



PAN-AFRICAN UNIVERSITY
INSTITUTE FOR WATER AND ENERGY SCIENCES
(Including CLIMATE CHANGE)

Master Dissertation

Submitted in partial fulfillment of the requirements for the Master degree in

Water engineering

Presented by:

Abdelhak TAICHA

**Photocatalytic degradation of pharmaceutical contaminants in
wastewater assisted by ecofriendly synthesized ZnO nanoparticles**

Defended on 04/2024 Before the Following Committee:

Chair	First and Last Name	Title	Affiliation (Institution only)
Supervisor	Khedidja BENOUIS	Pr	(University of SBA, Algeria)
Co supervisor	M. ElAmin SAID	Dr	(University of SBA, Algeria)
External Examiner	First and Last Name	Title	Affiliation (Institution only)
Internal Examiner	First and Last Name	Title	Affiliation (Institution only)

DECLARATION

STUDENT'S DECLARATION

I, Abdelhak TAICHA, hereby declare that this thesis titled "Photocatalytic degradation of pharmaceutical contaminants in wastewater assisted by ecofriendly synthesized ZnO nanoparticles" is my original work to the best of my knowledge and has not been submitted to a University or any other institute or published earlier for the award of any degree or diploma. I also declare that all the informations, materials and results from other works presented in this thesis have been duly cited and recognized as required of academic rules and ethics.

Name: Abdelhak TAICHA

Date: 28/03/2024

Signature:



SUPERVISOR'S DECLARATION

I, Professor Khedidja BENOUIS, hereby declare that I supervised the preparation of this Master thesis submitted therein in accordance with the guidelines on supervision of Master thesis laid down by the Pan African University Institute of Water and Energy Sciences, Algeria.

Name: Pr. Khedidja BENOUIS

Date: 28/03/2024

Signature:



ACKNOWLEDGEMENT

First and foremost, I would like to thank God Almighty for giving me the strength and knowledge to finalize this work.

I am profoundly grateful to my supervisor, Professor Khedidja BENOUIS and my co-supervisor, Associate Professor Mohammed ElAmin SAÏD, from the University of Sidi Bel Abbes, Algeria for their helpful support, encouragement, valuable guidance, patience, suggestions, and professional advice throughout the realization of the master thesis. I greatly appreciate their deep knowledge in various disciplines, which contributed a lot to this project. I am privileged to have been under their supervision.

I would also like to thank all the staff members of the Laboratory of Process Engineering, Materials and Environment of the University of Sidi Bel Abbes, Algeria with a special thanks to Anes GHAZI for his helpful support, encouragement, and valuable guidance throughout my thesis work.

My sincere gratitude goes to the African Union Commission (AUC) for awarding me a full scholarship for my master studies at the Pan African University Institute of Water and Energy Sciences (PAUWES) as well as a research grant for conducting this study. In addition, I thank all PAUWES staff members for all their help during the past two past years. I would like to express my appreciation and sincere thanks to all the students of Pauwes especially the Water Engineering class for the wonderful time we spent together. I would also like to thank Professor Mohamed BOUAZIZ, from the University of Sfax, Tunisia, for accepting and allowing me to do my internship in the Laboratory of Electrochemistry and Environment, and for his professional advice, help, and encouragement. I want to express my heddle thanks to my whole family, my dad and mom without forgetting my two sisters and my brother. This accomplishment would not have been possible without them. Thank you very much from the bottom of my heart.

ABBREVIATIONS AND ACRONYMS

A. P	Adequate Precision
ANOVA	Analysis of variance
AOPs	Advanced oxidation processes
ARVs	Antiretroviral medicines
BBD	Box-Behnken design
C.V	Coefficient of Variation
CFM	Cefotaxime
CIP	Ciprofloxacin
EDX	Energy Dispersive X-ray Spectroscopy
EMA	European Medicines Agency
FDA	US Food and Drug Administration
FESEM	Field Emission Scanning Electron Microscopy
NPs	Nanoparticles
PEG	Polyethylene glycol
PPCPs	Pharmaceuticals and Personal Care Products
PVC	Polyvinyl chloride
RSM	Response Surface Methodology
S. D	Standard Deviation
TEM	Transmission Electron Microscope
UV	Ultraviolet

VAN	Vancomycin
WHO	World Health Organization
WWTPs	Wastewater Treatment Plant
XRD	X-Ray Diffraction
ZnONPs	Zinc Oxide Nanoparticles

TABLE OF CONTENTS

DECLARATION	I
ACKNOWLEDGEMENT	II
ABBREVIATIONS AND ACRONYMS.....	III
TABLE OF CONTENTS	V
LISTE OF TABLES.....	VIII
LISTE OF FIGURES.....	IX
1. INTRODUCTION	1
1.1 Introduction:	1
1.2 Problem Statement:.....	2
1.3 Research Questions:	2
1.4 Main Objective:.....	3
1.5 Specific Objectives:	3
1.6 Thesis Organization:.....	3
2. LITERATURE REVIEW	4
2.1 The Pharmaceutical Industry: Global Overview and the Case of Algeria	4
2.1.1 Worldwide Assessment of the Pharmaceutical Industry	4
2.1.2 Key Players and Market Trends Worldwide	4
2.1.3 Challenges in the Global Pharmaceutical Sector	4
2.1.4 Financialization and Evolution of the Pharmaceutical Industry.....	5
2.1.5 The Pharmaceutical Industry in Algeria: Current Status and Challenges.....	5
2.1.6 Government Policies and Initiatives in Algeria's Pharmaceutical Sector	5
2.1.7 Opportunities and Growth Potential for the Pharmaceutical Industry in Algeria	6
2.2 The Impact of Pharmaceuticals on Water Quality: Occurrence, Fate, and Removal Strategies	6
2.2.1 Sources of Water Pollution.....	6
2.2.2 Emergence of Pharmaceuticals in Water Systems	6
2.2.3 Fate of pharmaceuticals in wastewater treatment plants	8
2.2.4 Health Impacts of Pharmaceutical Contamination	9
2.2.5 Challenges in Analysis and Detection	9
2.2.6 Degradation and Persistence of Pharmaceuticals.....	10

2.2.7	Treatment Methods for Pharmaceutical Removal.....	11
2.3	Generality on nanotechnology	13
2.4	Nanomaterial	14
2.5	Classification of nanomaterials.....	15
2.5.1	Classification Nanomaterials according to their dimensionality.....	15
2.5.2	Classification Nanomaterials by type	16
2.6	Nanoparticles (NPs)	17
2.6.1	Definition	17
2.6.2	Synthesis of nanoparticles.....	18
2.7	Zinc Oxide Nanoparticles (ZnONPs)	19
2.7.1	Definition	19
2.7.2	Green synthesis of ZnONPs	20
3.	MATERIAL AND METHODS	22
3.1	Chemicals and instrumentation.....	22
3.2	Preparation of the aqueous extract of banana peels.....	22
3.3	Biosynthesis of ZnONPs	23
3.4	Dosage of phenolic compounds.....	24
3.4.1	Total phenol dosages.....	25
3.4.2	Flavonoid dosage	26
3.4.3	Dosage of flavonols	27
3.4.4	Dosage of condensed tannins	27
3.4.5	Dosage of hydrolysable tannins	28
3.5	RSM optimization study.....	29
3.6	Experimental design.....	29
3.7	Antibiotics solutions preparation	30
4.	RESULTS AND DISCUSSION	33
4.1	Characterization of the banana peel extract	33
4.2	Statistical analysis	35
4.3	Process optimization.....	42
4.4	Analysis of the degradation of antibiotics using response surface methodology	43
5.	CONCLUSION AND RECOMMENDATIONS.....	49
5.1	Conclusions	49

5.2	Recommendations	50
6.	REFERENCES	51
7.	APPENDIX	63

LISTE OF TABLES

Material and Methods

Table 3. 1: <i>Experimental range and levels of variables</i>	30
--	----

Results and Discussions

Table 4. 1 : <i>Polyphenol compounds percentage in the banana peel extract before and after synthesis of nanoparticles</i>	35
Table 4. 2 : <i>Experimental Setup for factors and Responses</i>	35
Table 4. 3 : <i>Model Summary Statistics</i>	38
Table 4. 4 : <i>ANOVA for quadratic model (Y1)</i>	39
Table 4. 5 : <i>ANOVA for quadratic model (Y2)</i>	39
Table 4. 6 : <i>ANOVA for quadratic model (Y3)</i>	40
Table 4. 7 : <i>Statistical parameters for responses Y1, Y2, and Y3</i>	41
Table 4. 8: <i>Predicted and validated results of the optimized variables</i>	43

LISTE OF FIGURES

Literature Review

Figure 2. 1: Schematic diagram of source, environmental, and health effects of pharmaceuticals ⁵⁵	10
Figure 2. 2: Summary of removal methods for pharmaceuticals ⁵⁵	12
Figure 2. 3 : Schematic illustration showing how nanotechnology and its nanotools, nanomaterials, and nanodevices are impacting our world ⁸³	15
Figure 2. 4 : Types of nanomaterials by size ⁸⁵	16
Figure 2. 5 : Summary diagram of different types of nanomaterials ⁸⁹	17
Figure 2. 6 : Size range of nanoparticles compared to those of major chemical and biological structures ⁹¹	18
Figure 2. 7 : Synthetic routes for nanophotocatalysts ⁵⁵	19

Material and Methods

Figure 3. 1 : Formation of White Precipitates (ZnONPs).....	23
Figure 3. 2 : Schematic illustration of ZnO nanoparticles (ZnONPs) biosynthesis.....	24
Figure 3. 3: Schematic illustration of total phenol dosage.....	25
Figure 3. 4: Schematic illustration of flavonoid dosage.....	26
Figure 3. 5: Schematic illustration of flavonols dosage.....	27
Figure 3. 6: Schematic illustration of condensed tannins dosage.....	28
Figure 3. 7: Schematic illustration of hydrolysable tannins dosage.....	29
Figure 3. 8: Schematic illustration of Photocatalysis Process.....	31
Figure 3. 9 : Photocatalysis Chamber.....	32

Results and Discussions

Figure 4. 2 : Phenolic compounds content in the banana peel extract.....	34
Figure 4. 3 : Predicted vs actual plot for CFM degradation (a); CIP degradation (b) and VAN degradation (c).....	41
Figure 4. 4 : 2D response surface plot for (a,b,c): CFM degradation, (d,e,f): CIP degradation, (g,h,i): VAN degradation.....	45
Figure 4. 5: Response surface 3D-plot for CFM degradation.....	45
Figure 4. 6: Response surface 3D-plot for CIP degradation.....	46
Figure 4. 7: Response surface 3D-plot for VAN degradation.....	47
Figure 4. 8 : Predicted solution obtained by numerical optimization (CFM).....	47
Figure 4. 9: Predicted solution obtained by numerical optimization (CIP).....	47
Figure 4. 10 : Predicted solution obtained by numerical optimization (VAN).....	48

Appendix

A. 1 : Total phenols calibration curve.....	63
A. 2 : Flavonoid's calibration curve.....	63
A. 3 : Flavonols calibration curve.....	64

A. 4 : <i>Hydrolysable tannins calibration curve</i>	64
A. 5 : <i>Condensed tannins calibration curve</i>	65

ABSTRACT/ RESUME

Abstract:

In this study, the photocatalytic degradation of cefotaxime (CFM), ciprofloxacin (CIP), and vancomycin (VAN) in aqueous solution was investigated using the green nanophotocatalyst ZnONPs, synthesized from banana peels. A predictive mathematical model based on response surface methodology was developed to explore this degradation process. Three independent variables were investigated: the initial concentration of the antibiotic, the dose of ZnONPs nanocatalyst, and the irradiation time. The response studied was the percentage degradation of the antibiotic. A Box-Behnken design was adopted, revealing an optimal level of degradation for CFM, CIP, and VAN, reaching 40.41%, 61.51%, and 53.22%, respectively. This optimum was observed at initial antibiotic concentrations of 10.78 mg/L, 29.99 mg/L, and 29,92 mg/L, a dose of ZnONPs nanocatalyst of 0.78 g/L, 0.70 g/L, and 0.77 g/L, and irradiation times of 89.46 minutes, 89.99 minutes, and 60 minutes, respectively.

Key words: Cefotaxime, Ciprofloxacin, Vancomycin, Pharmaceutical, ZnO, Nanoparticles, Banana Peels, Photocatalysis, Response Surface Methodology.

Résumé :

Dans cette étude, La photocatalyse de dégradation en solution aqueuse de la cefotaxime (CFM), de la ciprofloxacine (CIP) et de la vancomycine (VAN) en utilisant le nanophotocatalyseur vert ZnONPs, synthétisé à partir des pelures de banane, a été explorée, en développant un modèle mathématique prédictif basé sur la méthodologie de la surface de réponse. Trois variables indépendantes ont été étudiées : la concentration initiale de l'antibiotique, la dose du nanocatalyseur ZnO NP et la durée d'irradiation. La réponse étudiée était le pourcentage de dégradation de l'antibiotique. Une conception Box-Behnken a été adoptée, révélant un niveau optimal de dégradation pour le CFM, la CIP et la VAN, atteignant respectivement 40,41 %, 61,51 % et 53,22 %. Cet optimum a été observé à des concentrations initiales d'antibiotiques de 10,78 mg/L, 29,99 mg/L et 29,92 mg/L, une dose de nanocatalyseur ZnONPs de 0,78 g/L, 0,70 g/L et 0,77 g/L, et des durées d'irradiation de 88,58 minutes, 89,99 minutes et 34,77 minutes, respectivement.

Mots clés : Céfotaxime, Ciprofloxacine, Vancomycine, Pharmaceutique, ZnO, Nanoparticules, Pelures De Banane, Photocatalyse, Méthodologie De Surface De Réponse.

1. INTRODUCTION

1.1 Introduction:

In recent years, the escalating concern regarding the presence of pharmaceutical residues in wastewater, particularly antibiotics, has become a pressing environmental and public health issue. These contaminants, originate from various sources including medical facilities, agriculture, and households, pose significant challenges due to their persistence and potential adverse effects on aquatic ecosystems and human health. The widespread use of antibiotics, exacerbated by the COVID-19 pandemic for preventing and treating bacterial infections, has further intensified this concern¹⁻⁴.

Despite the implementation of various wastewater treatment methods, including physical, chemical, and biological processes, conventional approaches often struggle to achieve complete degradation of pharmaceutical residues. This inadequacy stems from the complex nature of these contaminants, their resistance to degradation, and the limitations of existing treatment technologies. Consequently, there is a growing demand for more effective and sustainable treatment strategies capable of addressing the persistent presence of pharmaceutical residues in wastewater.

Recently, advanced oxidation processes (AOPs) have garnered significant attention due to their demonstrated efficacy in degrading organic pollutants present in pharmaceutical wastewater. In response to this challenge, photocatalytic degradation has emerged as a promising approach for eliminating pharmaceutical contaminants from wastewater. These innovative methods offer efficient and sustainable solutions to address the persistent presence of pharmaceutical residues, thereby contributing to the advancement of wastewater treatment technologies and the preservation of environmental quality⁵⁻⁷. Heterogeneous photocatalysis, a process that harnesses the energy of photons to initiate chemical reactions on the surface of photocatalysts, offers several advantages, including high efficiency, versatility, and environmental compatibility⁸⁻¹⁰. Furthermore, the utilization of eco-friendly synthesized ZnO nanoparticles as photocatalysts enhances the sustainability and applicability of this method.

This study focuses on investigating the efficacy of photocatalytic degradation assisted by eco-friendly synthesized ZnO nanoparticles for eliminating three commonly found antibiotics in wastewater: cefotaxime, ciprofloxacin, and vancomycin. By evaluating the degradation efficiency, environmental sustainability, and feasibility of implementing this approach, this research aims to contribute to the development of innovative and sustainable solutions for addressing pharmaceutical contamination in wastewater treatment systems.

Through a comprehensive examination of the effectiveness and implications of photocatalytic degradation, this study aims to advance our understanding of sustainable wastewater treatment practices and pave the way for the implementation of environmentally friendly technologies in mitigating the environmental impacts of pharmaceutical residues.

1.2 Problem Statement:

The persistence of pharmaceutical residues, particularly antibiotics, in wastewater presents a significant challenge to wastewater treatment systems. Conventional treatment methods often struggle to completely degrade these contaminants, leading to their accumulation in water bodies and potential adverse effects on the environment and human health. Despite advancements in treatment technologies, the presence of pharmaceutical residues persists, necessitating more effective and sustainable solutions for their removal from wastewater.

1.3 Research Questions:

- What are the primary challenges associated with the presence and degradation of antibiotics in wastewater?
- How effective is the photocatalytic degradation method assisted by eco-friendly synthesized ZnO nanoparticles in degrading antibiotic contaminants such as cefotaxime, ciprofloxacin, and vancomycin?

1.4 Main Objective:

The main objective of this study is to investigate the efficacy of using eco-friendly synthesized ZnO nanoparticles as photocatalysts for the degradation of three commonly found antibiotics in wastewater.

1.5 Specific Objectives:

- To assess the degradation efficiency of cefotaxime, ciprofloxacin, and vancomycin in wastewater using photocatalytic degradation assisted by eco-friendly synthesized ZnO nanoparticles.
- To evaluate the environmental sustainability and feasibility of implementing this photocatalytic degradation method in wastewater treatment systems.
- To compare the performance of this approach with conventional treatment methods in terms of degradation efficiency, cost-effectiveness, and environmental impact.

1.6 Thesis Organization:

- Introduction: Provides an overview of the research background, problem statement, research questions, main and specific objectives.
- Literature Review: Explores previous studies on pharmaceutical residues in wastewater, conventional treatment methods, photocatalytic degradation, and the synthesis of eco-friendly ZnO nanoparticles.
- Methodology: Describes the experimental setup, materials, and procedures used for evaluating the photocatalytic degradation of antibiotics in wastewater using eco-friendly synthesized ZnO nanoparticles.
- Results and Discussion: Presents and discusses the findings of the experimental investigation, including the degradation efficiency of antibiotics, environmental sustainability, and comparison with conventional treatment methods.
- Conclusion and Recommendations: Summarize the key findings and draw conclusions, followed by recommendations for future research or practical applications.

2. LITERATURE REVIEW

2.1 The Pharmaceutical Industry: Global Overview and the Case of Algeria

2.1.1 Worldwide Assessment of the Pharmaceutical Industry

The pharmaceutical industry is an essential global activity encompassing the prescription, innovation, production, and circulation of various pharmaceuticals and medications. This industry has vital role in healthcare by providing life-saving treatments for numerous illnesses and health problems¹¹. Over the years, the global pharmaceutical industry has prevailed for multiple reasons. One of these reasons is the rise in spending on healthcare systems and the emergence of medicine and vaccines industries as lucrative markets. Additionally, the rise in aging populations and the prevalence of specific chronic diseases have contributed to the industry's growth within various regions¹². In addition, there is an increase in the aging of societies and specific chronic diseases affecting the population of a given county.

2.1.2 Key Players and Market Trends Worldwide

The industry's key players are Pfizer, Roche, Novartis, Johnson & Johnson, and Merck & Co. These multinational corporations not only sponsor the research and development of new, previously unidentified drugs, but also take charge of their advertising and distribution to ensure that these products reach as many patients as possible¹³. The pharmaceutical sector is distinguished by three significant patterns: further biology, personalized medicine, and emerging markets¹⁴.

2.1.3 Challenges in the Global Pharmaceutical Sector

The pharmaceutical industry operates within a multinational regulatory framework with strict guidelines regulating various aspects of drug design, manufacturing, promotion, and safety, enforced by organizations such as the US Food and Drug Administration (FDA), the European Medicines Agency (EMA), and the World Health Organization (WHO). Policies and guidelines have been implemented to maintain quality, efficacy, and safety standards. However, the industry faces obstacles including increased regulatory

oversight, patent expirations, market competition from generic drugs, and higher costs associated with pharmaceutical research and development^{15,16}.

2.1.4 Financialization and Evolution of the Pharmaceutical Industry

The pharmaceutical industry illustrates the growing influence of financial markets. Expanding in both size and financial sophistication, these markets have developed complex mechanisms for allocating billions of dollars and redistributing them to investors, especially big Wall Street banks. Over the past six decades, the pharmaceutical industry has undergone significant transformation¹⁷. Pharmaceutical companies, as well as oil and transportation companies, are now among the world's largest global corporations. Government funding of research and health insurance programs represents two examples of "public trust" in the pharmaceutical industry. However, these industries have sometimes become excessively profit-oriented entities serving shareholders rather than the well-being of patients, often as a result of this public trust¹⁸.

2.1.5 The Pharmaceutical Industry in Algeria: Current Status and Challenges

Algeria's pharmaceutical sector plays a significant role in both the nation's economic landscape and healthcare infrastructure. This industry encompasses a blend of indigenous pharmaceutical enterprises and foreign entities engaged in the Algerian market¹⁹. Algeria has undertaken initiatives to bolster domestic pharmaceutical production and reduce dependence on imported drugs. Nevertheless, the sector encounters obstacles including limited research and development capacities, insufficient infrastructure, regulatory hurdles, and the prevalence of counterfeit pharmaceuticals²⁰.

2.1.6 Government Policies and Initiatives in Algeria's Pharmaceutical Sector

The Algeria government has implemented various policies and programs to encourage growth in the pharmaceutical sector and improve access to healthcare services. These measures include incentives for investment, tax exemptions, and financial support for local pharmaceutical producers. Additionally, the government has initiatives to promote research and innovation²¹. It has also enforced regulations on prices with the aim of

ensuring accessibility of pharmaceutical products to the population. Furthermore, price controls have been enforced to make pharmaceutical goods more affordable²².

2.1.7 Opportunities and Growth Potential for the Pharmaceutical Industry in Algeria

Despite facing challenges, Algeria's pharmaceutical industry shows significant promise for growth and improvement. The country's large population and increasing healthcare needs create a demand for a wide range of medicines²³. Moreover, Algeria's strategic location in North African and its involvement in regional trade agreements give pharmaceutical exporters access to neighboring markets²⁴.

2.2 The Impact of Pharmaceuticals on Water Quality: Occurrence, Fate, and Removal Strategies

2.2.1 Sources of Water Pollution

Water quality suffers from increased human activities. These activities generate both organic and inorganic contaminants, as well as microbes. Ultimately, these contaminants end up discharged into water sources²⁵. Pharmaceuticals enter water bodies through various pathways including agricultural runoff, hospital, and household disposal, pharmaceutical industry waste, urban runoff, pharmaceutical products themselves, metabolites, and human/animal excretions²⁶ (Figure 2.1). Other sources also contribute to water pollution, such as untreated wastewater leaking from septic systems, manure application, or sludge use as fertilizers in agriculture²⁷.

2.2.2 Emergence of Pharmaceuticals in Water Systems

2.2.2.1 Pharmaceuticals and Personal Care Products (PPCPs) as Pollutants

Personal care and pharmaceutical products (PPCPs) are considered emerging pollutants due to their potential to have harmful effects even at very low concentrations²⁸. They cover a broad spectrum of compounds, some of which are often found in prescription, over-the-counter, and veterinary pharmaceuticals and have pharmacological effects. Pharmaceuticals are widely used in both human and animal healthcare, and as a result,

they frequently end up in the environment as metabolites or in their unusable form. Their low volatility and strong polarity let them stay in water bodies²⁹.

