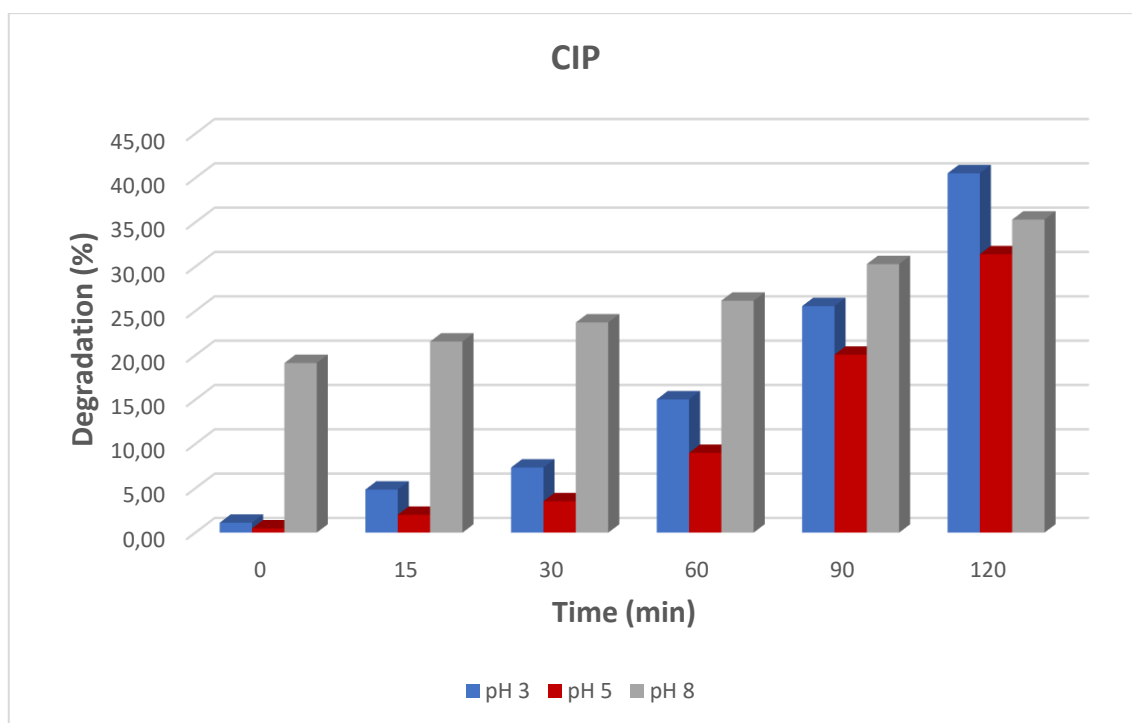
FigureIV.7 Calibration curve of CIP.

TableIV.3 Measurement of CIP absorption during photodegradation over different time periods and at different pH levels.

The Concentration values at each pH			
Time	pH 3	pH 5	pH 8
t0	19,78	19,91	16,17
t1	19,03	19,60	15,69
t2	18,53	19,29	15,26
t3	17,00	18,20	14,77
t4	14,89	15,99	13,94
t5	11,89	13,72	12,94

TableIV.4 The removal and photocatalytic degradation efficiency of CIP by TiO₂ over different time periods and at different pH levels.

The Photocatalytic degradation efficiency (%)			
Time	pH 3	pH 5	pH 8
t0	1,11	0,47	19,13
t1	4,86	2,01	21,55
t2	7,34	3,54	23,70
t3	15,02	8,99	26,16
t4	25,55	20,07	30,29
t5	40,53	31,40	35,31

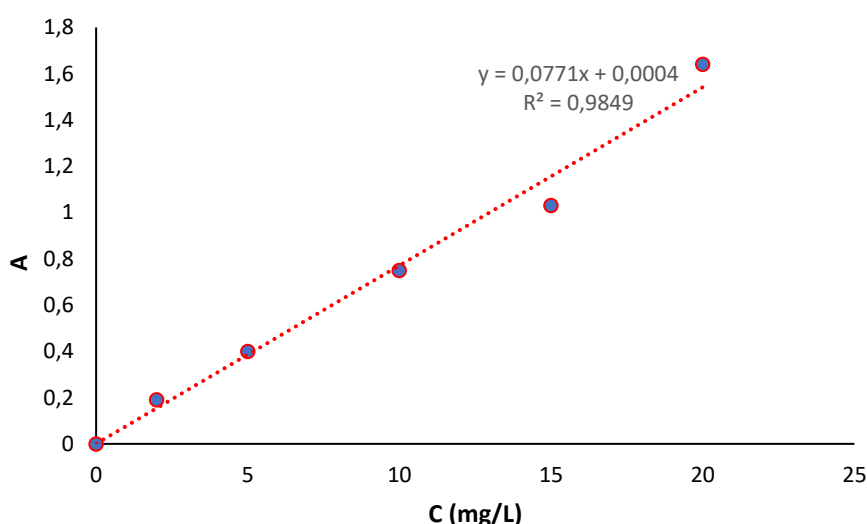


FigureIV.8 A graph showing the rate of photodegradation of CIP by TiO_2 over different time periods and at different pH levels under UV light.