2.2.2.2 Detection and Distribution in Water Systems

Pharmaceuticals have been found in a variety of water system components, such as surface waters, groundwater, raw drinking water, and both influents and effluents of wastewater treatment plants³⁰. Due to their widespread use, removing medications from contaminated groundwater poses significant challenges. As a result, it's important to identify the sources and implement efficient control measures at the site of release³¹. Furthermore, compared to wastewater systems, larger amounts of pharmaceuticals are usually found in downstream samples, suggesting that wastewater treatment plant discharges play a major role in pharmaceutical contamination in surface waters³². Furthermore, pharmaceutical residues can contaminate the environment and groundwater at amounts of up to 10 ng/L when used as land application of municipal wastewater onto authorized property³³. Pharmaceuticals find their way into surface and groundwater sources due to wastewater treatment plants inability to fully remove them, particularly from agricultural areas that use sludge and urban wastewater²⁷.

2.2.2.3 Examples of Pharmaceuticals Occurrence in Water Systems

In wastewater influents and effluents, pharmaceuticals have been found in concentrations ranging from hundreds of ng/L to $\mu\text{g/L}$ ³⁴. For example, high amounts of the drug gemfibrozil, which is used to treat aberrant blood lipid levels, have been found in groundwater and the influent and effluent of wastewater treatment facilities³⁵. However, elimination rates vary from one drug to another ; for instance, gemfibrozil has a higher rate of elimination than carbamazepine³⁶. Interestingly, several medications are common pollutants in water systems, including aspirin, nalidixic acid, ciprofloxacin, sulfamethoxazole, and antiretroviral medicines³⁷. Antiretroviral medicines (ARVs) including zidovudine and nevirapine have been detected at quantities of 3214–3336 ng/L, 0.9–2.1 ng/L, and 95–510 $\mu\text{g/kg}$, respectively, in wastewater of Wastewater Treatment Plants (WWTPs) effluent, surface water, and river sediments³⁸. Since most people in African countries consume untreated river water, the presence of ARVs in water bodies,

particularly river water, is a cause for worry. Southern Africa is home to surface water ARV hotspots in this respect. For instance, in Zambia and South Africa, respectively, efavirenz values of 140 µg/L and 119 µg/L have been reported³⁹. Aquatic systems frequently include antihypertensive drugs such metoprolol, enalapril, losartan, irbesartan, and valsartan⁴⁰. For instance, it was found that antihypertensive drugs accounted for 20% of the pharmaceuticals found in Spanish river water⁴¹. Metformin, The most widely used anti-diabetic drug in the world, has been detected in various concentrations worldwide: 1.79–59 µg/L in Iceland⁴², 70–325 µg/L in Portugal⁴³, 3.585–9.228 µg/L in South Africa⁴⁴, and 158–2100 µg/L in Germany³⁰. The reason for the high quantities of metformin in water bodies worldwide is probably due to that the drug has not been completely eliminated by biological breakdown mechanisms

2.2.3 Fate of pharmaceuticals in wastewater treatment plants

Several investigations show that WWTPs are not very effective at eliminating the majority of medicines⁴⁵. An efficacious and efficient wastewater treatment plant's capacity to eliminate pollutants is determined by the physical, chemical, and microbiological characteristics of both its influent and effluent wastewater. The efficiency of WWTPs is influenced by operational factors such as homogenization, sludge age, temperature, pH, and hydraulic retention time⁴⁶. The behavior of medicines in wastewater is therefore influenced by water properties such as electrical conductivity, chemical oxygen demand, turbidity, suspended particles, coliforms, total phosphorus, inorganic carbon, total nitrogen, total carbon, and total organic carbon⁴⁷. The WWTP effluents included acetaminophen and carbamazepine were also discovered in surface and groundwater⁴⁸. Moreover, it was discovered that sulfamethoxazole and ciprofloxacin remained in raw drinking water⁴⁹.

Pharmaceuticals have the ability to bioaccumulate and subsequently impact public health due to their resistance to clearance from the aquatic environment. Therefore, it's critical to evaluate and pinpoint ineffective approaches as well as those requiring scalability in order to remove drugs.

2.2.4 Health Impacts of Pharmaceutical Contamination

The presence of different medications in water can have a variety of negative effects on both human and animal health. For example, analgesics like paracetamol increase the risk of asthma, liver damage, and kidney cancer⁵⁰, while antibiotics like sulfamethoxazole cause genetic alterations and have long-term consequences even at low doses⁵¹. Overall, disturbance of the endocrine system and the development of antimicrobial resistance in environmental systems are among the negative impacts of medicines⁵².

2.2.5 Challenges in Analysis and Detection

Since many medications are often found in water bodies at extremely low quantities, it can be difficult to analyze, identify, and remove them from aquatic systems⁵³. Due to a lack of reliable and sensitive methodologies, the primary obstacle to understanding the presence and destiny of medicines is the difficulty of quantitative analysis and detection⁵⁴.

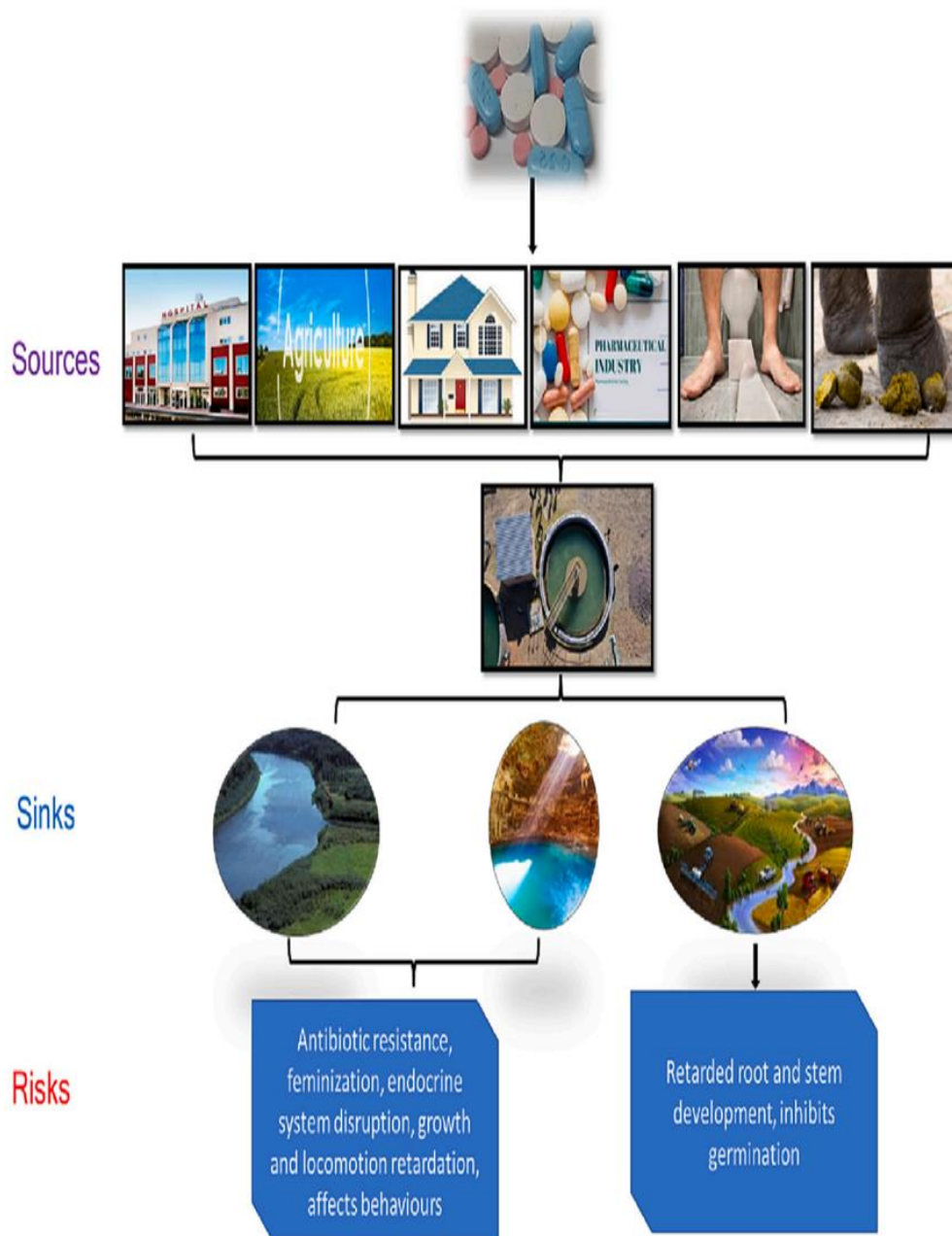


Figure 2. 1: Schematic diagram of source, environmental, and health effects of pharmaceuticals⁵⁵.

2.2.6 Degradation and Persistence of Pharmaceuticals

Because they are bioactive and persistent by nature, pharmaceutical substances continue to have therapeutic effects. Some medications degrade easily when released into the environment, whereas others remain intact⁵⁶. While biodegradation reduces the quantities of these substances in the receiving waters, their metabolites remain and are difficult for standard chemical and biological treatment methods to eliminate⁵⁷.

2.2.7 Treatment Methods for Pharmaceutical Removal

Pharmaceuticals in aquatic systems have been eliminated using a variety of treatment techniques. These include UV light, phytoremediation, peroxy-photo electrocoagulation, activated carbon filtration, enhanced ozonation, biodegradation, adsorption, electrochemical degradation, microfiltration, and conventional treatment methods⁵⁸.

2.2.7.1 Conventional Wastewater Treatment Plants

Pharmaceuticals cannot be completely removed by conventional WWTPs, which typically include pretreatment, primary settling, and biological treatment with activated sludge⁵⁷ (Figure 2.1). Biological treatment units are crucial to the adsorption and biodegradation of medicines in conventional treatment facilities. Therefore, the most plausible mechanism for pharmacological clearance in WWTPs is biological transformation.

2.2.7.2 Adsorption as a Removal Technique

Adsorbents have also been employed to remove various contaminants from wastewater; however, the slow kinetics, poor adsorption capacity, and limited recycling potential of typical adsorbents restrict their application⁵⁹. Environmentally friendly adsorbents, such as dendrimers, offer a significant adsorption capacity⁶⁰. Additionally, Pollutants in multicomponent wastewater systems were eliminated with the application of polyacrylonitrile and metal-organic frameworks⁶¹.

2.2.7.3 Advanced Treatment Processes

According to one research, 50% of antibiotics, 20%–30% of beta blockers, analgesics, and anti-inflammatory medications were eliminated from wastewater polluted with pharmaceuticals using standard treatment methods⁶². Pharmaceuticals were shown to have a high adsorption capacity of 345 mg/g for carbon activated by phosphorus oxyacids, whereas pharmaceuticals in water were reported to be removed with removal efficiencies of 70–100% by gamma radiation, 40–99% by ozonation, and 20–100% by Ultraviolet (UV) radiation [50]. Electrochemical oxidation has proven a viable method for the removal of analgesic and anti-inflammatory medicines⁶³.

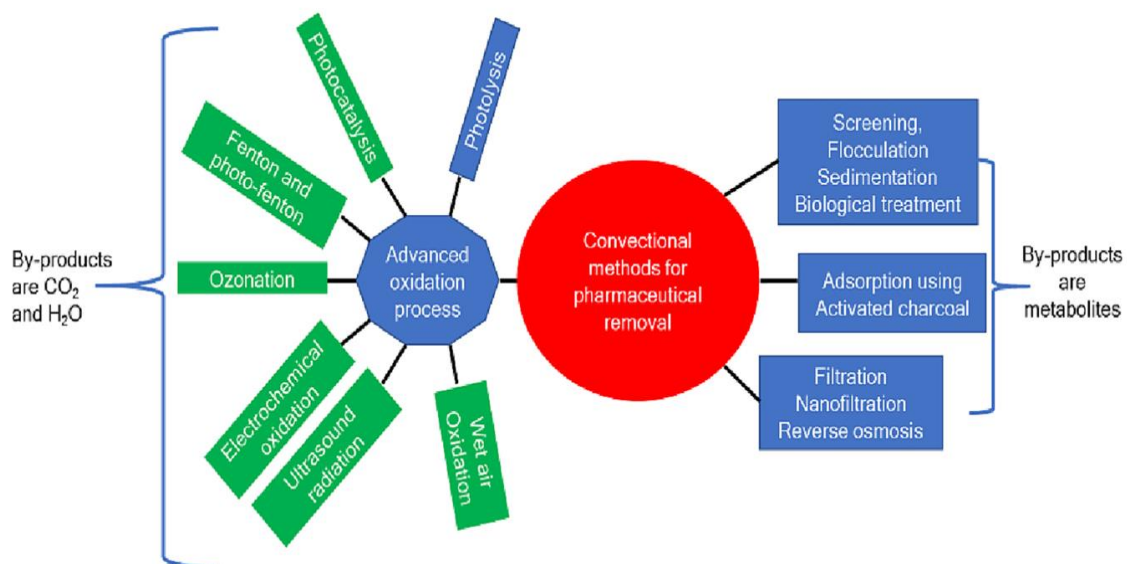


Figure 2. 2: Summary of removal methods for pharmaceuticals⁵⁵.

2.2.7.4 Photocatalysis: A Unique Approach

The most innovative, successful, and promising method for treating wastewater contaminants, including persistent and resistant micropollutants, is heterogeneous semiconductor photocatalysis⁶⁴. This is due to its powerful oxidizing species, such as:

2.2.7.5 Nano-enabled Photocatalysts for Pharmaceutical Removal

Photocatalysis mineralizes organic molecules without the need for additional reagents, in contrast to comparable reactions such as the Fenton process and ozonation^{65,66}. Over the past decade, several nano-enabled photocatalysts have been employed to degrade different medications when exposed to UV and visible light. The main variables that influence photocatalytic treatment and performance include the light source, pH, photocatalyst dose or concentration, ideal degradation reaction time, and pollutant concentration⁶⁷.

2.2.7.6 Challenges in Real-world Application

Few investigations on photocatalysts utilizing actual wastewater samples have reported varying efficiency of usage, with minimum and maximum reported durations of one hour and twenty-five days, respectively. Concerningly, some studies have found deactivation of the nano photocatalyst to be an issue⁶⁸. However, the process of nanophotocatalyst

deactivation in actual water systems remains largely unknown. There is comparatively little research on the removal of pharmaceuticals from wastewater using low-cost nano-enabled photocatalysts, despite the fact that there is a sizable body of literature on the occurrence, fate, and removal of pharmaceuticals in wastewater.

2.3 Generality on nanotechnology

The study, imaging, measurement, modeling, and manipulation of matter at sizes between one and one hundred nanometers (nm) are all included in nanotechnology. It is a highly interdisciplinary area that incorporates elements from engineering, biology, chemistry, materials science, colloidal science, and applied physics. After just thirty years of research, any technology that operates on the nanoscale and has practical applications utilizing single atoms and molecules to construct functional structures is referred to as nanotechnology⁶⁹. In the discipline of nanotechnology, systems with structural characteristics ranging from single atoms or molecules to submicron dimensions are created and used, and the resulting nanostructures are then integrated into larger systems^{70,71}.

The term "technology on the nanoscale" sums up nanotechnology the simplest. However, different meanings of nanotechnology have emerged over time. Additional work is needed to refine this first description, including defining what is meant by "nanoscale." Therefore, without first defining "nanoscale," or a scale ranging from 1 to 100 nm, we are unable to describe nanotechnology accurately. "Atomically precise technology" or "atomic precision engineering" is a succinct definition of nanotechnology⁷². Nanotechnology encompasses systems and materials that, due to their nanoscale dimension, reflect innovative, greatly enhanced chemical, physical, and biological qualities, processes, and phenomena. The dictionary definition of nanotechnology is "The design, characterization, manufacture, shape and size-controlled application of matters in the nanoscale"⁷³.

"The careful and controlled manipulation, precision placement, modeling, measurement, and production of materials at the nanoscale to make matters, systems, and devices by fundamentally novel properties and functions"⁷³ is an alternative meaning taken from the

same dictionary. The study of phenomena at the nanoscale is the scope of nanotechnology, an area of knowledge that falls under the subclassification of technology in colloidal science, chemistry, physics, biology, and other scientific domains⁷⁴. Numerous economic sectors and society are already being impacted by nanotechnology, and these effects will only grow as the number of its products rises and their commercialization intensifies⁷⁵.

Many consumer goods and professional products, such as athletic equipment, cosmetics, sunscreen lotions, food and food packaging materials, apparel, household appliances, electronics, disinfectants, paints, furniture varnishes, building materials, and medications, now contain materials derived from nanotechnology. It is anticipated that nanotechnology will impact almost every sector of the economy and every aspect of our lives. It will serve as the foundation for innovations in zero-pollution transportation technology^{76,77}, drastically improved diagnostic and therapeutic technologies that could improve human health and longevity⁷⁸, and sophisticated sensors for military and environmental protection applications⁷⁹.

2.4 Nanomaterial

Nanoparticles are very small particles with a huge surface area-to-volume ratio giving them increased thermal conductivity, chemical stability, non-linear optical performance, and catalytic reactivity. Nanomaterials are particles at the nanoscale. Because of their antibacterial properties, nanoparticles (NPs) are now considered as nano antibiotics. Due to the widespread use of nanoparticles in consumer products related to health, food, feed, space, chemicals, and cosmetics, their synthesis must take environmental sustainability and sustainability into consideration⁸⁰.

A material created by manipulating and controlling individual atoms, small groups of atoms, or molecules is known as a nanomaterial. These materials, dubbed "nanotechnology," have special new features. They may appear in either macroscopic or microscopic forms. New physico-chemical characteristics, including as reactivity, resistance, biocompatibility, magnetism, and optical features, are created by their nanoscale structure⁸¹.

Nanomaterials have unique features starting at the nanoscale and can be created intentionally or naturally⁸².

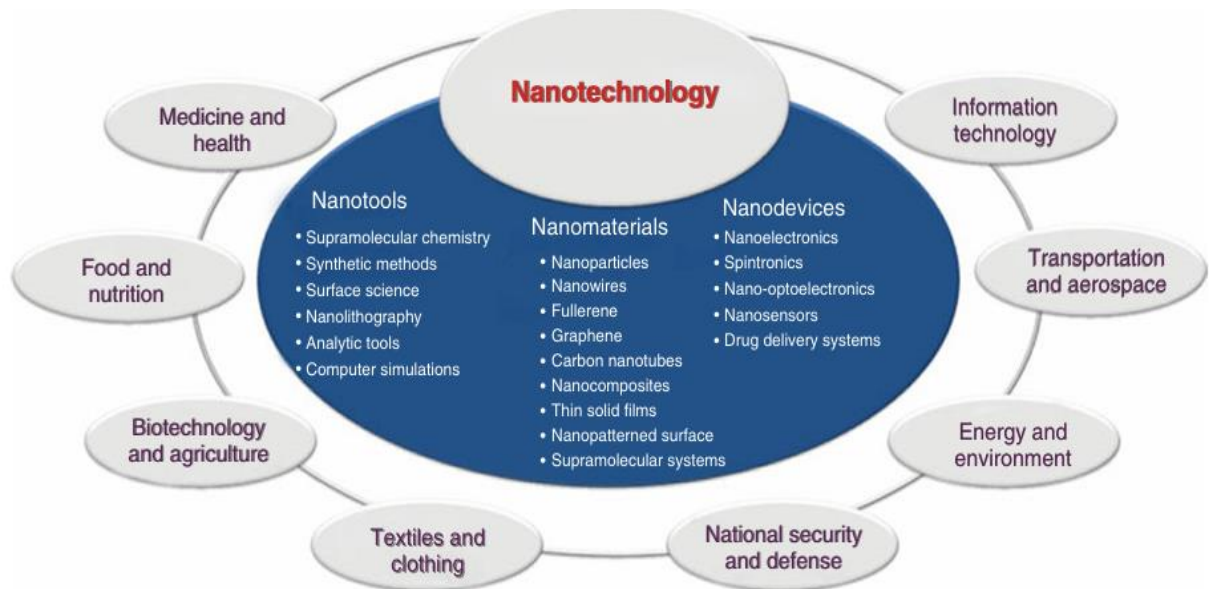


Figure 2. 3 : Schematic illustration showing how nanotechnology and its nanotools, nanomaterials, and nanodevices are impacting our world⁸³

2.5 Classification of nanomaterials

2.5.1 Classification Nanomaterials according to their dimensionality

2.5.1.1 Nanomaterials 0 d:

Materials in the form of aggregates, nanoparticles, quantum crystallites, nanocrystals, nanophases, ultrafine powders, highly dispersed media, etc.

2.5.1.2 Nanomaterials 1 d:

Materials in the form of nanotubes, nanofibers, etc.

2.5.1.3 Nanomaterials 2 d:

Thin film materials, nanostructured submicron coatings and membranes, exfoliated nano-clays.

2.5.1.4 Nanomaterials 3 d:

Materials in compact form as in nanostructured ceramics and metals, nanostructured thick coatings and membranes, nano-organized solids, nanoceramics, nanocomposites⁸⁴.

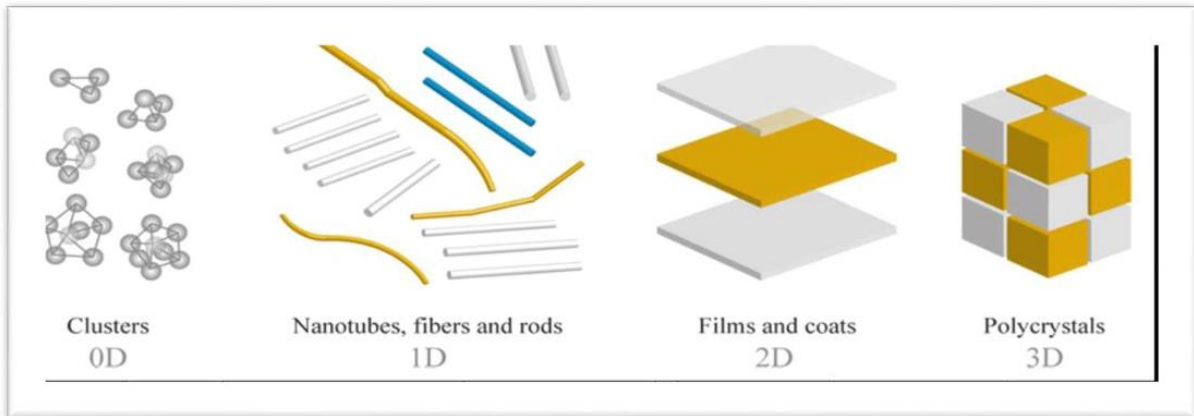


Figure 2. 4 : Types of nanomaterials by size⁸⁵

2.5.2 Classification Nanomaterials by type

There are two major families of nanomaterials⁸⁶:

2.5.2.1 Nano-objects:

are materials with one, two or three external dimensions in the nanoscale domain. Among these nano-objects, nanoparticles include their three dimensions in the nanoscale domain, nano-plates have one nanoscale dimension while nanofibers are nano-objects with two dimensions⁸⁷.

2.5.2.2 Nano-structured materials:

are materials, including aggregate, nano-composite and nanoporous that integrate nanoparticles in its structure, either superficially (surface treatment) or in its entire volume i.e.that has an internal or surface structure at the nanoscale⁸⁸.

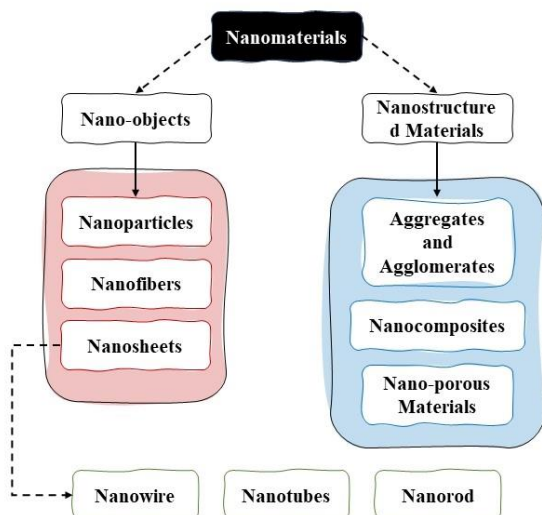


Figure 2. 5 : Summary diagram of different types of nanomaterials⁸⁹

2.6 Nanoparticles (NPs)

2.6.1 Definition

In the word nanoparticle, the term nano means 10^{-9} meters, nanoparticles are made up of no more than 10^6 atoms, and their properties are different from those of the same atoms bound together to form massive materials. They are generally considered to be a number of atoms or molecules connected with a radius of less than 100 nm⁹⁰.

Nanoparticles are a microscopic atomic or molecular assembly ranging in number from a few atoms (molecule) to a million atoms. They are connected to each other in an almost spherical manner and with a radius of 1 to 100 nanometers⁹¹.

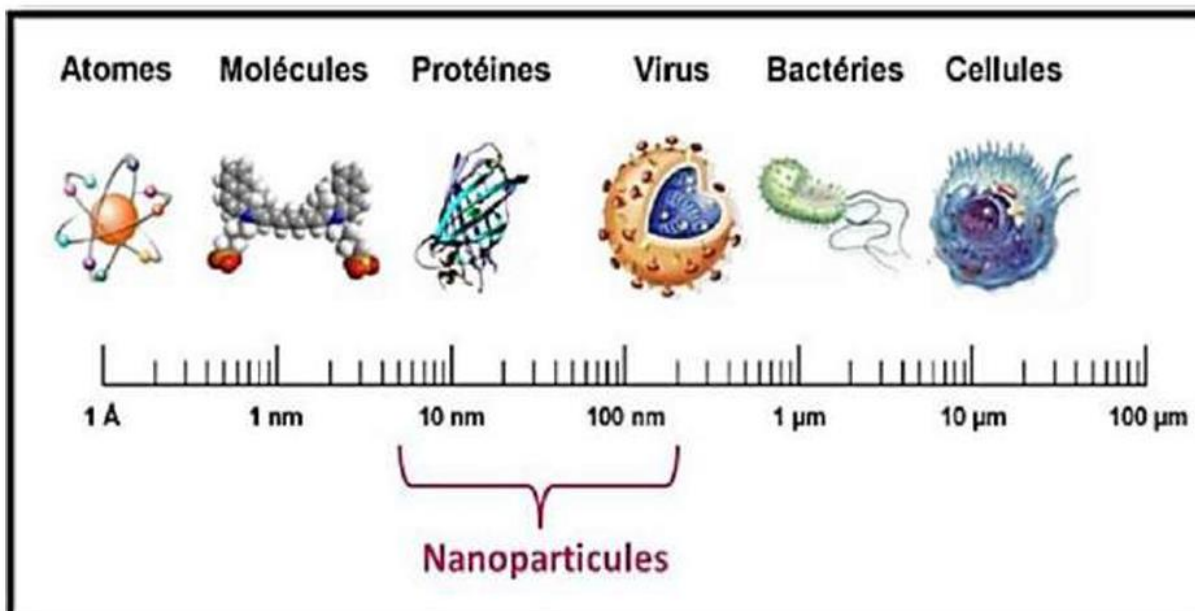


Figure 2. 6 : Size range of nanoparticles compared to those of major chemical and biological structures⁹¹.

2.6.2 Synthesis of nanoparticles

In general, there are two types of approaches in the literature (Figure 2.7) for the synthesis processes of nanostructured metal oxides including thin films, nanowires, nanoparticles⁹².

2.6.2.1 The top-down approach

This approach is based on miniaturization. It consists of fractionating a material until it holds an object of nanometric size by optimizing existing industrial technologies. This approach mainly involves the use of mechanical methods (mechanical synthesis, strong deformations, etc.) and essentially physical processes (high energy grinding, evaporation-condensation, laser ablation process, etc.)⁹³.

The top-down approach is becoming increasingly appealing. For example, the miniaturization of computer technology on silicon-based chips is quickly approaching its upper limit^{94,95}.

2.6.2.2 The bottom-up approach, or bottom up (chemical methods):

The bottom-up path, consists in assembling elementary components (atoms, molecules, aggregates) to make more complex structures, this pathway requires the use of three indispensable components A precursor, a reducing agent and a stabilizing agent. The assembly or arrangement of atoms, molecules or aggregates is carried out in a precise, controlled and exponential way, allowing the development of functional materials whose structure is completely controlled⁹⁶.

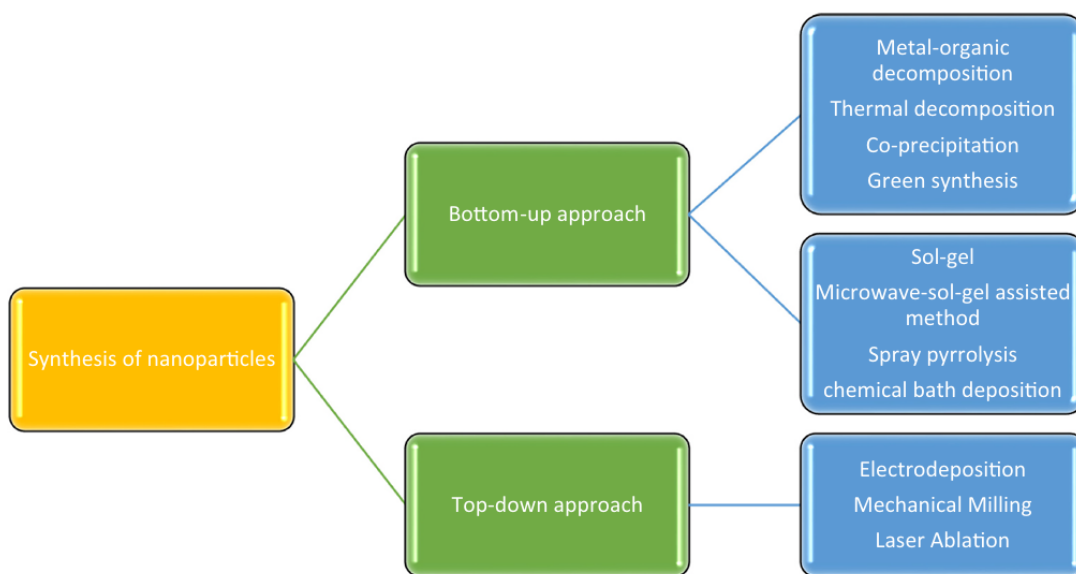


Figure 2. 7 : Synthetic routes for nanophotocatalysts⁵⁵

2.7 Zinc Oxide Nanoparticles (ZnONPs)

2.7.1 Definition

Zinc oxide nanoparticles, or ZnONPs, are widely employed in a variety of applications, including photocatalysts, solar cells, gas sensors, and cosmetics⁹⁷. ZnONPs represent an important semiconductor with distinctive optical and electrical characteristics^{98,99}. ZnONPs have been extensively studied for biomedical applications due to their low cost and good biocompatibility. The results of these experiments have shown that ZnONPs have antibacterial, antioxidant, and anti-inflammatory properties, as well as anticancer activity against a variety of cancer cells^{100–103}.

Due to its excellent band gap properties, zinc oxide (ZnO) has been recognized by the US Food and Drug Administration (US FDA) as one of the best semiconductors. Because of its

UV filtering properties, ZnO may be used in cosmetic products such as sunscreen lotions. Additionally, ZnO exhibits anti-cancer, anti-diabetic, antibacterial, and antifungal properties. Furthermore, zinc oxide (ZnO) is utilized for specific drug delivery despite its unresolved cytotoxicity restriction. Furthermore, ZnO is used in the production of rubber, paint, water purification, protein adsorption, and dentistry applications. ZnONPs possess both pyroelectric and piezoelectric qualities. They are employed in the removal of aquatic weeds that defies all known eradication methods, including mechanical, chemical, and physical ones.

Various morphologies of ZnONPs, including nano-flake, nano-flower, nano-belt, nano-rod, and nano-wire, have been described.

2.7.2 Green synthesis of ZnONPs

Zinc oxide nanoparticles (ZnONPs) are among the metal oxide nanoparticles that are very beneficial to humans. ZnONPs are employed in various fields, such as antibacterial agents, materials for solar cells, substances for cosmetics, and gas adsorbents for CO₂, NH₃, NO_x, and other gases¹⁰⁴. The advancement of ZnO NPs nowadays extends beyond its use; it also involves the synthesis of the nanoparticles to yield the required characteristics. There are several ways to synthesize ZnONPs, and one of them is chemical. Regrettably, this approach has drawbacks regarding the environmental effect of waste from the synthesis process that uses hazardous chemicals (such as PEG polymers, PVC, and anionic surfactants) as capping agents.

Furthermore, using chemical procedures necessitates high temperatures, a lengthy process, and hefty costs¹⁰⁵. The green approach is one of the eco-friendly alternatives to synthesize ZnONPs nanoparticles. In this procedure, natural materials such as plant extracts, algae, or mushrooms are used in place of chemicals as reducing or capping agents. Numerous studies have conducted green synthesis of ZnONPs utilizing various sources including *Bauhinia tomentosa* leaves¹⁰⁶, *Citrus sinensis* leaves¹⁰⁷, cavendish banana peels¹⁰⁸, *Sargassum* sp. leaves¹⁰⁹, and garlic peels¹¹⁰. However, banana peel waste also contains phytochemicals such flavonoids, phenols, and aldehydes^{111,112}, which have been shown to function as metal capping and reducing agents. Every day, fruit markets, the food industry, and households worldwide generate

tons of garbage from banana peels. Numerous researches have attempted to use this abundant biomass of banana peels for various purposes, including the production of a mycological medium¹¹³, bioenergy¹¹⁴, plant composition¹¹⁵, and pectin¹¹⁶. Furthermore, it has been reported that banana peel hot water extract may be utilized to create silver nanoparticles¹⁰⁵, while banana peel ethanol extract can be used as an anticorrosion additive to create iron NPs¹¹⁷. Other agricultural wastes were also utilized for green synthesis of nanoparticles, in addition to banana peel extract. For instance, green ZnO thin film was created using extract from the peel of *Citrus reticulata*¹¹⁸ [52]. Additionally, ZnONPs with antibacterial activity were synthesized using the fruit shell extract of *Aesculus hippocastanum*, commonly known as *horse chestnut*,¹¹⁹. Similarly, ZnONPs derived from the aqueous extract of *Punica granatum* shell were found to inhibit *Bacillus thuringiensis*, a Gram-positive bacterium¹²⁰.

3. MATERIAL AND METHODS

3.1 Chemicals and instrumentation

Banana peels were taken from the local market in Sidi Bel Abbes. The Pure Antibiotics and Chemicals used for the present study, including Cefotaxime Sodium, Vancomycin Hydrochloride, Ciprofloxacin, Zinc Acetate Dihydrate, Sodium Hydroxide, Folin-Ciocalteu reagent, Sodium Carbonate, Sodium Nitrite, Aluminum Trichloride, Sodium Acetate, Hydrochloric Acid, Vanillin, and Ferric Chloride were obtained from PROCHIMA-SIGMA. Double-distilled water and 96% ethanol were used for the experiments. All glassware was washed adequately with distilled water and air-dried.

Various techniques were used to characterize the prepared nanoparticle. General morphological features such as size, shape, and distribution were examined by Field Emission Scanning Electron Microscopy (FESEM), Energy Dispersive X-ray Spectroscopy (EDX) (Zeiss Sigma 300) and Transmission Electron Microscope (TEM) (JEOL JEM 1400 plus). The particle size was estimated by zeta potential in liquid suspension (Zetasizer Nano Range, Malvern Instruments) through the Dynamic Light Scattering method (DLS) at 173°. The nanoparticle structure was confirmed by X-Ray Diffraction (XRD) using a Bruker D8 instrument. Fourier Transform Infrared (FTIR) spectroscopy was employed to examine the chemical composition and purity of the prepared nanoparticle using an Agilent 650 spectrophotometer.

3.2 Preparation of the aqueous extract of banana peels

To prepare the extract from the dried banana peels, 10 g of peels were ground into a fine powder using a porcelain mortar. Then, the powder was mixed with 200 ml of distilled water. The mixture was heated to 70°C for 30 minutes to facilitate the extraction of the polyphenolic compounds from the peels. Subsequently, the solution was allowed to cool and stand. Finally, it was filtered using filter paper to obtain the desired extract. This method guarantees the extraction of high-quality extract from dried banana peels. This extract has been used as a precursor for the biosynthesis of Zinc Oxide Nanoparticles

(ZnONPs). For the dosage of phenolic compounds, 10 mL of this extract was taken (Extract 1).

3.3 Biosynthesis of ZnONPs

To synthesize ZnONPs, 80 mL of zinc acetate dihydrate solution ($\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}$) with a concentration of 0.01 M was combined with 20 mL of extract, followed by the addition of 1 N sodium hydroxide (NaOH) solution to adjust the pH to 10. The formation of zinc oxide was indicated by the appearance of a white precipitate (Figure 3.1). The resulting mixture was then incubated for 24 hours at room temperature while being continuously stirred. After incubation, the mixture underwent centrifugation at 4000 rpm for 10 minutes to isolate the precipitate, which was subsequently thoroughly washed with distilled water and 96% ethanol to eliminate impurities. From the remaining solution after centrifugation, 10 mL was taken for the dosage of phenolic compounds (Extract 2). The ZnONPs were further subjected to calcination in a tubular oven at 400°C for 2 hours. Finally, the synthesized ZnONPs were stored in an airtight container for future experimental applications. The synthesis steps are summarized in Figure 3.2.

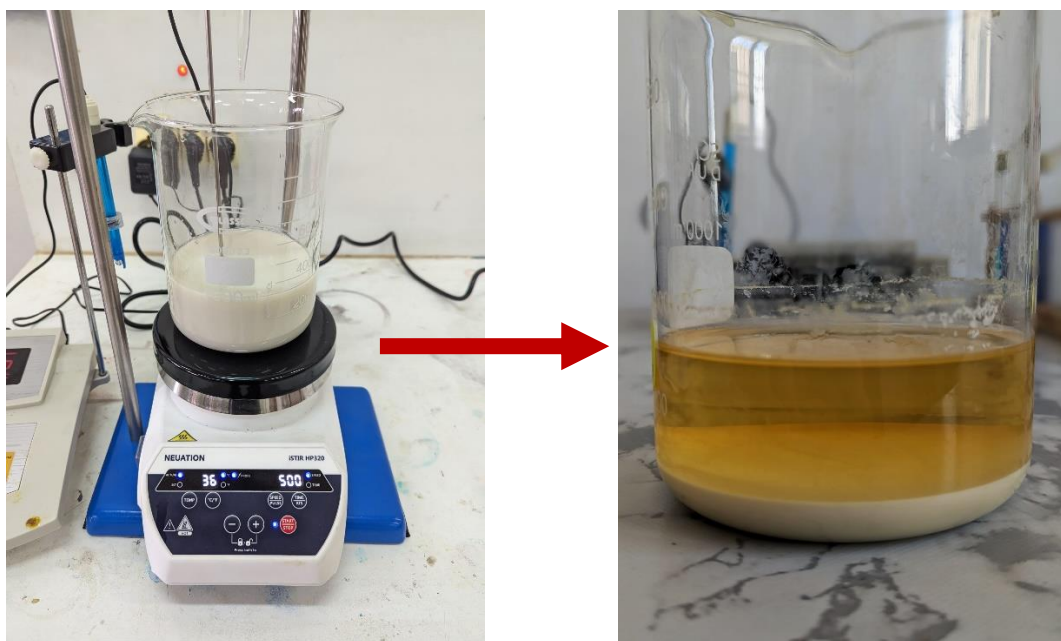


Figure 3. 1 : Formation of White Precipitates (ZnONPs)

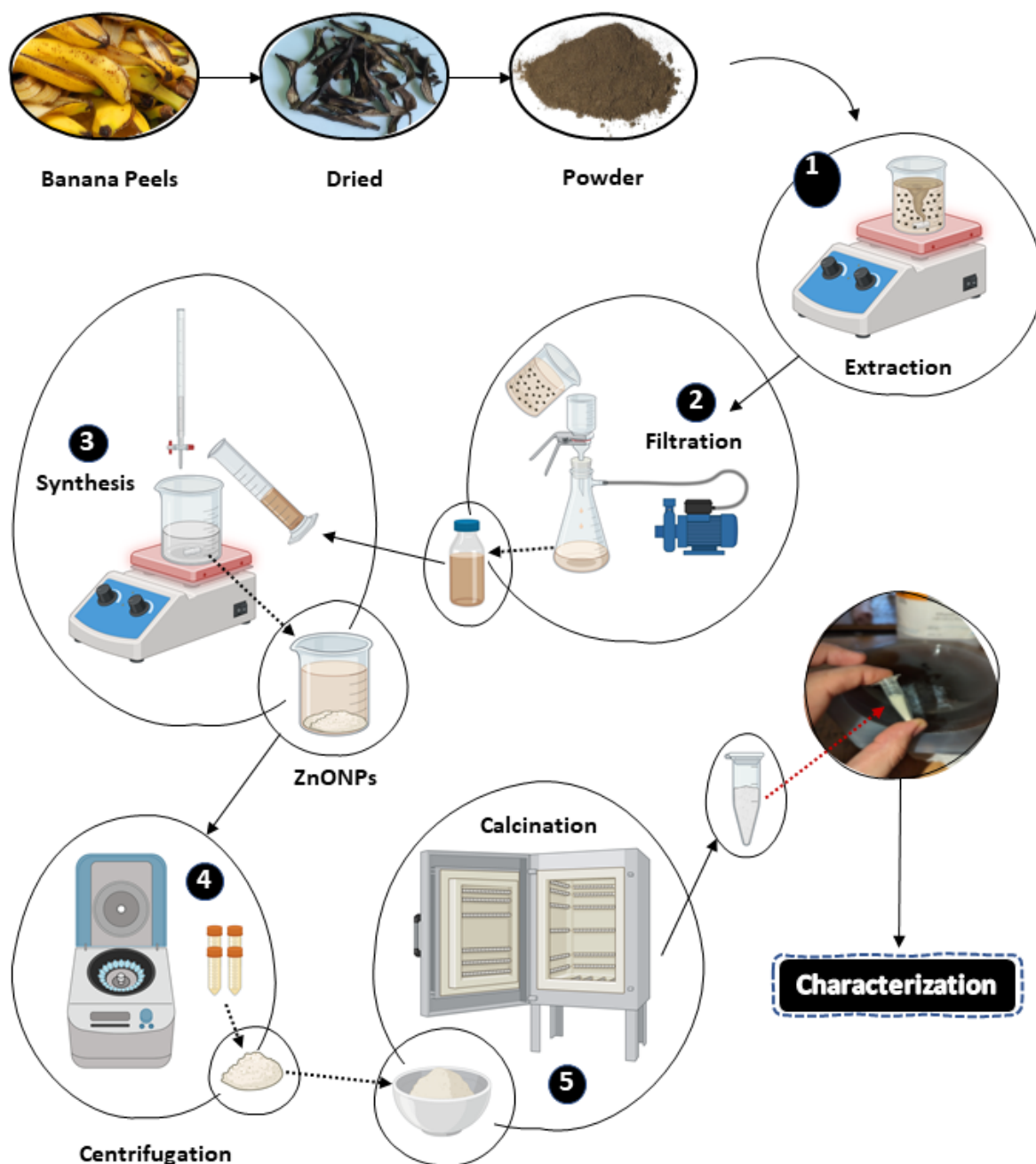


Figure 3. 2 : Schematic illustration of ZnO nanoparticles (ZnONPs) biosynthesis.

3.4 Dosage of phenolic compounds

Phenolic compounds of both extracts (extracts of banana peels before and after the biosynthesis of ZnONPs) can be quantified using various methods. One common approach is through the measurement of polyphenols using UV-Visible spectrophotometry.

3.4.1 Total phenol dosages

Polyphenols are secondary metabolites naturally found in plants, distinguished by the presence of more than one phenol group. Phenolic compounds or polyphenols are characterized by the presence of an aromatic ring carrying one or more hydroxyl groups. The quantification of total phenols using Folin-Ciocalteu reagent was described as early as 1965 by Singleton and Rossi¹²¹.

200 μl of Extract 1 and 200 μl of Extract 2 were each placed into separate test tubes. To each test tube, a mixture of 1000 μl of Folin Ciocalteu reagent (diluted 10 times) and 800 μl of sodium carbonate Na_2CO_3 (7.5 %) was added (Figure 3.3). After this, the mixtures were incubated for 30 minutes at room temperature in the dark. The observed coloration is proportional to the amount of phenolic acids present in the sample. The absorption is measured at 765 nm against a blank using a Jenway 7205 UV/VIS spectrophotometer. A calibration curve is performed in simultaneously under the same operating conditions using gallic acid as positive control. The results are expressed in milligrams (mg) of equivalent Gallic Acid per gram of extract (mg eq GA/g).

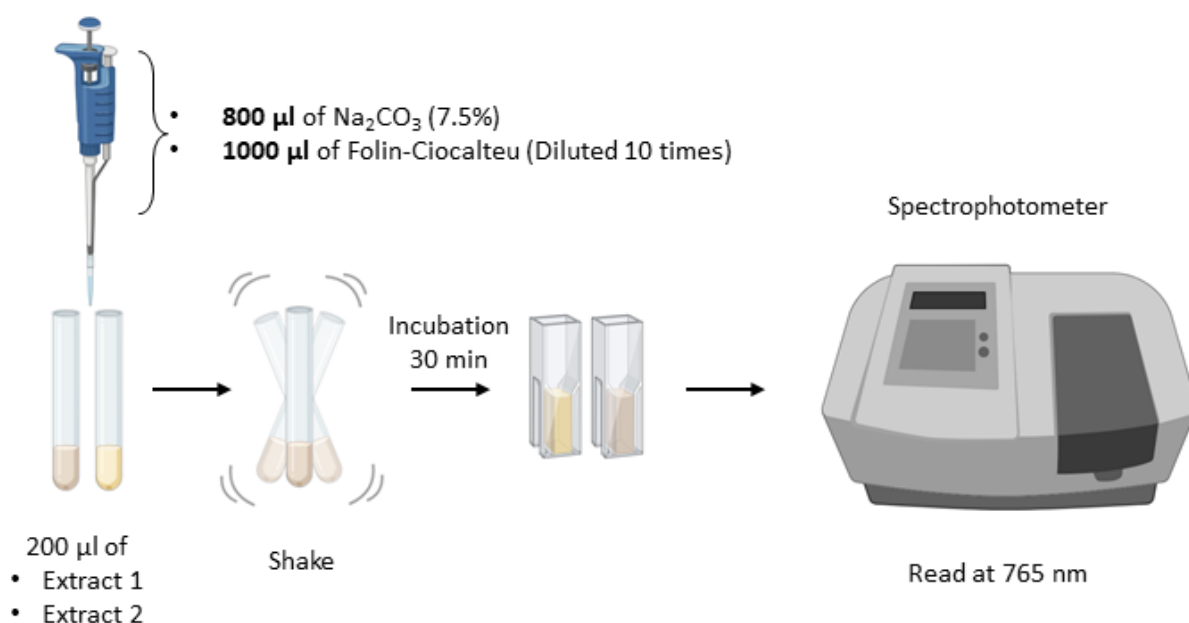


Figure 3. 3: Schematic illustration of total phenol dosage

3.4.2 Flavonoid dosage

To measure the flavonoid content in the extract, the aluminum trichloride and sodium hydroxide method described by Zhishen *et al.* ¹²² is used. Aluminum trichloride forms a yellow complex with the flavonoids, and sodium hydroxide forms a pink complex that absorbs into the visible light at 510 nm^{123,124}.