IV.5.2 Photocatalytic Degradation of NFL

The effect of medium pH and irradiation duration on the photocatalytic degradation of the antibiotic norfloxacin using bio-synthesized TiO_2 NPs was studied over various time intervals: 15 min, 30 min, 60 min, 90 min, and 120 min under UV light in three media with different pH levels, namely 3, 5, and 8. This was done after achieving adsorption-desorption equilibrium in the dark for 30 min. The results are shown in **TableIV.5** and **TableIV.6**, and the graph in **FigureIV.10** indicate a steady decrease in the amount of NFL in the solution with an increase in the rate of photodegradation over time, which peaked at 120 min, 33.08% at pH 8, 27.29% at pH 3, and 25.36% at pH 5. The slow rate of photodegradation can be explained by the lack of continuous stirring of the reaction solution, which is a key factor in determining the efficiency of photo-catalyzed degradation[6]. pH is also one of the most important factors influencing the photocatalytic degradation of NFL[6, 18] catalyzed by TiO_2 NPs. As shown in **TableIV.5** and **TableIV.6** and the graph in **FigureIV.10**, the highest rate of photodegradation occurred at pH 8, with a value of 33.08% after two hours of irradiation, while the lowest degradation efficiency was observed at pH 5, with a value of 25.36%. These results clearly indicate the extent of the pH's effect on the degradation of NFL. The higher degradation rate in the weakly alkaline medium can be explained by the fact that NFL molecules exist in their anionic

form due to the loss of a proton from the carboxyl group[18]. Although the TiO_2 surface is also negative in this medium (since the zeta potential of titanium dioxide is around pH 6 [17]), the abundance of hydroxyl ions (OH^-) in this medium significantly enhanced the production of highly reactive hydroxyl radicals ($\bullet\text{OH}$), as the latter can overcome electrostatic repulsion, facilitating photodegradation. Conversely, NFL molecules become positively charged at pH 3, leading to electrostatic repulsion with the positively charged surface of the TiO_2 NPs. Meanwhile, this antibiotic exists as a doubly charged ion at pH 5, which reduces the attractive forces between it and the positively charged surface of the TiO_2 NPs, potentially reducing the efficiency of degradation.



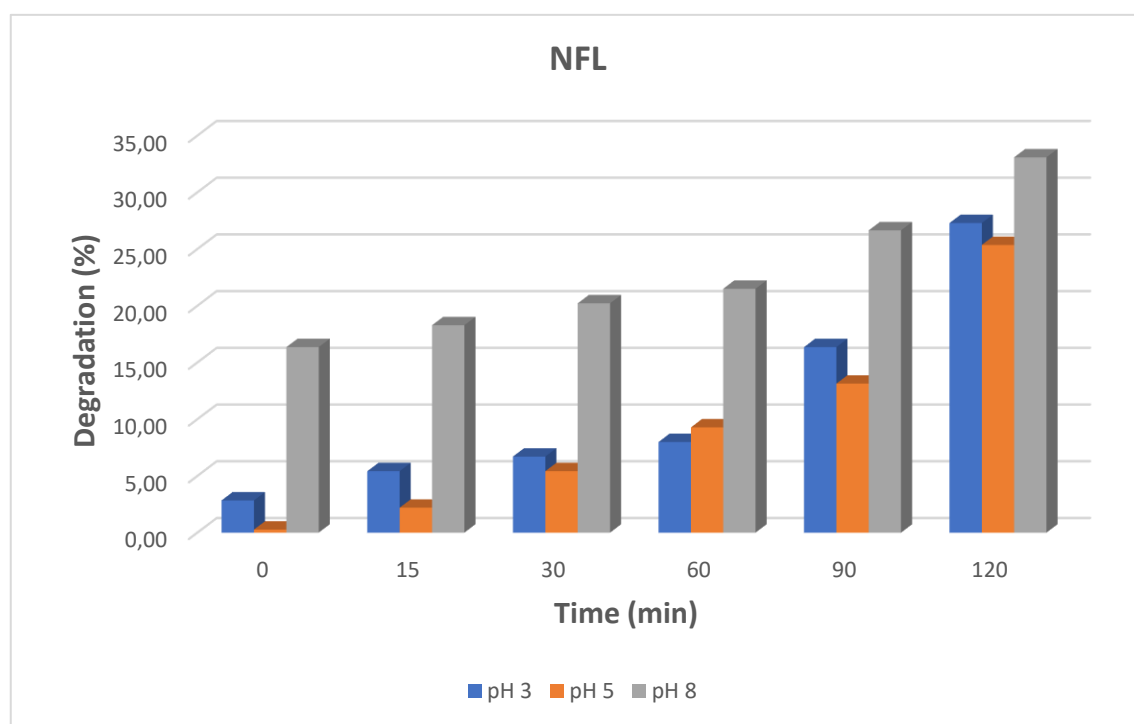
FigureIV.9 Calibration curve of NFL.