Initially, 500 μ l of extracts are mixed with 1500 μ l distilled water, followed by the addition of 150 μ l of sodium nitrite (NaNO_2) at a concentration of 5 %. After 5 min, 150 μ l of aluminum trichloride (AlCl_3) at 10 % is added to the mixture. After 6 minutes of incubation at room temperature, 500 μ l of sodium hydroxide (NaOH) at 4% is added (Figure 3.4). Immediately, the mixture is completely shaken to homogenize the contents. The absorption of the resulting pink-colored solution is determined at 510 nm against a blank. An evaluation curve is performed in parallel under the same operating conditions using the catechin as a positive control. The total flavonoid content of the extracts is expressed in milligrams (mg) of equivalent Catechin per gram of extract (mg eq C/g).

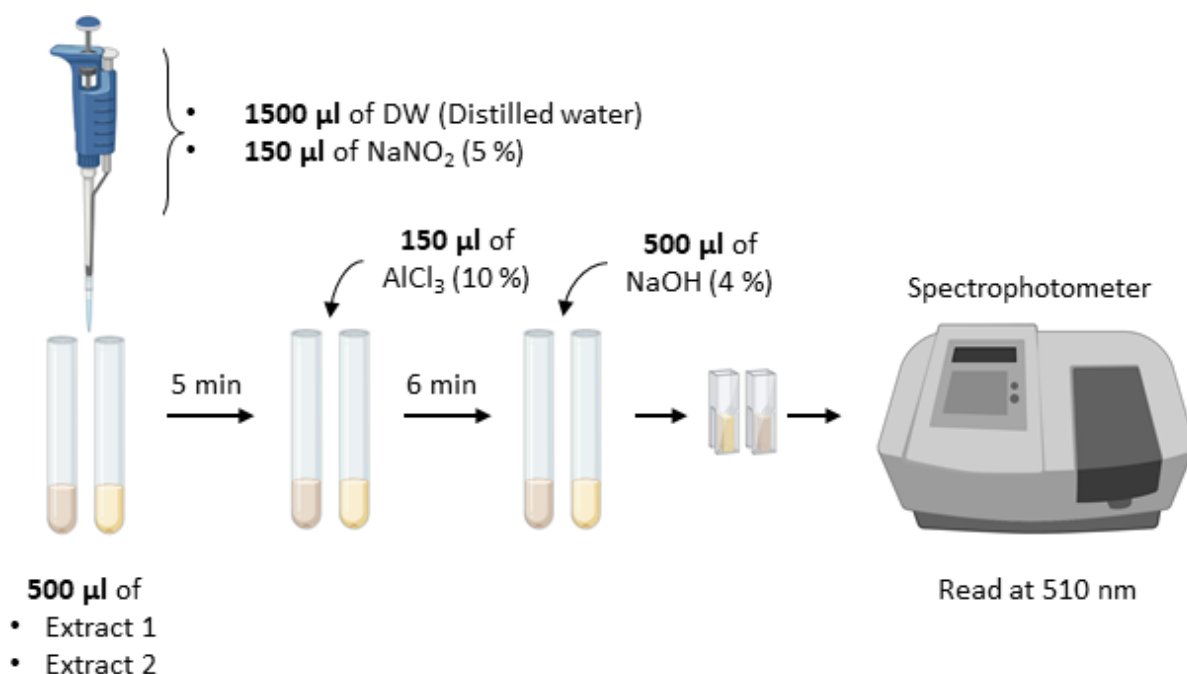


Figure 3. 4: Schematic illustration of flavonoid dosage

3.4.3 Dosage of flavonols

The flavonol-containing sample gives a green color when reacted with aluminum chloride and sodium acetate, and the samples absorbances are measured at 440 nm using a UV-Vis spectrophotometer¹²⁵.

For the analysis, 2 ml of each extract is mixed with 2 ml aluminum trichloride (2%) and 6 ml sodium acetate (5%) (Figure 3.5). The mixture was then incubated for 2h and 30 min at 20 °C before reading absorbance at 440 nm¹²⁶

A calibration curve was performed in parallel under the same operating conditions using myricetin as a positive control. The content of flavonols is expressed in milligrams of equivalent Myricetin per gram of extract (mg eq M/g).

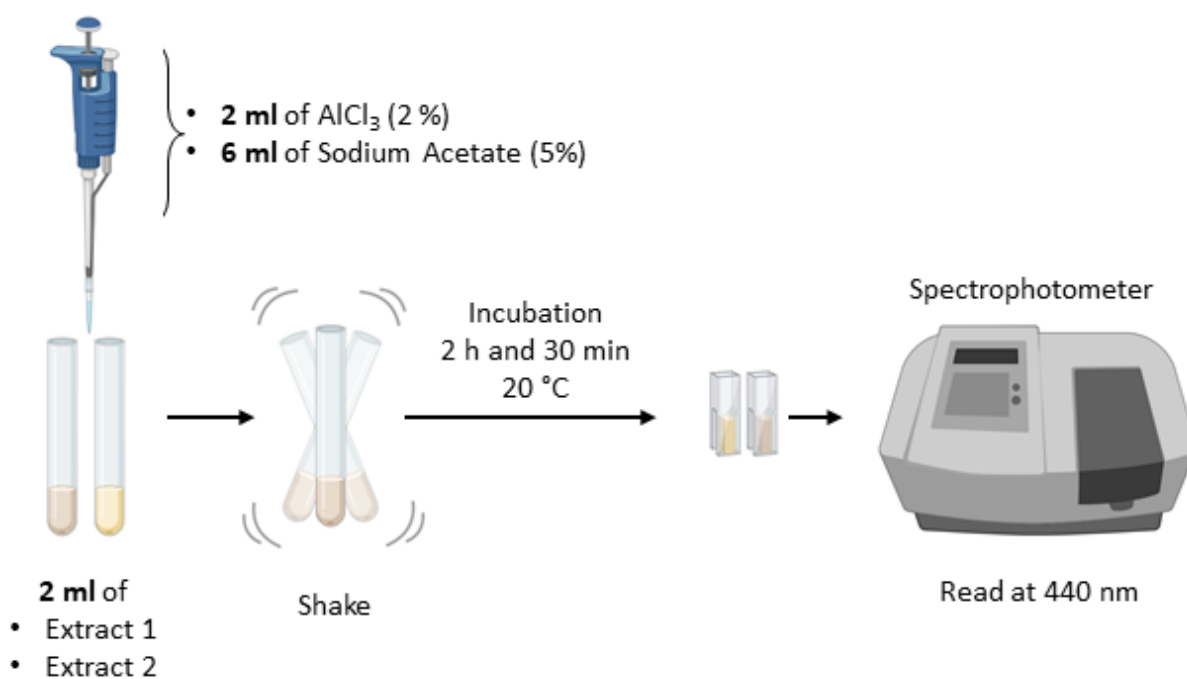


Figure 3. 5: Schematic illustration of flavonols dosage

3.4.4 Dosage of condensed tannins

The principle of this dosage depends on the reaction of vanillin with units of condensed tannins in an acidic medium (HCl). The absorption is measured at 550 nm against a blank. The reactivity of vanillin with tannins implies only the first unit of the polymer¹²⁷.

To perform the assay, 50µl extract is mixed with 1500 µl of a vanillin solution (4% in methanol), and 750µl concentrated hydrochloric acid. After incubation for 20 minutes,

the absorption was measured at 550nm (Figure 3.6). Concentrations of condensed tannins are determined from the calibration curve established using catechin as standard and are expressed in milligrams (mg) of equivalent Catechin per gram of extract (mg eq C/g).

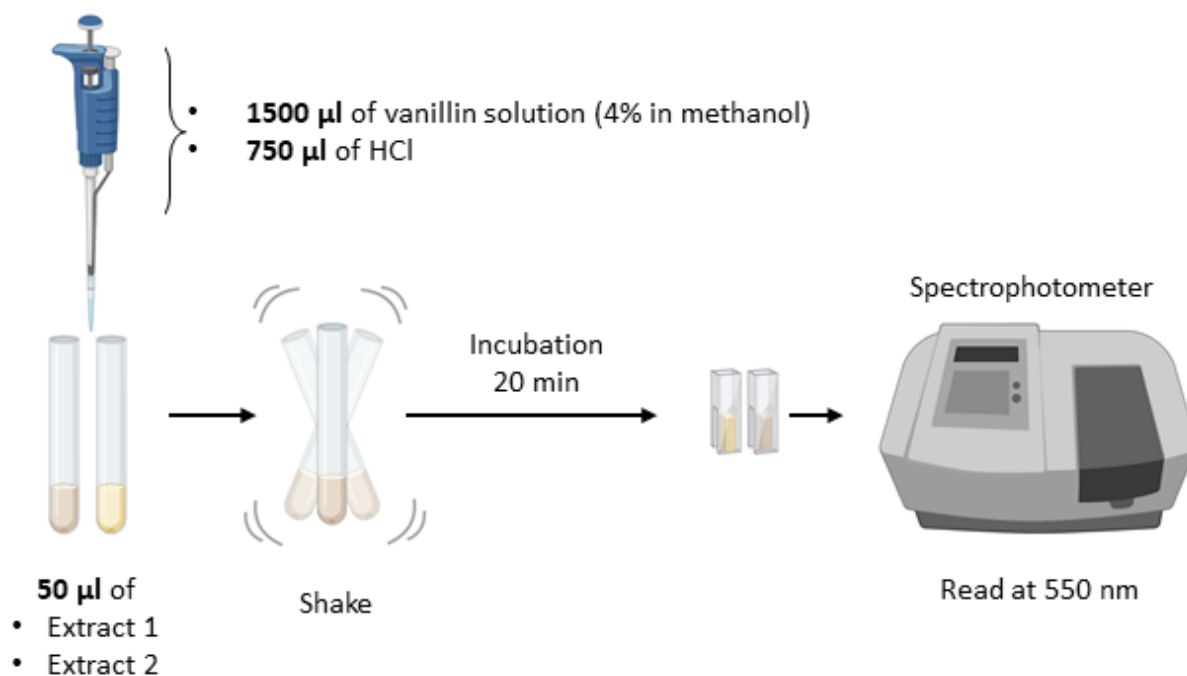


Figure 3. 6: Schematic illustration of condensed tannins dosage

3.4.5 Dosage of hydrolysable tannins

Hydrolysable tannins are composed of simple phenolic molecules. As their name suggests, they are easily hydrolysable by the acids and enzymes resulting in the formation of pyrogallol. The dosage of hydrolysable tannins is based on their reaction with ferric chlorides, producing brown-colored complexes¹²⁸.

Initially, 500 µl of each studied extract is mixed with 3.5 ml of a ferric chloride solution (0.01 M of FeCl₃, in 0.001 M of HCl). The absorption of the mixtures was measured immediately at 660nm (Figure 3.7)¹²⁹. The concentrations of hydrolysable tannins are deduced from a calibration curve established using tannic acid as standard and are expressed in milligrams of equivalent Tannic Acid per gram of extract (mg eq TA/g).

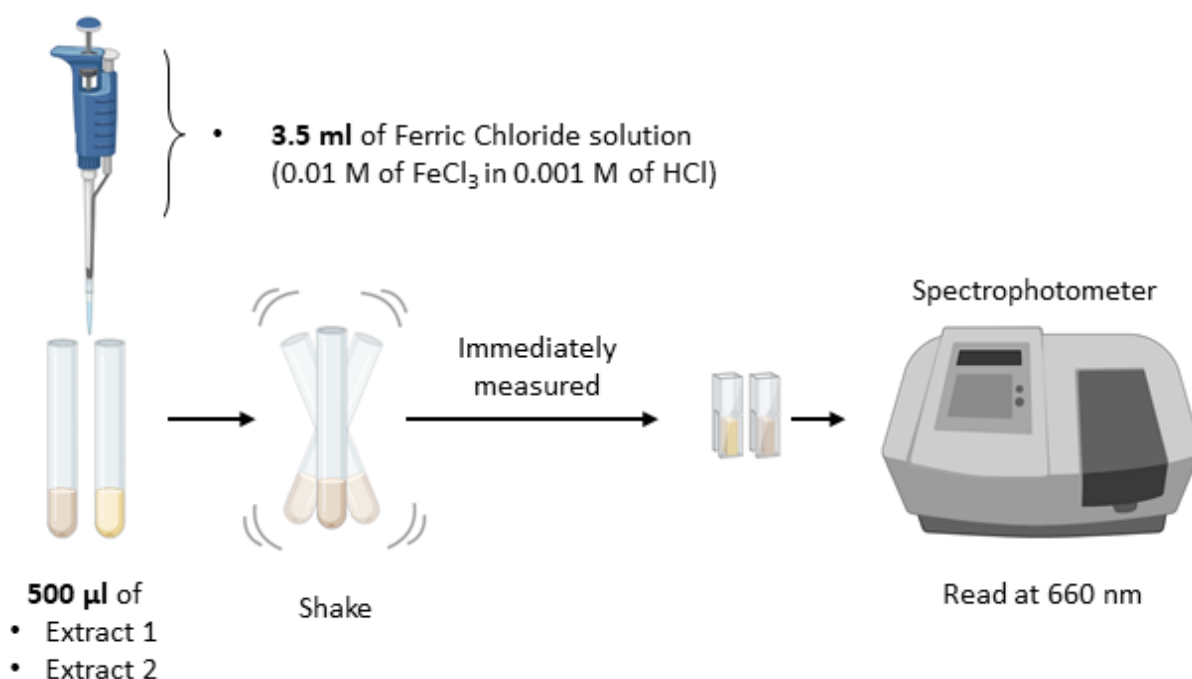


Figure 3. 7: Schematic illustration of hydrolysable tannins dosage

3.5 RSM optimization study

In order to scrutinize the significant parameters influencing the synthesis of ZnONPs, Response Surface Methodology (RSM) was applied in the present study using DesignExpert® 13.0. Mathematical modeling was applied using the RSM technique via Box–Behnken design, with the independent predicted variable, i.e., peel extract, precursor concentration, and nanoparticle production conditions¹³⁰. It is well established that statistically designed experiments with several different factors are more effective when more than one factors are investigated together¹³¹.

3.6 Experimental design

Response Surface Methodology (RMS) was employed to analyze the interplay of selected operating parameters via the Box-Behnken design (BBD) in Design-Expert® software. The study investigated how three variable factors: antibiotic concentration (X1), ZnONPs mass (X2), and degradation time (X3) affect the degradation efficiency of CFM (Y1), CIP (Y2), and VAN (Y3).

Ranges of the variables were determined based on previous experiments. The values are presented in Table 3.1:

Coded factors	Factors	Unit	Coded levels		
			-1	0	1
X1	Antibiotic Concentration	mg/L	10	20	30
X2	ZnONP Catalyst	g/L	0.2	0.5	0.8
X3	Irradiation time	min	30	60	90

Table 3. 1: Experimental range and levels of variables

The number of tests (n) required to generate BoxBehnken Design is specified as follows:

$$n = 2f(f - 1) + Cp \quad (1)$$

f and Cp denote the number of factors and the number of central points, respectively.

Experiments were carried out randomly to reduce systematic error; three center replicates were inserted to evaluate the pure experimental error and improve the model's design. A total of 15 experiments were performed, and the obtained and predicted responses achieved are shown in Table 4.1. The relationships between the responses, the independent variables and the prediction of optimal coagulation-flocculation process conditions were established using an empirical second-order polynomial model (equation 2).

$$Y = a_0 + \sum_{i=1}^k a_i X_i + \sum_{i=1}^k a_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^k a_{ij} X_i X_j + e \quad (2)$$

Design-Expert software was used to design the experiment, estimate the coefficients, analyze data, and plot graphs.

3.7 Antibiotics solutions preparation

Stock solutions of Cefotaxime, Ciprofloxacin, and Vancomycin (100 mg/L) were prepared. From each stock solution, daughter solutions of 10, 20, and 30 mg/L were prepared and utilized for the 15 trials.

The specific amount of synthesized ZnONPs and the 100 mL of each assay of the antibiotic solutions (10, 20, and 30 mg/L) were mixed in beakers and placed in a dark room under stirring. The UV light source utilized in the process was a PL-L 36W/10/4P lamp. This lamp emits UV-A radiation with wavelengths ranging from 350 to 400 nm. Moreover, the lamp has a very low UV-B/UV-A ratio, less than 0.1%, ensuring minimal UV-B radiation (280 - 315 nm) output (Figure 3.8 and 3.9). The conditions such as initial concentration (10–30 mg/L), and ZnONPs dose (0.2, 0.5, and 0.8 g) were maintained throughout the experiment. After specified time intervals (30, 60, 90 min), 5 ml of samples were taken for centrifugation at 4000 rpm for 10 min, then the absorption specter of the solution was recorded using a UV-Vis Spectrophotometer (Agilent - Cary 60). The degradation rate was observed in terms of the change in intensity at λ_{\max} of the CFM, CIP and VAN solutions. The degradation efficiency (%) was calculated using the standard formula proposed by Sen and Mondal, (2019).¹³⁰.

$$\text{Degradation Efficiency (\%)} = (1-A/A_0) * 100 \quad (3)$$

Where A and A₀ are, respectively, the λ_{\max} intensity of the solution after the photodegradation and the initial λ_{\max} intensity

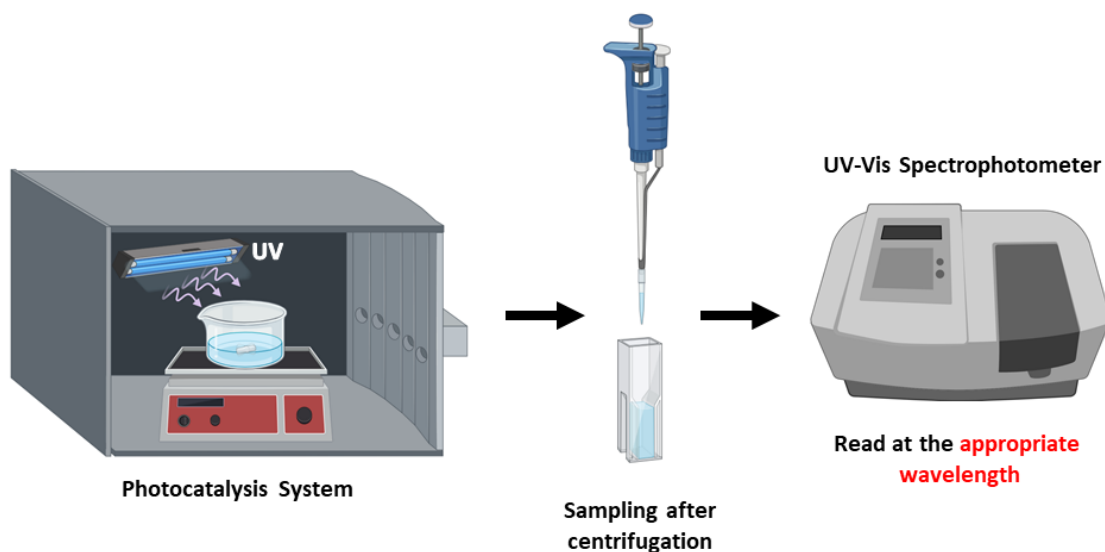


Figure 3. 8: Schematic illustration of Photocatalysis Process

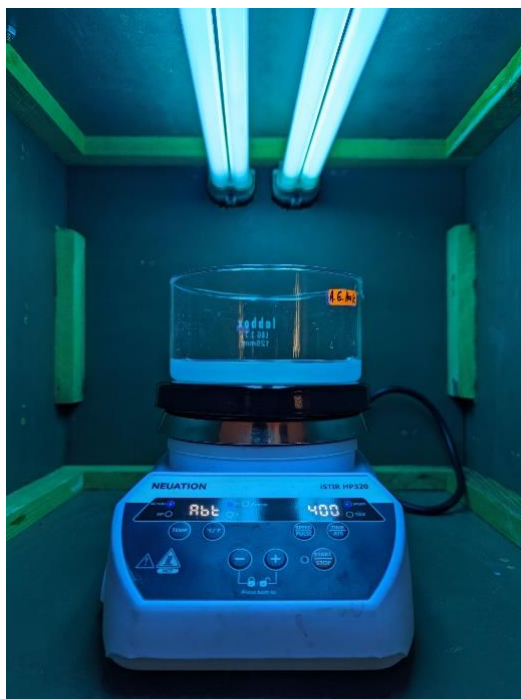


Figure 3. 9 : Photocatalysis Chamber

4. RESULTS AND DISCUSSION

4.1 Characterization of the banana peel extract

The quantitative study of crude extracts by spectrophotometry aims to determine the content of phenolic compounds. These compounds were chosen principally due to their role in the green synthesis of nanoparticles¹³². Total phenols are quantified by the Folin-Ciocalteu reagent method, flavonoids are determined using by aluminum trichloride and sodium hydroxide, flavonol by employing aluminum chloride and sodium acetate, condensed tannins by utilizing vanillin under acidic conditions, and hydrolysable tannins by ferric chloride.

Calibration curves are performed under the same operating conditions, using phenolic standards (see appendix). The results are calculated graphically from the equations of the linear regression lines of the calibration curves, and expressed in milligrams of equivalents gallic acid per gram of extract (mg GA eq/g) for total phenols, in milligram equivalents of myricetin per gram of extract (mg M eq/g) for flavonol, in milligram equivalents of tannic acid per gram of extract (mg TA eq/g) for hydrolysable tannins and in milligram equivalents of catechin per gram of extract (mg C eq/g) for flavonoids and condensed tannins.

The results of phenolic compounds analysis in the banana peel extract before and after the biosynthesis of ZnO nanoparticles are presented in the Figure 4.1.

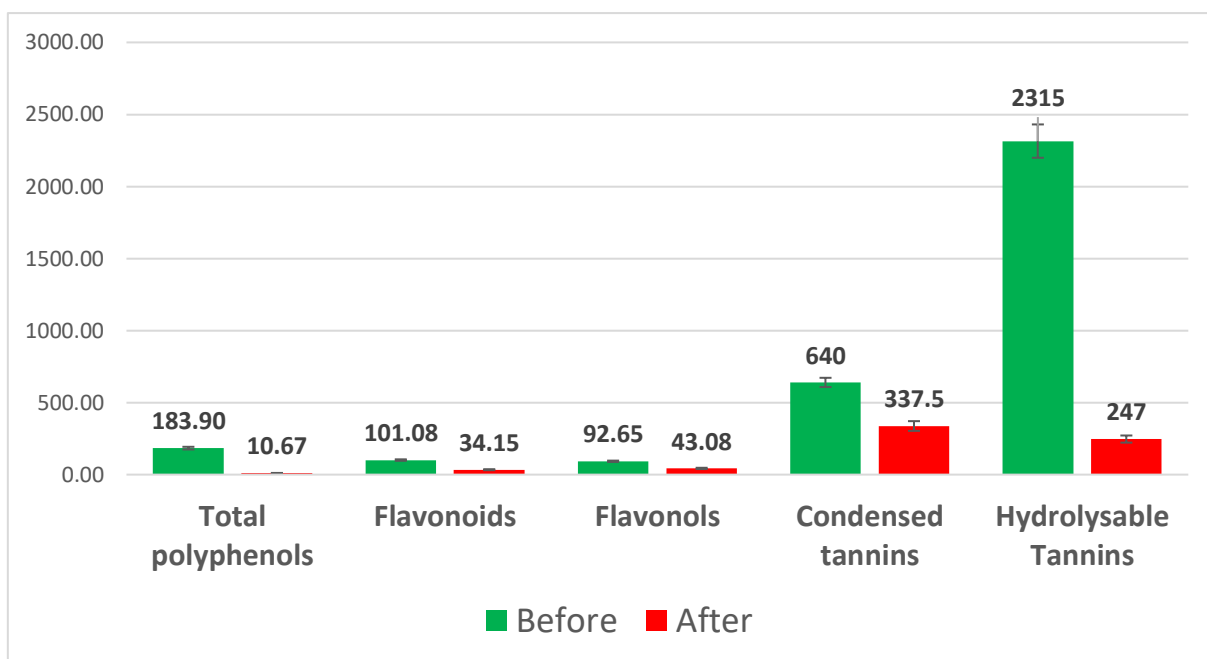


Figure 4. 1 : Phenolic compounds content in the banana peel extract

The analysis reveals significant contents of hydrolysable and condensed tannin content in our extract, with values of 2315 mg TA eq/g and 640 mg C eq/g respectively. Total polyphenols content is approximately 184 mg GA eq/g, while flavonoids and flavanols are comparatively lower, with values of 101,08 mg C eq/g and 92,65 mg M eq/g respectively. These results highlight the abundance of polyphenolic compounds in banana peel extract, which has great potential for nanoparticle synthesis.

Previous research confirms the high polyphenol content in banana peels, as shown in the study conducted by Omar Hisham *et al.* which reported that the total phenolic contents ranged from 235 to 240 GA eq/g in banana peels¹³³. Another study indicated that the flavonoid content in the banana peels extract is 196 mg C eq/g¹³⁴. Furthermore, other studies have shown that the flavonol contents in banana peels ranges from 0,31 to 973 mg M eq/g¹³⁵. Finally, the content of condensed tannins ranged from 0,12 to 3,34 mg C eq/g in the study of Yasmeeen *et al.* while the study of Youssef *et al.* reported the content of hydrolysable tannins as 22 mg TA eq/g^{136,137}.

The differences between our results and those reported in the literature can be attributed to various factors influencing the extraction of phenolic compounds. These factors include the extraction method used, the choice of solvent, the extraction time, the temperature and the plant state (fresh or dried), etc.^{138,139}.

After the synthesis of nanoparticles, there is a clear decrease in the content of all polyphenols, as shown in Figure 4.1 and Table 4.1.

Table 4. 1 : Polyphenol compounds percentage in the banana peel extract before and after synthesis of nanoparticles

	Before (%)	After (%)
	Reacted quantity	Remaining quantity
Total polyphenols	95	5
Flavonoids	75	25
Flavanols	68	32
Condensed tannins	65	35
Hydrolysable tannins	90	10

This reduction ranges from 65% to 95% depending on the compound confirming that these molecules are directly involved in the biosynthesis of ZnO nanoparticles. Many studies have reported the use of banana peel, along with other waste materials such as date seed, orange peel etc., as both reducing and capping agents in the reaction with zinc acetate^{140–143}.

4.2 Statistical analysis

Table 4. 2 : Experimental Setup for factors and Responses

N°	Factors			Responses					
	X1: Concentration of Antibiotic (mg/L)	X2: Catalyst ZnONPs Dose (g/L)	X3: Irradiation Time (min)	Y1: CFM Degradation (%)		Y2: CIP Degradation (%)		Y3: VAN Degradation (%)	
1	10	0.8	60	27.73	27.2	51.2	49.12	38.06	37.36
2	20	0.5	60	23.59	23.95	23.06	24.36	36.28	35.81

3	20	0.2	30	28.31	28.53	13.49	11.18	36	35.72
4	20	0.2	90	28.15	27.41	19.13	20.15	36.23	36.83
5	20	0.5	60	24.6	23.95	25.86	24.36	35.94	35.81
6	10	0.2	60	26.34	26.33	37.17	36.38	31.83	30.81
7	20	0.8	90	40.18	39.96	34.89	37.2	35.98	36.26
8	20	0.5	60	23.65	23.95	24.16	24.36	35.22	35.81
9	30	0.2	60	24.88	25.42	16.01	18.1	51.37	52.07
10	20	0.8	30	11.02	11.76	24.27	23.25	43.36	42.76
11	10	0.5	90	31.63	32.38	48.11	47.88	28.03	28.45
12	30	0.5	30	15.71	14.96	19.74	19.97	49.51	49.09
13	30	0.8	60	20.32	20.33	33.7	34.49	35.98	36.26
14	10	0.5	30	20.64	20.43	60.3	63.4	29.32	30.62
15	30	0.5	90	29.88	30.09	61.51	58.41	47.15	45.86

Analysis of variance (ANOVA) was employed to investigate the interplay among various factors, potential interactions, and their influence on the responses. It facilitated the validation and assessment of the developed model's significance. ANOVA results indicated the considerable significance of the quadratic model for all three variables Y1, Y2, and Y3. Summary statistics of the models are gathered in Table 4.3 for easy reference.

Furthermore, the model F-values exceeding 99.64, 41.44 and 54.66, alongside the model p-values below 0.05, underscore the robustness and significance of the conducted analysis. Tables 4.3, 4.4 and 4.5 provide a comprehensive overview of the factors significantly influencing the Removal efficiency of CFM, CIP, and VAN, respectively. Notably, for CFM (Y1), CIP (Y2) and VAN (Y3) removal, terms such as (X1), (X2), (X3), (X1X2), (X1X3), (X2X3), (X1²), (X2²), and (X3²) exhibit significant impacts, as evidenced by their p-values being less than 0.05.

Equations 3, 4, and 5 represent the quadratic equations employed to elucidate the removal processes of antibiotics CFM (Y1), CIP (Y2), and VAN (Y3), respectively. These

equations underwent adjustments by eliminating statistically insignificant terms. Subsequently, the resulting mathematical expressions were used to assess the goodness of fit.

$$\begin{aligned} \text{Degradation of CFM} = & 23.9467 - 1.94375 * \text{Concentration of Antibiotic} - 1.05375 * \\ & \text{Catalyst ZnONPs Dose} + 6.77 * \text{Irradiation Time} - 1.4875 * \text{Concentration of Antibiotic} * \\ & \text{Catalyst ZnONPs Dose} + 0.795 * \text{Concentration of Antibiotic} * \text{Irradiation Time} + 7.33 * \\ & \text{Catalyst ZnONPs Dose} * \text{Irradiation Time} - 0.789583 * (\text{Concentration of Antibiotic})^2 + \\ & 1.66042 * (\text{Catalyst ZnONPs Dose})^2 + 1.30792 * (\text{Irradiation Time})^2 \quad \mathbf{(3)} \end{aligned}$$

$$\begin{aligned} \text{Degradation of CIP} = & 24.36 - 8.2275 * \text{Concentration of Antibiotic} + 7.2825 * \text{Catalyst} \\ & \text{ZnONPs Dose} + 5.73 * \text{Irradiation Time} + 0.915 * \text{Concentration of Antibiotic} * \text{Catalyst} \\ & \text{ZnONPs Dose} + 13.49 * \text{Concentration of Antibiotic} * \text{Irradiation Time} + 1.245 * \text{Catalyst} \\ & \text{ZnONPs Dose} * \text{Irradiation Time} + 17.315 * (\text{Concentration of Antibiotic})^2 - 7.155 * \\ & (\text{Catalyst ZnONPs Dose})^2 + 5.74 * (\text{Irradiation Time})^2 \quad \mathbf{(4)} \end{aligned}$$

$$\begin{aligned} \text{Degradation of VAN} = & 35.8133 + 8.97 * \text{Concentration of Antibiotic} + 1.6175 * \text{Catalyst} \\ & \text{ZnONPs Dose} - 1.35 * \text{Irradiation Time} - 1.6575 * \text{Concentration of Antibiotic} * \text{Catalyst} \\ & \text{ZnONPs Dose} - 0.2675 * \text{Concentration of Antibiotic} * \text{Irradiation Time} - 1.9025 * \text{Catalyst} \\ & \text{ZnONPs Dose} * \text{Irradiation Time} + 3.92708 * (\text{Concentration of Antibiotic})^2 + 3.31708 * \\ & (\text{Catalyst ZnONPs Dose})^2 - 1.23792 * (\text{Irradiation Time})^2 \quad \mathbf{(5)} \end{aligned}$$

The forecasted and observed values of Y1, Y2 and Y3 showcased in Figure 4.2 demonstrate a strong concordance. Moreover, the distribution of data points closely follows the 45° axis, suggesting that the models constructed for CFM, CIP and VAN removal effectively establish a connection between the independent and dependent variables.

The coefficient of determination (R^2) for the regression models corresponding to responses Y1, Y2 and Y3 was determined to be 0.9915, 0.9868 and 0.9899, respectively (as shown in Table 4.7). This signifies that the developed models account for 99.15%, 98.68% and 98.99% of the total variation, indicating a robust relationship between the dependent and independent variables. However, there remains a residual portion of approximately 0.85%, 1.32% and 1.01% of the total variation unexplained by the models.

The estimated R^2 values for responses Y1, Y2, and Y3 were found to be 0.9248, 0.8041, and 0.8489, respectively (as presented in Table 4.7). These values closely align with the R^2 values of 0.9915, 0.9868, and 0.9899, respectively. Furthermore, they demonstrate good consistency with the RMS adjusted R^2 values of 0.9763, 0.9630, and 0.9718 for the removal of CFM (Y1), CIP (Y2), and VAN (Y3), respectively, with differences less than 0.2.

The Adequate Precision (A.P) serves as a metric for the signal-to-noise ratio in experiments, with a preference for a ratio exceeding 4. For Y1, Y2 and Y3, the AP scores were 40.5302, 21.078 and 22.581, respectively (as illustrated in Table 4.7), indicating robust signal strength and suggesting excellent model performance.

Additionally, the Standard Deviation (S.D) and Coefficient of Variation (C.V) are indicators of experimental precision. Lower S.D values (1.04 for Y1, 3.03 for Y2 and 1.28 for Y3) and C.V values (3.39% for Y1, 9.24 for Y2 and 3.28% for Y3) suggest high precision and reliability of the experiments, indicating that the models are reasonably reproducible. (Table 4.7)

Table 4. 3 : Model Summary Statistics

	Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Y1: CFM Degradation (%)	Linear	0.0114	0.0116	0.516	0.1927	
	2FI	0.0002	0.0824	0.9393	0.8451	
	Quadratic	0.0196	0.2532	0.9845	0.9248	Suggested
	Cubic	0.2532		0.9931		Aliased
Y2: CIP Degradation (%)	Linear	0.1725	0.0079	0.1765	-0.3229	
	2FI	0.3399	0.0079	0.2385	-1.0536	
	Quadratic	0.0003	0.1268	0.963	0.8041	Suggested
	Cubic	0.1268		0.992		Aliased
Y3: VAN Degradation (%)	Linear	0.0001	0.0192	0.7875	0.6806	
	2FI	0.6204	0.0158	0.763	0.4396	
	Quadratic	0.003	0.1052	0.9718	0.8489	Suggested
	Cubic	0.1052		0.995		Aliased

Table 4. 4 : ANOVA for quadratic model (Y1)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	651.16	9	72.35	99.64	< 0.0001	significant
X1: Concentration of Antibiotic	30.23	1	30.23	41.62	0.0013	
X2: Catalyst ZnONPs Dose	8.88	1	8.88	12.23	0.0173	
X3: Irradiation Time	366.66	1	366.66	504.94	< 0.0001	
X1X2	8.85	1	8.85	12.19	0.0174	
X1X3	2.53	1	2.53	3.48	0.1211	
X2X3	214.92	1	214.92	295.96	< 0.0001	
X1 ²	2.3	1	2.3	3.17	0.1351	
X2 ²	10.18	1	10.18	14.02	0.0134	
X3 ²	6.32	1	6.32	8.7	0.0319	
Residual	3.63	5	0.7262			
Lack of Fit	2.99	3	0.9962	3.1	0.2532	not significant
Pure Error	0.6421	2	0.321			
Cor Total	654.8	14				

Table 4. 5 : ANOVA for quadratic model (Y2)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3434.33	9	381.59	41.44	0.0004	significant
X1: Concentration of Antibiotic	541.53	1	541.53	58.81	0.0006	
X2: Catalyst ZnONPs Dose	424.28	1	424.28	46.07	0.0011	
X3: Irradiation Time	262.66	1	262.66	28.52	0.0031	
X1X2	3.35	1	3.35	0.3637	0.5728	

X1X3	727.92	1	727.92	79.05	0.0003	
X2X3	6.2	1	6.2	0.6733	0.4492	
X1 ²	1106.99	1	1106.99	120.21	0.0001	
X2 ²	189.02	1	189.02	20.53	0.0062	
X3 ²	121.65	1	121.65	13.21	0.015	
Residual	46.04	5	9.21			
Lack of Fit	42.06	3	14.02	7.05	0.1268	not significant
Pure Error	3.98	2	1.99			
Cor Total	3480.38	14				

Table 4. 6 : ANOVA for quadratic model (Y3)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	807.16	9	89.68	54.66	0.0002	significant
X1: Concentration of Antibiotic	643.69	1	643.69	392.28	< 0.0001	
X2: Catalyst ZnONPs Dose	20.93	1	20.93	12.76	0.016	
X3: Irradiation Time	14.58	1	14.58	8.89	0.0308	
X1X2	10.99	1	10.99	6.7	0.049	
X1X3	0.2862	1	0.2862	0.1744	0.6935	
X2X3	14.48	1	14.48	8.82	0.0311	
X1 ²	56.94	1	56.94	34.7	0.002	
X2 ²	40.63	1	40.63	24.76	0.0042	
X3 ²	5.66	1	5.66	3.45	0.1224	
Residual	8.2	5	1.64			
Lack of Fit	7.62	3	2.54	8.67	0.1052	not significant
Pure Error	0.5859	2	0.2929			
Cor Total	815.37	14				

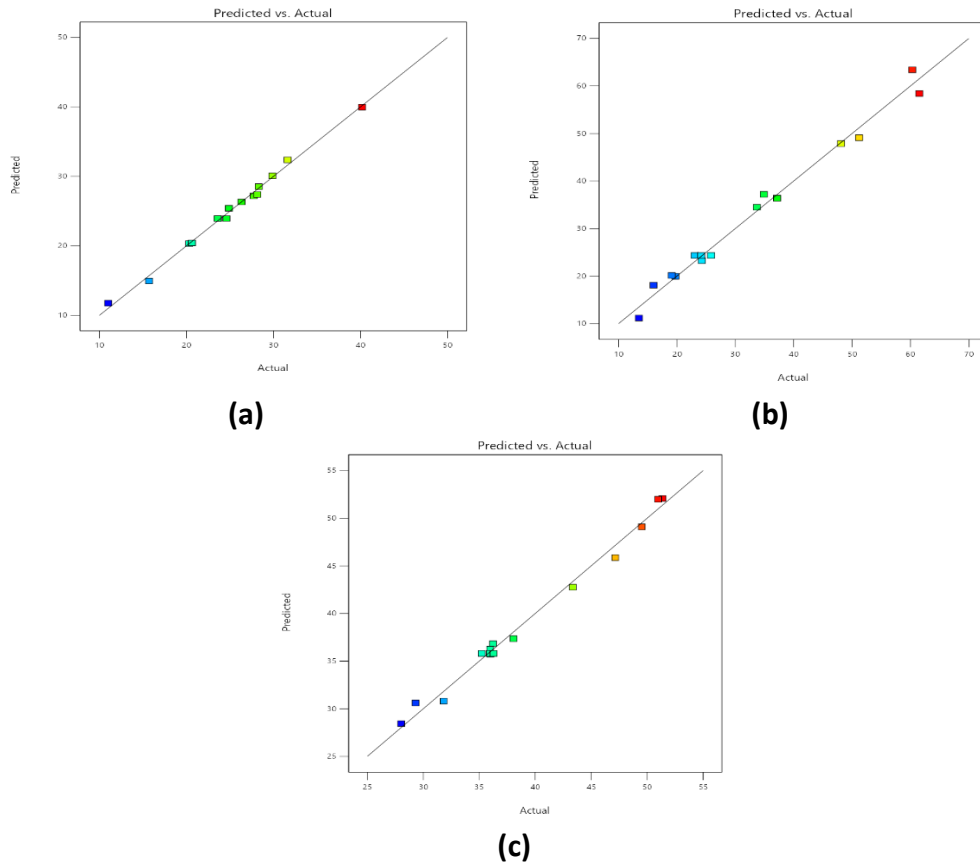


Figure 4. 2 : Predicted vs actual plot for CFM degradation (a); CIP degradation (b) and VAN degradation (c)

Table 4. 7 : Statistical parameters for responses Y1, Y2, and Y3

	Y1: CFM Degradation (%)	Y2: CIP Degradation (%)	Y3: VAN Degradation (%)
R²	0.9915	0.9868	0.9899
Adjusted R²	0.9763	0.963	0.9718
Predicted R²	0.9248	0.8041	0.8489
Adequate Precision A.P	40.5302	21.0779	22.5809
Standard Deviation S.D	1.04	3.03	1.28

Coefficient of Variation C.V. %	3.39	9.24	3.28
--	------	------	------

4.3 Process optimization

The optimization criteria encompass two key aspects: ensuring that all independent parameters fall within the specified range and maximizing the response for all antibiotics. The elements that were evaluated encompass three independent factors, and four responses are taken into account to meet the optimization criteria. Furthermore, the optimization underwent a thorough study to assess the suitability of the optimization outcome.

Table 4.8 presents a summary of the optimization results, validation experiments, and analysis. It should be noted that the optimization of degradation performance varies depending on the specific antibiotics used.

To achieve an optimal predicted degradation rate of 40.41% for antibiotic CFM, it is necessary to have an initial concentration of 10.78 mg/L, an irradiation time of 88.58 minutes, and a dosage of ZnONPs nanocatalyst of 0.78 g/L. Similarly, to reach an optimal predicted degradation rate of 61.51% for antibiotic CIP, an initial concentration of 29.99 mg/L, an irradiation time of 89.99 minutes, and a dose of ZnONPs nanocatalyst of 0.70 g/L are required. The optimal parameters for antibiotic VAN involve an initial concentration of 29.92 mg/L, an irradiation duration of 34.77 minutes, and a dosage of ZnONPs nanocatalyst of 0.77 g/L. These settings result in an optimal maximum degradation rate of 53.22 %.

Validation experiments were carried out with three replications to confirm these optimization results. Under the improved photocatalysis conditions, the average degradation rates for CFM, CIP, and VAN are 44.9 %, 57.40 %, and 50.18 % respectively.

Table 4. 8: Predicted and validated results of the optimized variables

Variables	X1: Concentration of Antibiotic (mg/L)	X2: Catalyst ZnONP Dose (g/L)	X3: Irradiation Time (min)	X1	X2	X3	X1	X2	X3
Objective	In range			In range			In range		
Optimized Value	10.78	0.78	88.58	29.99	0.7	89.99	29.92	0.77	34.77
Response	Y1: CFM Degradation (%)			Y2: CIP Degradation (%)			Y3: VAN Degradation (%)		
Objective	Maximize			Maximize			Maximize		
Model Prediction	40.41			61.51			53.22		
Validation	44.9			57.4			50.18		
Error	4.49			4.11			3.04		
Desirability	1								

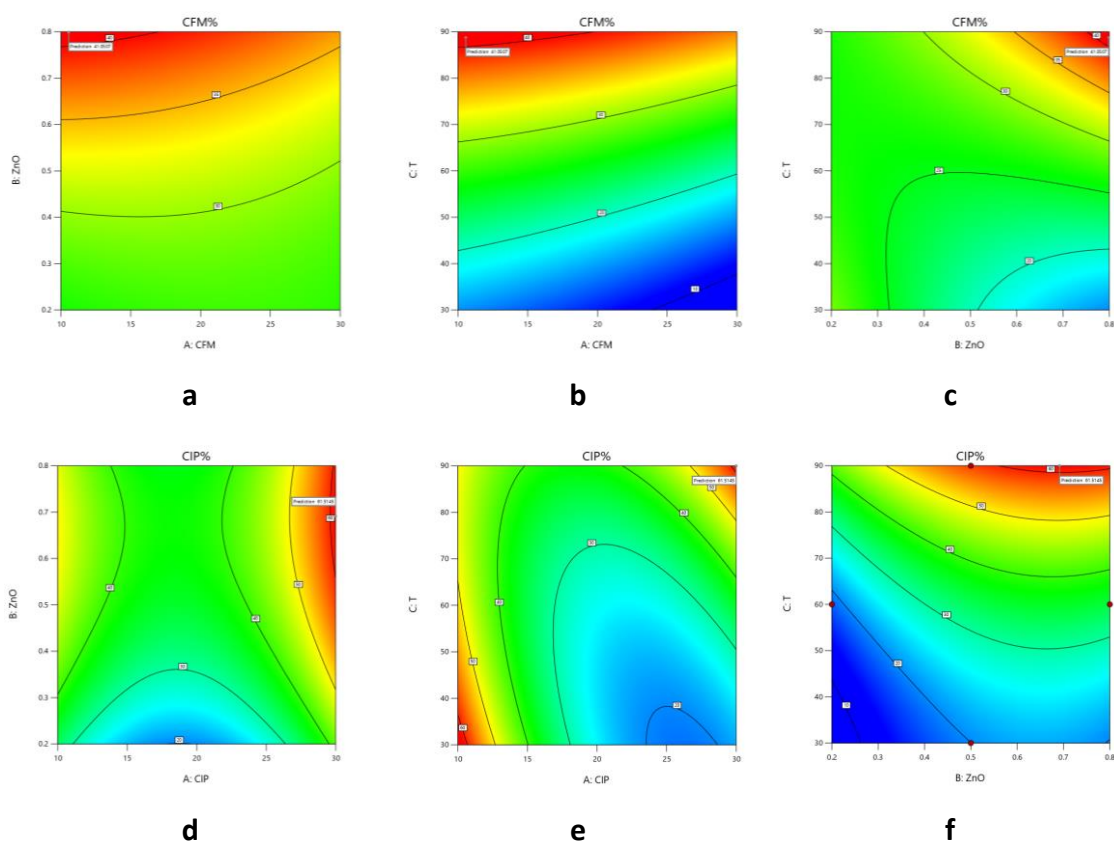
4.4 Analysis of the degradation of antibiotics using response surface methodology

The response surface contour plots for CFM, CIP, and VAN degradation are presented in Figure 4.3. The construction of these plots is derived from the interaction plots Concentration of Antibiotic, Catalyst ZnONPs Dose, and Irradiation Time, which were generated using the empirical models outlined in equations (3) to (5).

In order to analyze the contour plots, it is important to note that red regions represent degradation values that are comparatively greater, whilst blue regions show degradation values that are substantially lower.

The response surface plots analysis reveals a dissimilarity in surface profiles among the three antibiotics (Figure 4.3). This may be attributed to each antibiotic's specific characteristics and degradation behavior under photocatalytic conditions using a ZnONPs

nanocatalyst. This dissimilarity may stem from a complex interplay of factors, including molecular structure differences, catalyst reactivity, photophysical properties, and sensitivity to oxidation. Figure 4.4, 4.5 and 4.6 depict three-dimensional plots illustrating the response surfaces for CFM, CIP and VAN degradation, with independent variables X1, X2, and X3. The concentration of antibiotic (X1) and the presence of the catalyst ZnO (X2) positively influence rate of degradation. Furthermore, there's an interaction between these factors, indicating that the impact of one depends on the other in the degradation process.