TableIV.5 Measurement of NFL absorption during photodegradation over different time periods and at different pH levels.

The Concentration values at each pH			
Time	pH 3	pH 5	pH 8
t0	19,43	19,95	16,73
t1	18,92	19,56	16,34
t2	18,66	18,92	15,96
t3	18,40	18,14	15,70
t4	16,73	17,37	14,67
t5	14,54	14,93	13,38

TableIV.6 The removal and photocatalytic degradation efficiency of NFL by TiO₂ over different time periods and at different pH levels.

The Photocatalytic degradation efficiency (%)			
Time	pH 3	pH 5	pH 8
t0	2,84	0,27	16,36
t1	5,42	2,20	18,29
t2	6,70	5,42	20,22
t3	7,99	9,28	21,50
t4	16,36	13,14	26,65
t5	27,29	25,36	33,08

**FigureIV.10** A graph showing the rate of photodegradation of NFL by TiO₂ over different time periods and at different pH levels under UV light.

The results of the photocatalytic degradation of both ciprofloxacin and norfloxacin using bio-derived TiO₂ NPs show similar kinetic behavior in terms of time dependence, contrasted by a marked difference in degradation efficiency with pH changes, reflecting a close correlation between the catalyst's surface charge and the structural properties of each antibiotic. Both antibiotics exhibited a gradual and continuous decrease in their

concentration over time during the light irradiation period, reaching peak degradation 120 minutes after UV exposure. The results for both compounds also showed the lowest photodegradation efficiency at a moderately acidic pH of 5, with rates of 31.40% for CIP and 25.36% for NFL. This general relative inactivity in the degradation efficiency of both antibiotics, as mentioned earlier, may be attributed to the absence of a continuous stirring mechanism for the mixture during the reaction; since stirring is a critical factor in enhancing mass transfer and facilitating the access of contaminant molecules to the active sites on the surface of the nanocatalyst, as well as its role in renewing the diffusion of dissolved oxygen that captures electrons. The fundamental difference between the two catalysts lies in the optimal pH value for achieving the highest degradation efficiency, as the CIP compound exhibited maximum response in a strongly acidic medium (pH 3) at a rate of 40.53%, whereas the NFL compound performed optimally in a weakly basic medium (pH 8) at a rate of 33.08%. This discrepancy can be explained as follows:

- **In an acidic medium (pH 3):** The surface of TiO_2 nanoparticles becomes positively charged due to the protonation of surface hydroxyl groups (TiOH_2^+). Under these conditions, the CIP compound clearly outperforms the others, achieving the highest degradation rate (40.53%). This can be structurally explained by the presence of a cyclopropyl group at position 1 of the quinoline ring on the nitrogen atom; this group confers higher hydrophobic properties on the molecule compared to the linear structure of NFL. This hydrophobicity facilitated the approach of CIP molecules and their better orientation toward the surface by penetrating the hydrodynamic layer surrounding the catalyst, concurrently with the abundance of H^+ ions in the medium, which accelerate secondary reactions and promote the breakdown of intermediate compounds, altering their chemical nature to make them more susceptible to attack by positive H^+ holes. In contrast, the NFL compound exhibited low efficiency at this value (27.29%); it exists as a cation resulting from the protonation of the piperazine ring amine, and due to its possession of a linear “ethyl” (Ethyl group) at position 1 of the quinoline ring on the nitrogen atom, the molecule was unable to overcome the strong electrostatic repulsive forces with the positive catalyst surface, which hindered the surface adsorption process necessary to initiate decomposition.