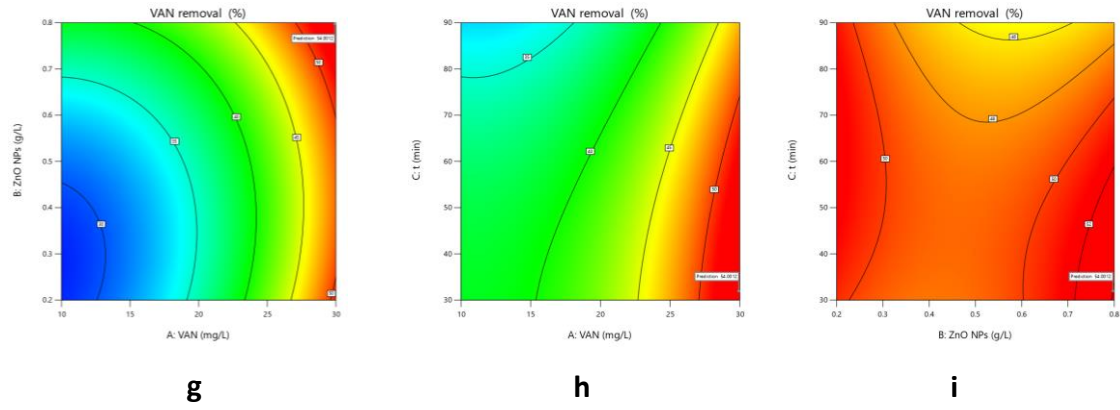


Figure 4. 3 : 2D response surface plot for (a,b,c): CFM degradation, (d,e,f): CIP degradation, (g,h,i): VAN degradation

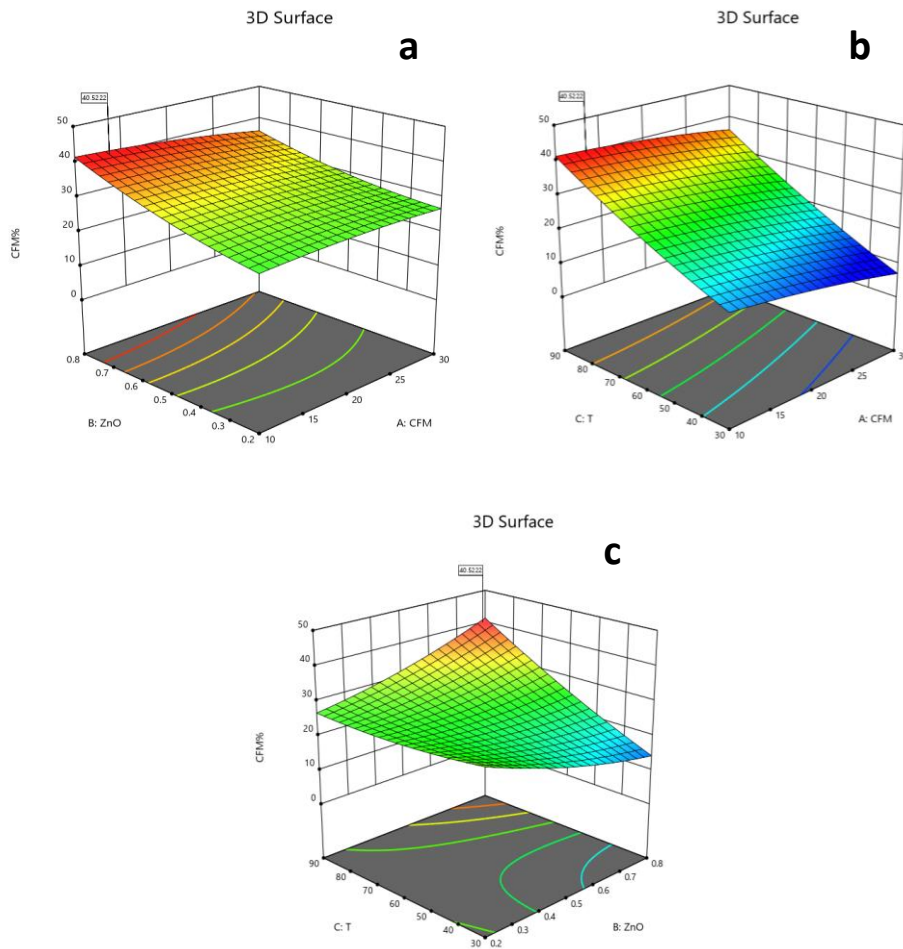


Figure 4. 4: Response surface 3D-plot for CFM degradation

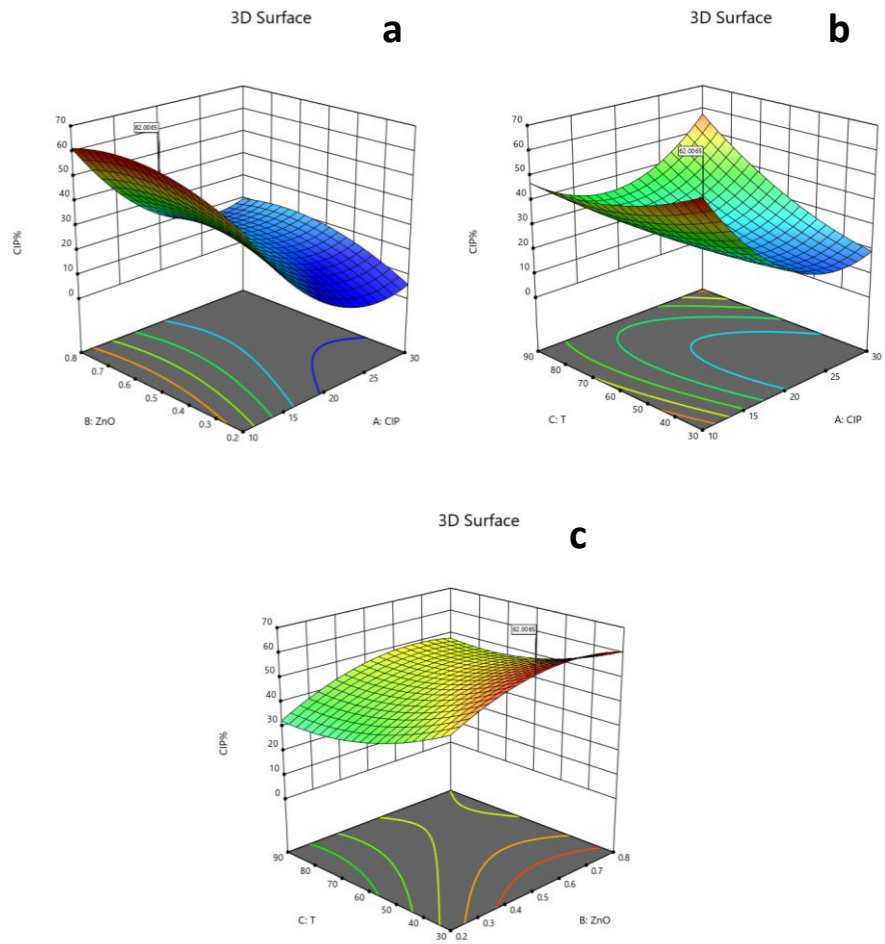
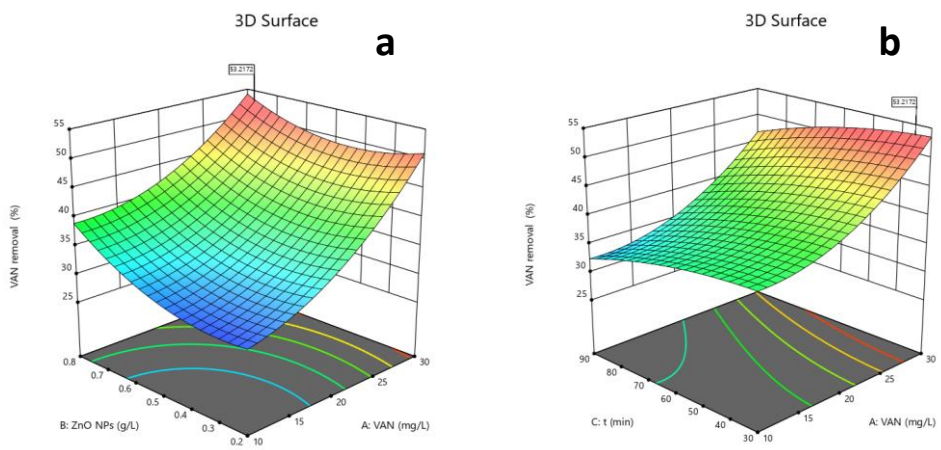


Figure 4. 5: Response surface 3D-plot for CIP degradation



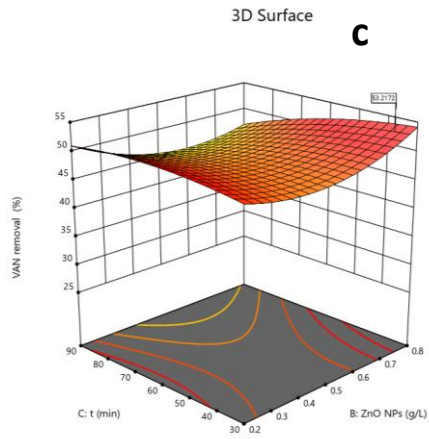


Figure 4. 6: Response surface 3D-plot for VAN degradation

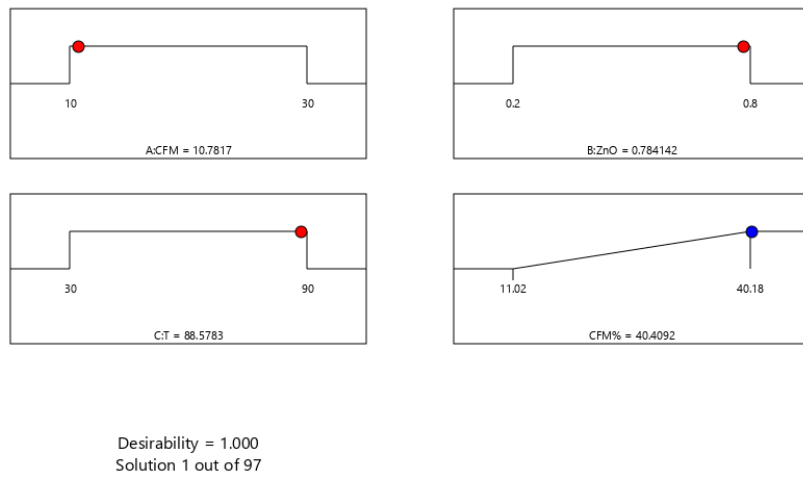


Figure 4. 7 : Predicted solution obtained by numerical optimization (CFM)

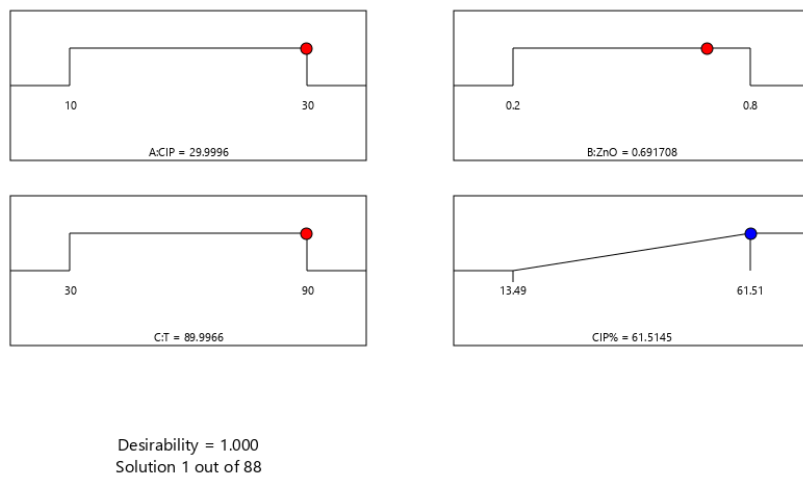
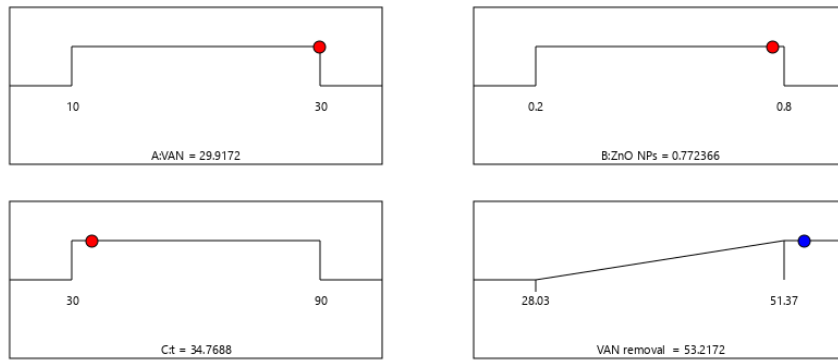


Figure 4. 8: Predicted solution obtained by numerical optimization (CIP)



Desirability = 1.000
 Solution 1 out of 100

Figure 4. 9 : Predicted solution obtained by numerical optimization (VAN)

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

The study focused on using green ZnO nanoparticles synthesized from dried banana peels to photocatalyst the degradation of three antibiotics commonly detected in pharmaceutical wastewater: cefotaxime (CFM), ciprofloxacin (CIP), and vancomycin (VAN). An optimization of photocatalysis parameters that impact antibiotic degradation was conducted using a response surface methodology and a Box-Behnken design (RSM-BBD). The quadratic model was found to be highly relevant for the three CFM, CIP, and VAN responses, as indicated by the analysis of variance (ANOVA).

The phenolic assays reveal significant contents of total polyphenols, flavonoids, flavonols, and hydrolysable and condensed tannins in our extract, with values of 184 mg GA eq/g, 101.08 mg C eq/g, 92.65 mg M eq/g, 2315 mg TA eq/g, and 640 mg C eq/g respectively. The abundance of polyphenolic compounds in banana peel extract has great potential for nanoparticle synthesis, as confirmed by the results of UV-Visible analysis after the synthesis of nanoparticles. These results demonstrate a clear decrease in the content of polyphenols, with percentages ranging from 65% to 95%, confirming that these molecules are directly involved in the biosynthesis of ZnO nanoparticles.

The coefficient of determination (R^2) indicates that the developed models represent 99.15%, 98.68%, and 98.99% of the total variation, revealing a robust relationship between the studied photocatalysis factors and antibiotic degradation rates. An optimal degradation level for CFM, CIP, and VAN, reaching 40.41%, 61.51%, and 53.22%, respectively, was observed. These optimal results were obtained for the initial antibiotic concentrations of 10.78 mg/L, 29.99 mg/L, and 29.92 mg/L; the doses of ZnO NP nanocatalyst were 0.78 g/L, 0.70 g/L, and 0.77 g/L, and irradiation durations were 88.58 minutes, 89.99 minutes, and 34.77 minutes, respectively. These findings highlight the potential of green ZnO NP-based nano-photocatalysts derived from banana waste in environmental applications, particularly for the effective degradation of pharmaceutical residues.

5.2 Recommendations

In the future, further research can build on the results of this study to attempt to improve antibiotic degradation, explore other drug classes, evaluate the long-term stability of green ZnO nanoparticles under various environmental conditions, and conduct comprehensive cost-benefit analyses to assess economic feasibility and scalability for larger-scale implementation.

6. REFERENCES

- (1) Cheng, D.; Ngo, H. H.; Guo, W.; Liu, Y.; Chang, S. W.; Nguyen, D. D.; Nghiem, L. D.; Zhou, J.; Ni, B. Anaerobic Membrane Bioreactors for Antibiotic Wastewater Treatment: Performance and Membrane Fouling Issues. *Bioresour. Technol.* **2018**, *267*, 714–724. <https://doi.org/10.1016/j.biortech.2018.07.133>.
- (2) Zhao, L.; Lv, Z.; Lin, L.; Li, X.; Xu, J.; Huang, S.; Chen, Y.; Fu, Y.; Peng, C.; Cao, T.; Ke, Y.; Xia, X. Impact of COVID-19 Pandemic on Profiles of Antibiotic-Resistant Genes and Bacteria in Hospital Wastewater. *Environ. Pollut.* **2023**, *334*, 122133. <https://doi.org/10.1016/j.envpol.2023.122133>.
- (3) Wang, Q.; Liu, C.; Sun, S.; Yang, G.; Luo, J.; Wang, N.; Chen, B.; Wang, L. Enhance Antibiotic Resistance and Human Health Risks in Aerosols during the COVID-19 Pandemic. *Sci. Total Environ.* **2023**, *871*, 162035. <https://doi.org/10.1016/j.scitotenv.2023.162035>.
- (4) Bai, H.; He, L.-Y.; Gao, F.-Z.; Wu, D.-L.; Yao, K.-S.; Zhang, M.; Jia, W.-L.; He, L.-X.; Zou, H.-Y.; Yao, M.-S.; Ying, G.-G. Airborne Antibiotic Resistome and Human Health Risk in Railway Stations during COVID-19 Pandemic. *Environ. Int.* **2023**, *172*, 107784. <https://doi.org/10.1016/j.envint.2023.107784>.
- (5) Cincinelli, A.; Martellini, T.; Coppini, E.; Fibbi, D.; Katsoyiannis, A. Nanotechnologies for Removal of Pharmaceuticals and Personal Care Products from Water and Wastewater. A Review. *J. Nanosci. Nanotechnol.* **2015**, *15* (5), 3333–3347. <https://doi.org/10.1166/jnn.2015.10036>.
- (6) Adeoye, J. B.; Tan, Y. H.; Lau, S. Y.; Tan, Y. Y.; Chiong, T.; Mubarak, N. M.; Khalid, M. Advanced Oxidation and Biological Integrated Processes for Pharmaceutical Wastewater Treatment: A Review. *J. Environ. Manage.* **2024**, *353*, 120170. <https://doi.org/10.1016/j.jenvman.2024.120170>.
- (7) Ghosh, S.; Pourebrahimi, S.; Malloum, A.; Ajala, O. J.; AlKafaas, S. S.; Onyeaka, H.; Nnaji, N. D.; Oroke, A.; Bornman, C.; Christian, O.; Ahmadi, S.; Wani, M. Y. A Review on Ciprofloxacin Removal from Wastewater as a Pharmaceutical Contaminant: Covering Adsorption to Advanced Oxidation Processes to Computational Studies. *Mater. Today Commun.* **2023**, *37*, 107500. <https://doi.org/10.1016/j.mtcomm.2023.107500>.
- (8) Mishra, S.; Sundaram, B. A Review of the Photocatalysis Process Used for Wastewater Treatment. *Mater. Today Proc.* **2023**. <https://doi.org/10.1016/j.matpr.2023.07.147>.
- (9) Tahir, M. B.; Sohaib, M.; Sagir, M.; Rafique, M. Role of Nanotechnology in Photocatalysis. In *Encyclopedia of Smart Materials*; Olabi, A.-G., Ed.; Elsevier: Oxford, 2022; pp 578–589. <https://doi.org/10.1016/B978-0-12-815732-9.00006-1>.
- (10) Meijide, J.; Lama, G.; Pazos, M.; Sanromán, M. A.; Dunlop, P. S. M. Ultraviolet-Based Heterogeneous Advanced Oxidation Processes as Technologies to Remove Pharmaceuticals from Wastewater: An Overview. *J. Environ. Chem. Eng.* **2022**, *10* (3), 107630. <https://doi.org/10.1016/j.jece.2022.107630>.

- (11) Darrow, J. J.; Avorn, J.; Kesselheim, A. S. FDA Approval and Regulation of Pharmaceuticals, 1983-2018. *JAMA* **2020**, *323* (2), 164–176. <https://doi.org/10.1001/jama.2019.20288>.
- (12) cycles, T. text provides general information S. assumes no liability for the information given being complete or correct D. to varying update; Text, S. C. D. M. up-to-D. D. T. R. in the. *Topic: Global pharmaceutical industry*. Statista. <https://www.statista.com/topics/1764/global-pharmaceutical-industry/> (accessed 2024-03-29).
- (13) *Top 10 Global Pharma Companies 2022*. PharmaBoardroom. <https://pharmaboardroom.com/articles/top-10-global-pharma-companies-2022/> (accessed 2024-03-29).
- (14) Goetz, L. H.; Schork, N. J. Personalized Medicine: Motivation, Challenges, and Progress. *Fertil. Steril.* **2018**, *109* (6), 952–963. <https://doi.org/10.1016/j.fertnstert.2018.05.006>.
- (15) *Medicines*. <https://www.who.int/health-topics/medicines> (accessed 2024-03-29).
- (16) *FDA Registration - FDA Certificate - FDA Agent*. https://www.fdahelp.us/?gad_source=1&gclid=Cj0KQCjwqpSwBhCIARIsADIZ_TmEDtTJZjKSJIoYNyREGbgjxUVoL5L-KxyAaADsJz7R_XcBEYRGL8laAkcZELw_wcB (accessed 2024-03-29).
- (17) *1000 milliards d'euros de profits en vingt ans : comment les labos sont devenus des monstres financiers*. Basta! <https://basta.media/1000-milliards-d-euros-de-profits-en-vingt-ans-comment-les-labos-sont-devenus> (accessed 2024-03-29).
- (18) Nasreddine, A. The Algerian Pharmaceutical Market; Specifics and Characteristics. **2020**, *1*, 57–80.
- (19) Eddine, D. ALGERIA IMPORT SUBSTITUTION POLICY: THE CASE OF THE PHARMACEUTICAL INDUSTRY. **2021**, 37.
- (20) *Toumi M, Mercier G, Yahyaoui A. Algeria. In: Mombelli G, Ed. Pharmaceutical Markets and Health Systems of the BRICS: An International Perspective. Emerald Publishing Limited; 2016:137-154. Doi:10.1108/978-1-78560-791-320161011 (Référence 10) a Changer.*
- (21) Pieters, K. The Mediterranean countries (Morocco, Algeria, Tunisia, Libya, Egypt, Jordan, Syria and Lebanon); 2006; pp 391–432. https://doi.org/10.1007/978-90-6704-507-0_12.
- (22) Alsamara, T.; Farouk, G.; Adel, A. Public Health and the Legal Regulation of the Pharmaceutical Industry in Algeria. *Pan Afr. Med. J.* **2022**, *41*. <https://doi.org/10.11604/pamj.2022.41.86.31524>.
- (23) Lila ZIANI, L. Z. The Medicine's Industry in Algeria: State of Play and Constraints. *Rev. Abaad Iktissadia* **2021**.
- (24) SNOUSSI Zoulikha. MARCHÉ DES MÉDICAMENTS GÉNÉRIQUES EN ALGÉRIE : QUELLE RÉGULATION POUR QUELLE PROMOTION ? *Rev. Nouv. Econ.* **2012**.
- (25) Chaukura, N.; Marais, S. S.; Moyo, W.; Mbali, N.; Thakalekoala, L. C.; Ingwani, T.; Mamba, B. B.; Jarvis, P.; Nkambule, T. T. I. Contemporary Issues on the Occurrence and Removal of

Disinfection Byproducts in Drinking Water - A Review. *J. Environ. Chem. Eng.* **2020**, *8* (2), 103659. <https://doi.org/10.1016/j.jece.2020.103659>.

(26) Smýkalová; Sokolová; Foniok; Matějka; Praus. Photocatalytic Degradation of Selected Pharmaceuticals Using G-C₃N₄ and TiO₂ Nanomaterials. *Nanomaterials* **2019**, *9* (9), 1194. <https://doi.org/10.3390/nano9091194>.

(27) Paíga, P.; Santos, L. H. M. L. M.; Ramos, S.; Jorge, S.; Silva, J. G.; Delerue-Matos, C. Presence of Pharmaceuticals in the Lis River (Portugal): Sources, Fate and Seasonal Variation. *Sci. Total Environ.* **2016**, *573*, 164–177. <https://doi.org/10.1016/j.scitotenv.2016.08.089>.

(28) Oliveros, A. N.; Pimentel, J. A. I.; de Luna, M. D. G.; Garcia-Segura, S.; Abarca, R. R. M.; Doong, R.-A. Visible-Light Photocatalytic Diclofenac Removal by Tunable Vanadium Pentoxide/Boron-Doped Graphitic Carbon Nitride Composite. *Chem. Eng. J.* **2021**, *403*, 126213. <https://doi.org/10.1016/j.cej.2020.126213>.

(29) Kalyva, M. Fate of Pharmaceuticals in the Environment -A Review-.

(30) Pereira, A.; Silva, L.; Laranjeiro, C.; Lino, C.; Pena, A. Selected Pharmaceuticals in Different Aquatic Compartments: Part I—Source, Fate and Occurrence. *Molecules* **2020**, *25* (5). <https://doi.org/10.3390/molecules25051026>.

(31) Sui, Q.; Cao, X.; Lu, S.; Zhao, W.; Qiu, Z.; Yu, G. Occurrence, Sources and Fate of Pharmaceuticals and Personal Care Products in the Groundwater: A Review. *Emerg. Contam.* **2015**, *1* (1), 14–24. <https://doi.org/10.1016/j.emcon.2015.07.001>.

(32) Kang, Y.-M.; Kim, M.-K.; Kim, T.; Kim, T.-K.; Zoh, K.-D. Occurrence and Fate of Micropollutants in Private Wastewater Treatment Facility (WTF) and Their Impact on Receiving Water. *Environ. Manage.* **2019**, *64* (5), 650–660. <https://doi.org/10.1007/s00267-019-01211-5>.

(33) McEachran Andrew D.; Blackwell Brett R.; Hanson J. Delton; Wooten Kimberly J.; Mayer Gregory D.; Cox Stephen B.; Smith Philip N. Antibiotics, Bacteria, and Antibiotic Resistance Genes: Aerial Transport from Cattle Feed Yards via Particulate Matter. *Environ. Health Perspect.* **2015**, *123* (4), 337–343. <https://doi.org/10.1289/ehp.1408555>.

(34) Lindholm-Lehto, P. C.; Ahkola, H. S. J.; Knuutinen, J. S.; Herve, S. H. Widespread Occurrence and Seasonal Variation of Pharmaceuticals in Surface Waters and Municipal Wastewater Treatment Plants in Central Finland. *Environ. Sci. Pollut. Res.* **2016**, *23* (8), 7985–7997. <https://doi.org/10.1007/s11356-015-5997-y>.

(35) Fang, T.-H.; Lin, C.-W.; Kao, C.-H. Occurrence and Distribution of Pharmaceutical Compounds in the Danshuei River Estuary and the Northern Taiwan Strait. *Mar. Pollut. Bull.* **2019**, *146*, 509–520. <https://doi.org/10.1016/j.marpolbul.2019.06.069>.

(36) Kwon, J.-W.; Rodriguez, J. M. Occurrence and Removal of Selected Pharmaceuticals and Personal Care Products in Three Wastewater-Treatment Plants. *Arch. Environ. Contam. Toxicol.* **2014**, *66* (4), 538–548. <https://doi.org/10.1007/s00244-013-9979-0>.

- (37) Giebułtowicz, J.; Nałęcz-Jawecki, G.; Harnisz, M.; Kucharski, D.; Korzeniewska, E.; Płaza, G. Environmental Risk and Risk of Resistance Selection Due to Antimicrobials' Occurrence in Two Polish Wastewater Treatment Plants and Receiving Surface Water. *Molecules* **2020**, *25* (6). <https://doi.org/10.3390/molecules25061470>.
- (38) Adeola, A. O.; Forbes, P. B. C. Antiretroviral Drugs in African Surface Waters: Prevalence, Analysis, and Potential Remediation. *Environ. Toxicol. Chem.* **2022**, *41* (2), 247–262. <https://doi.org/10.1002/etc.5127>.
- (39) Al-Odaini, N. A.; Zakaria, M. P.; Yaziz, M. I.; Surif, S.; Abdulghani, M. The Occurrence of Human Pharmaceuticals in Wastewater Effluents and Surface Water of Langat River and Its Tributaries, Malaysia. *Int. J. Environ. Anal. Chem.* **2013**, *93* (3), 245–264. <https://doi.org/10.1080/03067319.2011.592949>.
- (40) Fonseca, E.; Hernández, F.; Ibáñez, M.; Rico, A.; Pitarch, E.; Bijlsma, L. Occurrence and Ecological Risks of Pharmaceuticals in a Mediterranean River in Eastern Spain. *Environ. Int.* **2020**, *144*, 106004. <https://doi.org/10.1016/j.envint.2020.106004>.
- (41) Huber, S.; Remberger, M.; Kaj, L.; Schlabach, M.; Jörundsdóttir, H. Ó.; Vester, J.; Arnórsson, M.; Mortensen, I.; Swartzson, R.; Dam, M. A First Screening and Risk Assessment of Pharmaceuticals and Additives in Personal Care Products in Waste Water, Sludge, Recipient Water and Sediment from Faroe Islands, Iceland and Greenland. *Sci. Total Environ.* **2016**, *562*, 13–25. <https://doi.org/10.1016/j.scitotenv.2016.03.063>.
- (42) de Jesus Gaffney, V.; Cardoso, V. V.; Cardoso, E.; Teixeira, A. P.; Martins, J.; Benoliel, M. J.; Almeida, C. M. M. Occurrence and Behaviour of Pharmaceutical Compounds in a Portuguese Wastewater Treatment Plant: Removal Efficiency through Conventional Treatment Processes. *Environ. Sci. Pollut. Res.* **2017**, *24* (17), 14717–14734. <https://doi.org/10.1007/s11356-017-9012-7>.
- (43) Archer, E.; Wolfaardt, G.; Van WYK, J. Pharmaceutical and Personal Care Products (PPCPs) as Endocrine Disrupting Contaminants (EDCs) in South African Surface Waters, 2017.
- (44) Tisler, S.; Zwiener, C. Formation and Occurrence of Transformation Products of Metformin in Wastewater and Surface Water. *Sci. Total Environ.* **2018**, *628–629*, 1121–1129. <https://doi.org/10.1016/j.scitotenv.2018.02.105>.
- (45) Martínez-Alcalá, I.; Guillén-Navarro, J. M.; Lahora, A. Occurrence and Fate of Pharmaceuticals in a Wastewater Treatment Plant from Southeast of Spain and Risk Assessment. *J. Environ. Manage.* **2021**, *279*, 111565. <https://doi.org/10.1016/j.jenvman.2020.111565>.
- (46) Kumar, R.; Sarmah, A. K.; Padhye, L. P. Fate of Pharmaceuticals and Personal Care Products in a Wastewater Treatment Plant with Parallel Secondary Wastewater Treatment Train. *J. Environ. Manage.* **2019**, *233*, 649–659. <https://doi.org/10.1016/j.jenvman.2018.12.062>.
- (47) Tran, N. H.; Li, J.; Hu, J.; Ong, S. L. Occurrence and Suitability of Pharmaceuticals and Personal Care Products as Molecular Markers for Raw Wastewater Contamination in Surface Water and Groundwater. *Environ. Sci. Pollut. Res.* **2014**, *21* (6), 4727–4740. <https://doi.org/10.1007/s11356-013-2428-9>.

- (48) Morasch, B.; Bonvin, F.; Reiser, H.; Grandjean, D.; de Alencastro, L. F.; Perazzolo, C.; Chèvre, N.; Kohn, T. Occurrence and Fate of Micropollutants in the Vidy Bay of Lake Geneva, Switzerland. Part II: Micropollutant Removal between Wastewater and Raw Drinking Water. *Environ. Toxicol. Chem.* **2010**, *29* (8), 1658–1668. <https://doi.org/10.1002/etc.222>.
- (49) Choueiri, T. K.; Je, Y.; Cho, E. Analgesic Use and the Risk of Kidney Cancer: A Meta-Analysis of Epidemiologic Studies. *Int. J. Cancer* **2014**, *134* (2), 384–396. <https://doi.org/10.1002/ijc.28093>.
- (50) Zhang, L.; Xu, T.; Zhao, X.; Zhu, Y. Controllable Synthesis of Bi₂MoO₆ and Effect of Morphology and Variation in Local Structure on Photocatalytic Activities. *Appl. Catal. B Environ.* **2010**, *98* (3), 138–146. <https://doi.org/10.1016/j.apcatb.2010.05.022>.
- (51) Bisognin, R. P.; Wolff, D. B.; Carissimi, E.; Prestes, O. D.; Zanella, R. Occurrence and Fate of Pharmaceuticals in Effluent and Sludge from a Wastewater Treatment Plant in Brazil. *Environ. Technol.* **2021**, *42* (15), 2292–2303. <https://doi.org/10.1080/09593330.2019.1701561>.
- (52) Fawzi Suleiman Khasawneh, O.; Palaniandy, P. Photocatalytic Degradation of Pharmaceuticals Using TiO₂ Based Nanocomposite Catalyst-Review. *Civ. Environ. Eng. Rep.* **2019**, *29* (3), 1–33. <https://doi.org/10.2478/ceer-2019-0021>.
- (53) Deblonde, T.; Cossu-Leguille, C.; Hartemann, P. Emerging Pollutants in Wastewater: A Review of the Literature. *Second Eur. PhD Stud. Workshop Water Health Cannes 2010* **2011**, *214* (6), 442–448. <https://doi.org/10.1016/j.ijheh.2011.08.002>.
- (54) López-Serna, R.; Jurado, A.; Vázquez-Suñé, E.; Carrera, J.; Petrović, M.; Barceló, D. Occurrence of 95 Pharmaceuticals and Transformation Products in Urban Groundwaters Underlying the Metropolis of Barcelona, Spain. *Environ. Pollut.* **2013**, *174*, 305–315. <https://doi.org/10.1016/j.envpol.2012.11.022>.
- (55) Ruziwa, D. T.; Oluwalana, A. E.; Mupa, M.; Meili, L.; Selvasembian, R.; Nindi, M. M.; Sillanpaa, M.; Gwenzi, W.; Chaukura, N. Pharmaceuticals in Wastewater and Their Photocatalytic Degradation Using Nano-Enabled Photocatalysts. *J. Water Process Eng.* **2023**, *54*, 103880. <https://doi.org/10.1016/j.jwpe.2023.103880>.
- (56) Luo, Y.; Guo, W.; Ngo, H. H.; Nghiem, L. D.; Hai, F. I.; Zhang, J.; Liang, S.; Wang, X. C. A Review on the Occurrence of Micropollutants in the Aquatic Environment and Their Fate and Removal during Wastewater Treatment. *Sci. Total Environ.* **2014**, *473–474*, 619–641. <https://doi.org/10.1016/j.scitotenv.2013.12.065>.
- (57) Zhang, Z.; Zhou, Z.; Cao, X.; Liu, Y.; Xiong, G.; Liang, P. Removal of Uranium(VI) from Aqueous Solutions by New Phosphorus-Containing Carbon Spheres Synthesized via One-Step Hydrothermal Carbonization of Glucose in the Presence of Phosphoric Acid. *J. Radioanal. Nucl. Chem.* **2014**, *299* (3), 1479–1487. <https://doi.org/10.1007/s10967-013-2830-2>.
- (58) Mahmoodi, N. M.; Ghezelbash, M.; Shabaniyan, M.; Aryanasab, F.; Saeb, M. R. Efficient Removal of Cationic Dyes from Colored Wastewaters by Dithiocarbamate-Functionalized Graphene Oxide Nanosheets: From Synthesis to Detailed Kinetics Studies. *J. Taiwan Inst. Chem. Eng.* **2017**, *81*, 239–246. <https://doi.org/10.1016/j.jtice.2017.10.011>.

- (59) Hayati, B.; Mahmoodi, N. M.; Arami, M.; Mazaheri, F. Dye Removal from Colored Textile Wastewater by Poly(Propylene Imine) Dendrimer: Operational Parameters and Isotherm Studies. *CLEAN – Soil Air Water* **2011**, *39* (7), 673–679. <https://doi.org/10.1002/clen.201000182>.
- (60) Oveisi, M.; Asli, M. A.; Mahmoodi, N. M. MIL-Ti Metal-Organic Frameworks (MOFs) Nanomaterials as Superior Adsorbents: Synthesis and Ultrasound-Aided Dye Adsorption from Multicomponent Wastewater Systems. *J. Hazard. Mater.* **2018**, *347*, 123–140. <https://doi.org/10.1016/j.jhazmat.2017.12.057>.
- (61) Liu, J.-L.; Wong, M.-H. Pharmaceuticals and Personal Care Products (PPCPs): A Review on Environmental Contamination in China. *Environ. Int.* **2013**, *59*, 208–224. <https://doi.org/10.1016/j.envint.2013.06.012>.
- (62) Feng, L.; van Hullebusch, E. D.; Rodrigo, M. A.; Esposito, G.; Oturan, M. A. Removal of Residual Anti-Inflammatory and Analgesic Pharmaceuticals from Aqueous Systems by Electrochemical Advanced Oxidation Processes. A Review. *Chem. Eng. J.* **2013**, *228*, 944–964. <https://doi.org/10.1016/j.cej.2013.05.061>.
- (63) Bartolomeu, M.; Neves, M. G. P. M. S.; Faustino, M. A. F.; Almeida, A. Wastewater Chemical Contaminants: Remediation by Advanced Oxidation Processes. *Photochem. Photobiol. Sci.* **2018**, *17* (11), 1573–1598. <https://doi.org/10.1039/c8pp00249e>.
- (64) Sewnet, A.; Abebe, M.; Asaithambi, P.; Alemayehu, E. Visible-Light-Driven g-C₃N₄/TiO₂ Based Heterojunction Nanocomposites for Photocatalytic Degradation of Organic Dyes in Wastewater: A Review. *Air Soil Water Res.* **2022**, *15*, 11786221221117266. <https://doi.org/10.1177/11786221221117266>.
- (65) Dominguez, S.; Ribao, P.; Rivero, M. J.; Ortiz, I. Influence of Radiation and TiO₂ Concentration on the Hydroxyl Radicals Generation in a Photocatalytic LED Reactor. Application to Dodecylbenzenesulfonate Degradation. *Photocatal. Sci. Appl.* **2015**, *178*, 165–169. <https://doi.org/10.1016/j.apcatb.2014.09.072>.
- (66) Villaluz, F. J. A.; de Luna, M. D. G.; Colades, J. I.; Garcia-Segura, S.; Lu, M.-C. Removal of 4-Chlorophenol by Visible-Light Photocatalysis Using Ammonium Iron(II) Sulfate-Doped Nano-Titania. *Process Saf. Environ. Prot.* **2019**, *125*, 121–128. <https://doi.org/10.1016/j.psep.2019.03.001>.
- (67) Yang, X.; Sun, H.; Li, G.; An, T.; Choi, W. Fouling of TiO₂ Induced by Natural Organic Matters during Photocatalytic Water Treatment: Mechanisms and Regeneration Strategy. *Appl. Catal. B Environ.* **2021**, *294*, 120252. <https://doi.org/10.1016/j.apcatb.2021.120252>.
- (68) Hernández, R.; Hernández-Reséndiz, J. R.; Cruz-Ramírez, M.; Velázquez-Castillo, R.; Escobar-Alarcón, L.; Ortiz-Frade, L.; Esquivel, K. Au-TiO₂ Synthesized by a Microwave- and Sonochemistry-Assisted Sol-Gel Method: Characterization and Application as Photocatalyst. *Catalysts* **2020**, *10* (9). <https://doi.org/10.3390/catal10091052>.
- (69) Kaehler, T. Nanotechnology: Basic Concepts and Definitions. *Clin. Chem.* **1994**, *40* (9), 1797–1799.

- (70) Daniel, M.-C.; Astruc, D. Gold Nanoparticles: Assembly, Supramolecular Chemistry, Quantum-Size-Related Properties, and Applications toward Biology, Catalysis, and Nanotechnology. *Chem. Rev.* **2004**, *104* (1), 293–346. <https://doi.org/10.1021/cr030698+>.
- (71) Rao, C. N. R.; Cheetham, A. K. Science and Technology of Nanomaterials: Current Status and Future Prospects. *J. Mater. Chem.* **2001**, *11* (12), 2887–2894. <https://doi.org/10.1039/B105058N>.
- (72) NATIONAL NANOTECHNOLOGY INITIATIVE - LEADING TO THE NEXT INDUSTRIAL REVOLUTION. *Microscale Thermophys. Eng.* **2000**, *4* (3), 205–212. <https://doi.org/10.1080/10893950050148160>.
- (73) Nasrollahzadeh, M.; Sajadi, S. M.; Sajjadi, M.; Issaabadi, Z. Chapter 1 - An Introduction to Nanotechnology. In *Interface Science and Technology*; Nasrollahzadeh, M., Sajadi, S. M., Sajjadi, M., Issaabadi, Z., Atarod, M., Eds.; Elsevier, 2019; Vol. 28, pp 1–27. <https://doi.org/10.1016/B978-0-12-813586-0.00001-8>.
- (74) Mansoori, G.; Fauzi Soelaiman, T. Nanotechnology — An Introduction for the Standards Community. *J. ASTM Int.* **2005**, *2* (6), 1–22. <https://doi.org/10.1520/JAI13110>.
- (75) Tasciotti, E.; Sakamoto, J.; Ferrari, M. Conference Scene: Nanotechnology and Medicine: The next Big Thing Is Really Small. *Nanomed.* **2009**, *4* (6), 619–621. <https://doi.org/10.2217/nnm.09.49>.
- (76) Cele, N.; Ray, S. S. Recent Progress on Nafion-Based Nanocomposite Membranes for Fuel Cell Applications. *Macromol. Mater. Eng.* **2009**, *294* (11), 719–738. <https://doi.org/10.1002/mame.200900143>.
- (77) Devanathan, R. Recent Developments in Proton Exchange Membranes for Fuel Cells. *Energy Environ. Sci.* **2008**, *1* (1), 101–119. <https://doi.org/10.1039/B808149M>.
- (78) *Nanobiotechnology: Concepts, Applications and Perspectives*; Niemeyer, C. M., Mirkin, C. A., Eds.; Wiley-VCH: Weinheim, 2004.
- (79) Bueno, C. C.; Garcia, P. S.; Steffens, C.; Deda, D. K.; de Lima Leite, F. 5 - Nanosensors. In *Nanoscience and its Applications*; Da Róz, A. L., Ferreira, M., de Lima Leite, F., Oliveira, O. N., Eds.; William Andrew Publishing, 2017; pp 121–153. <https://doi.org/10.1016/B978-0-323-49780-0.00005-3>.
- (80) Teragundi, A.; Nanjundeswaraswamy, D. Literature Review on Synthesis of ZnO Nano Particles Using Natural and Synthetic Methods. **2018**.
- (81) Gubala, V.; Johnston, L. J.; Liu, Z.; Krug, H.; Moore, C. J.; Ober, C. K.; Schwenk, M.; Vert, M. Engineered Nanomaterials and Human Health: Part 1. Preparation, Functionalization and Characterization (IUPAC Technical Report). **2018**, *90* (8), 1283–1324. <https://doi.org/10.1515/pac-2017-0101>.
- (82) Gaffet, E. " NanoMatériaux : Différentes Voies de Synthèse, Propriétés, Applications et Marchés " ". *ADSP Actual. Doss. En Santé Publique* **2008**, *64*, 18–23.

- (83) Iqbal, P.; Preece, J. A.; Mendes, P. M. Nanotechnology: The “Top-Down” and “Bottom-Up” Approaches. In *Supramolecular Chemistry*; 2012. <https://doi.org/10.1002/9780470661345.smc195>.
- (84) Farooq, S. A.; Raina, A.; Mohan, S.; Arvind Singh, R.; Jayalakshmi, S.; Irfan Ul Haq, M. Nanostructured Coatings: Review on Processing Techniques, Corrosion Behaviour and Tribological Performance. *Nanomaterials* **2022**, *12* (8). <https://doi.org/10.3390/nano12081323>.
- (85) Moustafa, E.-A.; Noah, A.; Beshay, K.; Sultan, L.; Essam, M.; Nouh, O. Investigating the Effect of Various Nanomaterials on the Wettability of Sandstone Reservoir. *World J. Eng. Technol.* **2015**, *03* (03), 116–126. <https://doi.org/10.4236/wjet.2015.33013>.
- (86) Gaffet, E. *Nanomaterials : Effects on the Environment and Human Health*; 2006.
- (87) Gaffet, E. Nanomaterials : A Review of the Definitions, Applications, Health Effects. How to Implement Secure Development Nanomatériaux : Une Revue Desdéfinitions, Des Applications, Des Effets Sanitaires et Des Moyens à Mettre En Oeuvre Pour Un Développement Sécurisé. *Comptes Rendus Académie Sci. - Phys.* **2011**, *12*, 648–658.
- (88) HARRAT Zouaoui Rabie. Analyse Du Comportement Mécanique Des Poutres Des Ponts En Béton Armé Renforcé Par Des Nanoparticules de Silice, 2021.
- (89) *Les nanomatériaux manufacturés - Brochure - INRS*. <https://www.inrs.fr/media.html?refINRS=ED%206050> (accessed 2024-03-30).
- (90) Chapter 1 - Basic Properties and Measuring Methods of Nanoparticles. In *Nanoparticle Technology Handbook (Third Edition)*; Naito, M., Yokoyama, T., Hosokawa, K., Nogi, K., Eds.; Elsevier, 2018; pp 3–47. <https://doi.org/10.1016/B978-0-444-64110-6.00001-9>.
- (91) Benzakour, M. Étude Structurale Par Spectroscopie d’absorption X Des Nanoparticules d’oxyde d’alcalinoterreux Réactifs : Application à La Destruction de Gaz Polluants et Toxiques, 2003.
- (92) Alhalili, Z. Metal Oxides Nanoparticles: General Structural Description, Chemical, Physical, and Biological Synthesis Methods, Role in Pesticides and Heavy Metal Removal through Wastewater Treatment. *Molecules* **2023**, *28* (7). <https://doi.org/10.3390/molecules28073086>.
- (93) Koch, C. C. Top-Down Synthesis Of Nanostructured Materials: Mechanical And Thermal Processing Methods. *Rev.Adv.Mater.Sci* **2003**, *5*, 91–99.
- (94) Matsui, S. Focused-Ion-Beam Chemical-Vapor-Deposition (FIB-CVD). In *Encyclopedia of Nanotechnology*; Bhushan, B., Ed.; Springer Netherlands: Dordrecht, 2012; pp 866–876. https://doi.org/10.1007/978-90-481-9751-4_230.
- (95) Wang, Y.; Xia, Y. Bottom-Up and Top-Down Approaches to the Synthesis of Monodispersed Spherical Colloids of Low Melting-Point Metals. *Nano Lett. - NANO LETT* **2004**, *4*. <https://doi.org/10.1021/nl048689j>.
- (96) Roopan, S. Devastated Crops: Multifunctional Efficacy for the Production of Nanoparticles,. *J. Nanomater.* **2013**, 2013. <https://doi.org/10.1155/2013/951858>.