- **In a basic medium (pH 8):** The surface of the nanocatalyst is negatively charged due to the removal of TiO^- protons, since the pH value is higher than the zeta potential equilibrium point ($pH_{pzc} = 6$). Despite the expected electrostatic repulsion between the negatively charged surface and the anionic form of NFL molecules (resulting from the loss of a carboxyl group proton), or CIP molecules that are mostly present in the form of zwitterionic/anionic ions, the decomposition remained active and with similar efficiencies (35.31% for CIP and 33.08% for NFL). This behavior is attributed to the high abundance of hydroxyl ions (OH^-) in the medium, which increases the hydrodynamic rate of free hydroxyl radical ($\cdot OH$) generation. These radicals possess sufficient oxidation energy to overcome repulsive forces and directly attack organic bonds in solution without the need for strong adsorption. Furthermore, the flexible ethyl group with lower steric hindrance in the NFL at this pH reduces the molecule's steric resistance as it approaches the catalyst's electron cloud, allowing the free radicals to penetrate the chemical structure and attack it successfully, resulting in a significant convergence in degradation efficiency between the two compounds and demonstrating that the free-radical attack mechanism in the basic medium partially neutralizes the steric differences caused by the substituents.

These results demonstrate that even the slightest differences in the steric structure of antibiotics (even if they belong to the same family) can affect their catalytic photodegradation efficiency.

References of Chapter Four

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Water pollution is one of the most pressing challenges facing the World Health Organization, including hospital waste and sewage in water, particularly antibiotic residues, which lead to the emergence of antibiotic-resistant bacterial strains that pose a serious threat to human health and the environment. Given that conventional water treatment methods cannot completely remove these compounds, there is an urgent need to develop more advanced and efficient technologies. In this context, the present study aimed to propose a scientific and practical approach involving the use of an innovative technique: the photodegradation of antibiotics using nanomaterials. The main objective of this work was to prepare titanium dioxide nanoparticles (TiO_2 NPs) in an environmentally friendly manner in the presence of the directing agent PEG300 and to use them in the photodegradation of antibiotics, owing to the method's efficiency and minimal negative environmental impact.

Leaves of the plant '*Beta vulgaris* subsp. *cicla*' were extracted using the decoction method, and $\text{TiO}(\text{OH})_2$ salt was added to the plant extract, and the directing agent PEG was added as a coating agent for the resulting titanium dioxide nanoparticles. These were then subjected to a drying and calcination process, yielding TiO_2 powder.

Characterization by FTIR, XRD, and EDX confirmed the chemical composition, morphological properties, and elemental composition of the TiO_2 powder, with results showing the purity of the synthesized particles and their freedom from impurities, and that the starting material had been almost completely reduced to TiO_2 nanoparticles. The XRD results confirmed the formation of the anatase phase of titanium dioxide, and the average size of the resulting nanoparticles was approximately 50nm, calculated according to the Debye-Scherrer equation from the most intense main peak of the (101) crystal plane. Tauc's equation was used to calculate the bandgap energy of the TiO_2 sample, which was found to be 2.4 eV. SEM results revealed a spherical morphology of the particles, with simple agglomerates permeated by pores.

As for the role of the PEG surfactant, the absence of its characteristic features in FTIR, XRD and EDX explains the agent's role in altering the surface morphology of the particles and preventing random agglomeration in the synthesis process. The reason for this is that the binding of PEG to the particle surface is a physical bond or self-aggregation; both types of bonding facilitate the easy separation of PEG from the particle

surface, particularly during the washing stage of the separation process or during its disappearance in the calcination process.

The photodegradation test of the antibiotic CIP showed a maximum response after two hours in a highly acidic medium (pH 3) at a rate of 40.53%, whilst for the antibiotic NFL, the degradation response was optimal in a weakly basic medium (pH 8) at a rate of 33.08%. These findings demonstrate that even the smallest changes in the spatial structure of antibiotics, even within the same family, can affect their photocatalytic efficiency.

In conclusion, this study represents an important step towards the replacement of traditional techniques for pollutant degradation with environmentally friendly techniques such as photodegradation using nanoparticles, and this opens up prospects for the development of this technology and the enhancement of its efficiency.

Future studies should prioritize the adaptation of this technique for sustainable industrial applications. We recommend continuous stirring during photodegradation to ensure full access of antibiotic molecules to the catalyst and to extend the reaction time, while investigating the effect of stirring speed on mass transfer and light distribution within the reactor. It is also essential to focus on studying the effect of different UV wavelengths (such as UV-A, UV-B, and UV-C) to determine the most effective light spectrum for breaking the bonds of these compounds. Intensive research must also be conducted on the effect of pH on the surface charge of nanoparticles and the molecular state of various antibiotics to accurately understand the mechanisms of adsorption and degradation, in addition to studying the effect of catalyst concentration on the initial concentration of the antibiotic. Practical tests must be conducted on real-world aqueous samples (hospital waste or wastewater) to study the effect of interference from salts and other organic materials on the nanoparticles' efficiency. It is also necessary to evaluate the stability of the nanoparticles and their recoverability and reusability over several consecutive cycles without significant loss of catalytic efficiency, in order to achieve economic sustainability.