- (97) Djurišić, A. B.; Chen, X.; Leung, Y. H.; Man Ching Ng, A. ZnO Nanostructures: Growth, Properties and Applications. *J. Mater. Chem.* **2012**, *22* (14), 6526. <https://doi.org/10.1039/c2jm15548f>.
- (98) Fan, Z.; Lu, J. G. Zinc Oxide Nanostructures: Synthesis and Properties. *J. Nanosci. Nanotechnol.* **2005**, *5* (10), 1561–1573. <https://doi.org/10.1166/jnn.2005.182>.
- (99) Kumar, S. S.; Venkateswarlu, P.; Rao, V. R.; Rao, G. N. Synthesis, Characterization and Optical Properties of Zinc Oxide Nanoparticles. *Int. Nano Lett.* **2013**, *3* (1), 30. <https://doi.org/10.1186/2228-5326-3-30>.
- (100) Jiang, J.; Pi, J.; Cai, J. The Advancing of Zinc Oxide Nanoparticles for Biomedical Applications. *Bioinorg. Chem. Appl.* **2018**, *2018*, 1062562. <https://doi.org/10.1155/2018/1062562>.
- (101) Sirelkhatim, A.; Mahmud, S.; Seeni, A.; Kaus, N. H. M.; Ann, L. C.; Bakhori, S. K. M.; Hasan, H.; Mohamad, D. Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism. *Nano-Micro Lett.* **2015**, *7* (3), 219–242. <https://doi.org/10.1007/s40820-015-0040-x>.
- (102) Nagajyothi, P. C.; Cha, S. J.; Yang, I. J.; Sreekanth, T. V. M.; Kim, K. J.; Shin, H. M. Antioxidant and Anti-Inflammatory Activities of Zinc Oxide Nanoparticles Synthesized Using Polygala Tenuifolia Root Extract. *J. Photochem. Photobiol. B* **2015**, *146*, 10–17. <https://doi.org/10.1016/j.jphotobiol.2015.02.008>.
- (103) Agarwal, H.; Nakara, A.; Shanmugam, V. K. Anti-Inflammatory Mechanism of Various Metal and Metal Oxide Nanoparticles Synthesized Using Plant Extracts: A Review. *Biomed. Pharmacother.* **2019**, *109*, 2561–2572. <https://doi.org/10.1016/j.biopha.2018.11.116>.
- (104) Basnet, P.; Inakhunbi Chanu, T.; Samanta, D.; Chatterjee, S. A Review on Bio-Synthesized Zinc Oxide Nanoparticles Using Plant Extracts as Reductants and Stabilizing Agents. *J. Photochem. Photobiol. B* **2018**, *183*, 201–221. <https://doi.org/10.1016/j.jphotobiol.2018.04.036>.
- (105) Ibrahim, H. M. M. Green Synthesis and Characterization of Silver Nanoparticles Using Banana Peel Extract and Their Antimicrobial Activity against Representative Microorganisms. *J. Radiat. Res. Appl. Sci.* **2015**, *8* (3), 265–275. <https://doi.org/10.1016/j.jrras.2015.01.007>.
- (106) Sharmila, G.; Muthukumar, C.; Sandiya, K.; Santhiya, S.; Pradeep, R. S.; Kumar, N. M.; Suriyanarayanan, N.; Thirumarimurugan, M. Biosynthesis, Characterization, and Antibacterial Activity of Zinc Oxide Nanoparticles Derived from Bauhinia Tomentosa Leaf Extract. *J. Nanostructure Chem.* **2018**, *8* (3), 293–299. <https://doi.org/10.1007/s40097-018-0271-8>.
- (107) Luque, P.; Soto-Robles, C. A.; Nava, O.; Gómez, C.; Castro-Beltrán, A.; Garrafa Galvez, H.; Vilchis-Nestor, A.; Olivas, A. Green Synthesis of Zinc Oxide Nanoparticles Using Citrus Sinensis Extract. *J. Mater. Sci. Mater. Electron.* **2018**, *29*. <https://doi.org/10.1007/s10854-018-9015-2>.
- (108) Kokila, T.; Ramesh, P. S.; Geetha, D. Biosynthesis of Silver Nanoparticles from Cavendish Banana Peel Extract and Its Antibacterial and Free Radical Scavenging Assay: A Novel Biological Approach. *Appl. Nanosci.* **2015**, *5* (8), 911–920. <https://doi.org/10.1007/s13204-015-0401-2>.

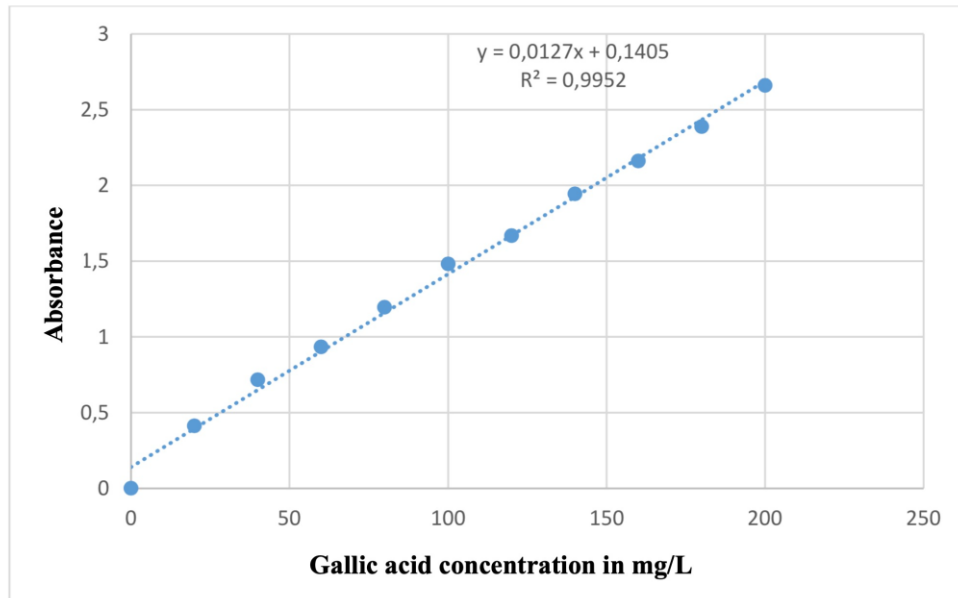
- (109) Lopez-Miranda, J. L.; Molina, G. A.; González-Reyna, M. A.; España-Sánchez, B. L.; Esparza, R.; Silva, R.; Estévez, M. Antibacterial and Anti-Inflammatory Properties of ZnO Nanoparticles Synthesized by a Green Method Using Sargassum Extracts. *Int. J. Mol. Sci.* **2023**, *24* (2). <https://doi.org/10.3390/ijms24021474>.
- (110) Slman, D. K.; Abdul jalill, R.; Abd, A. Biosynthesis of Zinc Oxide Nanoparticles by Hot Aqueous Extract of Allium Sativum Plants. *J. Pharm. Sci. Res.* **2018**, *10*, 1590–1596.
- (111) Doble, M.; Kruthiventi, A. K. *Green Chemistry and Processes*; Elsevier ; Academic Press: Amsterdam : Burlington, Mass, 2007.
- (112) Siddique, S.; Nawaz, S.; Muhammad, F.; Akhtar, B.; Aslam, B. Phytochemical Screening and In-Vitro Evaluation of Pharmacological Activities of Peels of Musa Sapientum and Carica Papaya Fruit. *Nat. Prod. Res.* **2018**, *32* (11), 1333–1336. <https://doi.org/10.1080/14786419.2017.1342089>.
- (113) Essien, J. P.; Akpan, E. J.; Essien, E. P. Studies on Mould Growth and Biomass Production Using Waste Banana Peel. *Bioresour. Technol.* **2005**, *96* (13), 1451–1456. <https://doi.org/10.1016/j.biortech.2004.12.004>.
- (114) Clarke, W. P.; Radnidge, P.; Lai, T. E.; Jensen, P. D.; Hardin, M. T. Digestion of Waste Bananas to Generate Energy in Australia. *Waste Manag.* **2008**, *28* (3), 527–533. <https://doi.org/10.1016/j.wasman.2007.01.012>.
- (115) Doran, I.; Sen, B.; Kaya, Z. The Effects of Compost Prepared from Waste Material of Banana on the Growth, Yield and Quality Properties of Banana Plants. *J. Environ. Biol.* **2005**, *26* (1), 7–12.
- (116) Happi Emaga, T.; Ronkart, S. N.; Robert, C.; Wathélet, B.; Paquot, M. Characterisation of Pectins Extracted from Banana Peels (Musa AAA) under Different Conditions Using an Experimental Design. *Food Chem.* **2008**, *108* (2), 463–471. <https://doi.org/10.1016/j.foodchem.2007.10.078>.
- (117) Ituen, E.; Mkpenie, V.; Ekemini, E. Adsorptive Fe-Nanoparticles Mediated by Musa Sapientum Peels Extract as Anticorrosion Additive for Aqueous Oilfield Descaling Solution. *Sci. Afr.* **2019**, *3*, e00075. <https://doi.org/10.1016/j.sciaf.2019.e00075>.
- (118) Durmuş, A.; Çolak, H.; Karaköse, E. Production and Examination of ZnO Thin Film for First Time Using Green Synthesized Method from Aqueous Citrus Reticulata Peel Extract. *J. Alloys Compd.* **2019**, *809*, 151813. <https://doi.org/10.1016/j.jallcom.2019.151813>.
- (119) Çolak, H.; Karaköse, E.; Duman, F. High Optoelectronic and Antimicrobial Performances of Green Synthesized ZnO Nanoparticles Using Aesculus Hippocastanum. *Environ. Chem. Lett.* **2017**. <https://doi.org/10.1007/s10311-017-0629-z>.
- (120) Karaköse, E.; Çolak, H.; Duman, F. Green Synthesis and Antimicrobial Activity of ZnO Nanostructures Punica Granatum Shell Extract. **2017**, *6* (3), 317–323. <https://doi.org/10.1515/gps-2016-0190>.

- (121) Wong, S. P.; Leong, L. P.; William Koh, J. H. Antioxidant Activities of Aqueous Extracts of Selected Plants. *Food Chem.* **2006**, *99* (4), 775–783. <https://doi.org/10.1016/j.foodchem.2005.07.058>.
- (122) Zhishen, J.; Mengcheng, T.; Jianming, W. The Determination of Flavonoid Contents in Mulberry and Their Scavenging Effects on Superoxide Radicals. *Food Chem.* **1999**, *64* (4), 555–559. [https://doi.org/10.1016/S0308-8146\(98\)00102-2](https://doi.org/10.1016/S0308-8146(98)00102-2).
- (123) Ribéreau-Gayon, Pascal. *Les composés phénoliques des végétaux*, Dunod. Paris.; 1968.
- (124) Christine Morand, M.-J. A.-C. *Les Polyphénols En Agroalimentaire*, Lavoisier 75008 Paris.; 2006.
- (125) Parimelazhagan, T. *Pharmacological Assays of Plant-Based Natural Products*; Progress in Drug Research; Springer International Publishing: Cham, 2016; Vol. 71. <https://doi.org/10.1007/978-3-319-26811-8>.
- (126) Miliauskas, G.; Venskutonis, P. R.; van Beek, T. A. Screening of Radical Scavenging Activity of Some Medicinal and Aromatic Plant Extracts. *Food Chem.* **2004**, *85* (2), 231–237. <https://doi.org/10.1016/j.foodchem.2003.05.007>.
- (127) Price, M. L.; Van Scoyoc, S.; Butler, L. G. A Critical Evaluation of the Vanillin Reaction as an Assay for Tannin in Sorghum Grain. *J. Agric. Food Chem.* **1978**, *26* (5), 1214–1218. <https://doi.org/10.1021/jf60219a031>.
- (128) Bruneton, J.; Poupon, E. *Pharmacognosie, phytochimie, plantes médicinales*, 5e éd.; Lavoisier Tec & doc: Paris, 2016.
- (129) Mole, S.; Waterman, P. G. A Critical Analysis of Techniques for Measuring Tannins in Ecological Studies. I. Techniques for Chemically Defining Tannins. *Oecologia* **1987**, *72* (1), 137–147.
- (130) Sen, K.; Datta, J. K.; Mondal, N. K. Glyphosate Adsorption by Eucalyptus Camaldulensis Bark-Mediated Char and Optimization through Response Surface Modeling. *Appl. Water Sci.* **2019**, *9* (7), 162. <https://doi.org/10.1007/s13201-019-1036-3>.
- (131) Almasi, A.; Mohammadi, M.; Baniamerian, F.; Berizi, Z.; Almasi, M. H.; Pariz, Z. Modeling of Antibiotic Degradation in Sonophotocatalytic Process, Increasing Biodegradability and Process Optimization by Response Surface Methodology (RSM). *Int. J. Environ. Sci. Technol.* **2019**, *16* (12), 8437–8448. <https://doi.org/10.1007/s13762-019-02307-5>.
- (132) Marfu'ah, S.; Rohma, S. M.; Fanani, F.; Hidayati, E. N.; Nitasari, D. W.; Primadi, T. R.; Ciptawati, E.; Sumari, S.; Fajaroh, F. Green Synthesis of ZnO Nanoparticles by Using Banana Peel Extract as Capping Agent and Its Bacterial Activity. *IOP Conf. Ser. Mater. Sci. Eng.* **2020**, *833* (1), 012076. <https://doi.org/10.1088/1757-899X/833/1/012076>.
- (133) Hamed, O. Fabrication of Zinc Oxide Nanoparticles and Films by Banana Peel Extract Food Waste and Investigation on Their Antioxidant and Antibacterial Activities.

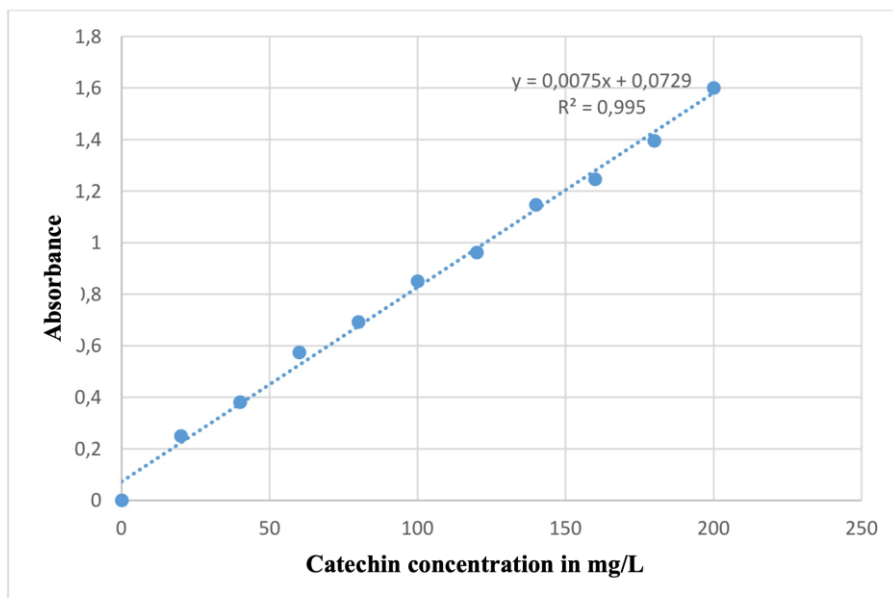
- (134) Anal, A. K.; Jaisanti, S.; Noomhorm, A. Enhanced Yield of Phenolic Extracts from Banana Peels (*Musa Acuminata* Colla AAA) and Cinnamon Barks (*Cinnamomum Varum*) and Their Antioxidative Potentials in Fish Oil. *J. Food Sci. Technol.* **2014**, *51* (10), 2632–2639. <https://doi.org/10.1007/s13197-012-0793-x>.
- (135) Mohd Zaini, H.; Roslan, J.; Saallah, S.; Munsu, E.; Sulaiman, N. S.; Pindi, W. Banana Peels as a Bioactive Ingredient and Its Potential Application in the Food Industry. *J. Funct. Foods* **2022**, *92*, 105054. <https://doi.org/10.1016/j.jff.2022.105054>.
- (136) Bashmil, Y. M.; Ali, A.; BK, A.; Dunshea, F. R.; Suleria, H. A. R. Screening and Characterization of Phenolic Compounds from Australian Grown Bananas and Their Antioxidant Capacity. *Antioxidants* **2021**, *10* (10). <https://doi.org/10.3390/antiox10101521>.
- (137) Youssef M.A; Eman A.; Abdel Khafar; Abeir M.F. Elbaz. Evaluation the Bioactive Compounds Extracted from Dried Banana (*Musa Sp.*) Peels Which Obtained by Different Drying Methods. *Curr. Sci. Int.* **2018**, *07* (02), Pages: 135-148.
- (138) Stalikas, C. D. Extraction, Separation, and Detection Methods for Phenolic Acids and Flavonoids. *J. Sep. Sci.* **2007**, *30* (18), 3268–3295. <https://doi.org/10.1002/jssc.200700261>.
- (139) Rhazi, N.; Hannache, H.; Oumam, M.; Sesbou, A.; Charrier, B.; Pizzi, A.; Charrier-El Bouhtoury, F. Green Extraction Process of Tannins Obtained from Moroccan Acacia Mollissima Barks by Microwave: Modeling and Optimization of the Process Using the Response Surface Methodology RSM. *Arab. J. Chem.* **2019**, *12* (8), 2668–2684. <https://doi.org/10.1016/j.arabjc.2015.04.032>.
- (140) Hussien, N. A.; Al Malki, J. S.; Al Harthy, F. A. R.; Mazi, A. W.; Al Shadadi, J. A. A. Sustainable Eco-Friendly Synthesis of Zinc Oxide Nanoparticles Using Banana Peel and Date Seed Extracts, Characterization, and Cytotoxicity Evaluation. *Sustainability* **2023**, *15* (13). <https://doi.org/10.3390/su15139864>.
- (141) Mahmoudi, G.; Sufimahmoudi, E.; Sajadi, S. M. Bioactive Metal Oxide Nanoparticles from Some Common Fruit Wastes and *Euphorbia Condylcarpa* Plant. *Food Sci. Nutr.* **2020**, *8* (10), 5521–5531. <https://doi.org/10.1002/fsn3.1853>.
- (142) Bhardwaj, K.; Singh, A. K. Bio-Waste and Natural Resource Mediated Eco-Friendly Synthesis of Zinc Oxide Nanoparticles and Their Photocatalytic Application against Dyes Contaminated Water. *Chem. Eng. J. Adv.* **2023**, *16*, 100536. <https://doi.org/10.1016/j.ceja.2023.100536>.
- (143) Okpara, E. C.; Fayemi, O. E.; Sherif, E.-S. M.; Junaedi, H.; Ebenso, E. E. Green Wastes Mediated Zinc Oxide Nanoparticles: Synthesis, Characterization and Electrochemical Studies. *Materials* **2020**, *13* (19). <https://doi.org/10.3390/ma13194241>.

7. APPENDIX

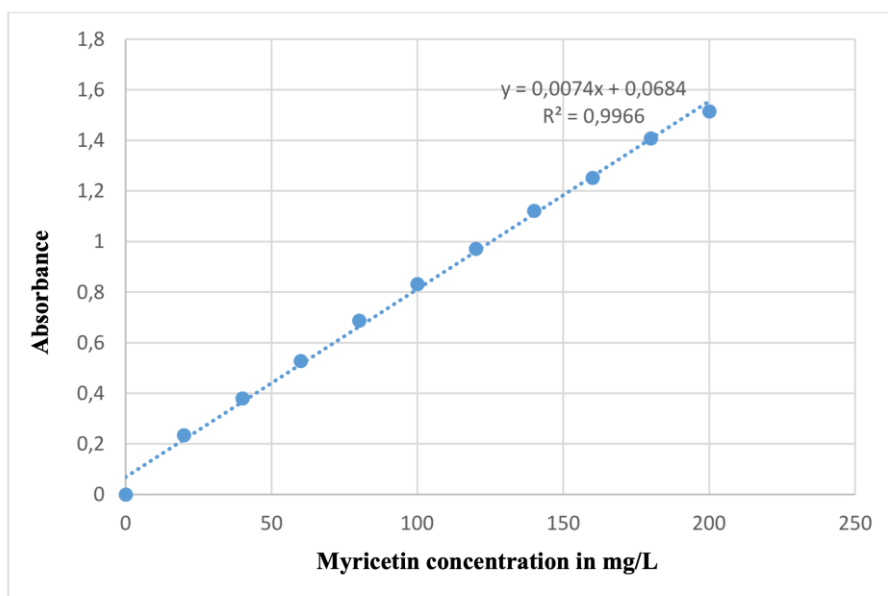
Appendix A: Phenolic compounds calibration curves



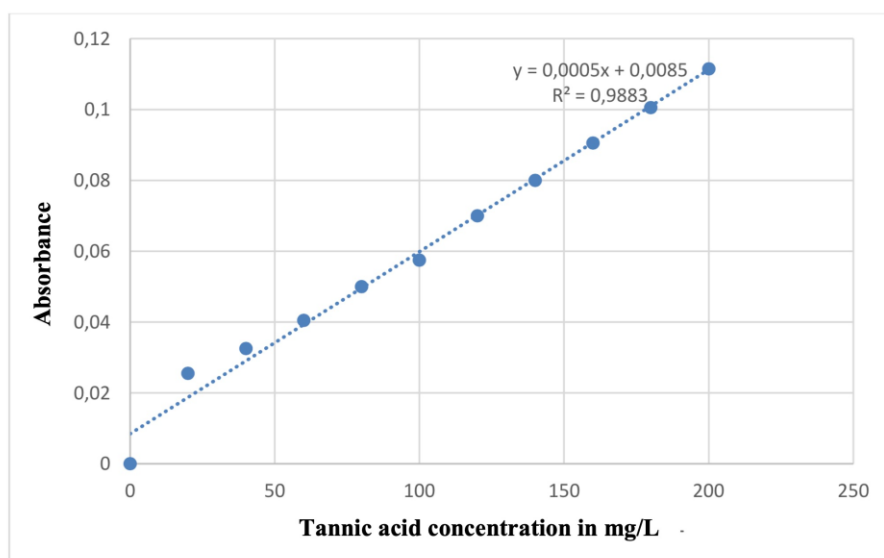
A. 1 : Total phenols calibration curve



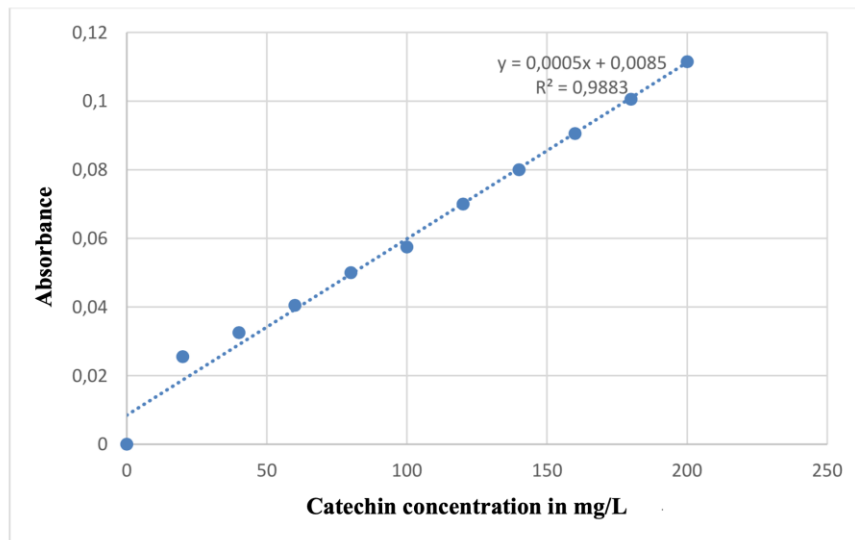
A. 2 : Flavonoid's calibration curve



A. 3 : Flavonols calibration curve



A. 4 : Hydrolysable tannins calibration curve



A. 5 : Condensed tannins calibration curve