

UNIVERSIDAD POLITÉCNICA DE MADRID
Escuela Técnica Superior de Ingenieros Industriales



Experimental research and development of
atmospheric plasma generators for pathogen
inactivation of surfaces and environments

DOCTORAL THESIS

Submitted for the degree of Doctor by:

Sonia Ruiz Trujillo

Bachelor's and Master's degree in Chemical Engineering

Madrid, 2025



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Under the supervision of:

Dr. Andrés Díaz Lantada

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Title: Experimental research and development of atmospheric plasma generators for pathogen inactivation of surfaces and environments

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Thesis Defense Date:

This thesis has been supported by CEDRION C.T.I. S.L and the Consejería de Investigación y Educación (Programa de Doctorados Industriales, Comunidad Autónoma de Madrid) through an industrial doctoral scholarship (IND2020/IND-17525), carried out together with Universidad Politécnica de Madrid.

A las personas que forman parte de mi felicidad.

"No hay revolución más silenciosa que la de una molécula alterada por una descarga."

Abstract

The transmission of microorganisms through airborne particles and contaminated surfaces represents a critical public health challenge, particularly in environments where individuals are in close proximity, such as hospitals, schools, and public transportation systems. Microbial pathogens, including bacteria, viruses, and fungi, significantly contribute to healthcare-associated infections and respiratory diseases, resulting in considerable morbidity, mortality, and economic costs worldwide. Current mitigation strategies, such as air filtration, ultraviolet germicidal irradiation (UVGI), and antimicrobial surface treatments, have demonstrated effectiveness but face limitations in terms of efficiency, scalability, and real-world application.

This thesis explores the development, modeling, and optimization of atmospheric non-thermal plasma (ANTP) systems as an innovative approach for microbial decontamination in air and on surfaces. This research focuses on studying and industrially developing ANTP devices and configurations generated via corona discharge. Initially, the systems are electrically, chemically, and fluid dynamically perspectives based on geometric and electrical parameters to determine the relationships between these parameters and the mechanisms for system optimization in open environments. Furthermore, the chemistry generated is simulated for closed systems, creating a framework that aligns with experimental data and facilitates system scalability within the operational range.

These findings are subsequently applied to the microbial inactivation of airborne and surface microorganisms, correlating the chemical compositions generated with the degree of inactivation achieved. The study also identifies and optimizes the required doses (species concentration $\times$ time) to achieve specific inactivation levels, reaching sterilization on surfaces made of different materials.

ANTP technology utilizes ionized gases generated by corona discharges to inactivate microorganisms by disrupting their cell membranes and genetic material. These discharges produce highly oxidizing chemical species, such as free radicals, ozone, and nitrogen dioxide, which are responsible for microbial inactivation. Simultaneously, the electrohydrodynamic (EHD) phenomenon generates a flow known as ionic wind, eliminating the need for mechanical components to produce airflow. This approach achieves high levels of inactivation in a single pass and enables the design of completely silent systems, with no moving parts and low energy consumption.

The findings aim to establish robust, energy-efficient, scalable, and versatile ANTP based solutions for infection control, contributing to the global effort to reduce the burden of infectious diseases and improve public health resilience.

Resumen

La transmisión de microorganismos a través de partículas en el aire y superficies contaminadas representa un desafío crítico para la salud pública, particularmente en entornos donde las personas están en estrecha proximidad, como hospitales, escuelas y sistemas de transporte público. Los patógenos microbianos, incluidos bacterias, virus y hongos, contribuyen significativamente a las infecciones asociadas a la atención sanitaria (IRAS) y a enfermedades respiratorias, generando una considerable carga de morbilidad, mortalidad y costos económicos a nivel mundial. Las estrategias actuales de mitigación, como la filtración de aire, la irradiación germicida ultravioleta (UVGI) y los tratamientos antimicrobianos para superficies, han demostrado eficacia, pero enfrentan limitaciones en términos de eficiencia, escalabilidad y aplicación práctica en entornos reales.

Esta tesis explora el desarrollo, modelado y optimización de sistemas de plasma atmosférico no térmico (ANTP) como un enfoque novedoso para la descontaminación microbiana en el aire y en superficies. En este trabajo de investigación, se estudian y desarrollan industrialmente dispositivos y configuraciones de ANTP generados mediante descarga corona. Inicialmente, los sistemas se analizan desde la perspectiva eléctrica, química y fluidodinámica, basándose en parámetros geométricos y eléctricos, con el objetivo de determinar las relaciones entre estos parámetros y los mecanismos de funcionamiento y optimización en sistemas abiertos. Además, la química generada se simula para sistemas cerrados, creando un modelo que permita ajustar los datos reales obtenidos y facilite su escalado dentro del rango de trabajo.

A partir de estas conclusiones, se aplican los sistemas desarrollados a la inactivación microbiana de microorganismos aerotransportados y en superficies, correlacionando las composiciones químicas generadas con el grado de inactivación alcanzado. Se estudian y optimizan las dosis necesarias (concentración de especies $\times$ tiempo) para lograr un grado específico de inactivación, alcanzando incluso la esterilización en superficies de diferentes materiales.

La tecnología ANTP utiliza gases ionizados generados mediante descargas tipo corona para inactivar microorganismos mediante la alteración de sus membranas celulares y material genético. Estas descargas producen especies químicas altamente oxidantes, como radicales libres, ozono y dióxido de nitrógeno, responsables de la inactivación microbiana. Simultáneamente, el fenómeno de dinámica electrohidrodinámica (EHD) genera un flujo conocido como viento iónico, que elimina la necesidad de componentes mecánicos externos para la generación de flujo de aire. Este enfoque permite alcanzar elevados grados de inactivación en una única pasada y posibilita el diseño de sistemas completamente silenciosos, sin partes móviles y con bajo consumo energético.

Los hallazgos buscan establecer soluciones robustas, eficientes en términos energéticos, escalables y versátiles basadas en ANTP para el control de infecciones, contribuyendo al esfuerzo global por reducir la carga de enfermedades infecciosas y mejorar la resiliencia en salud pública.

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Abbreviations and acronyms

AC	Alternating Current
ANTP	Atmospheric Non-Thermal Plasma
AUC	Area Under Curve
BHI	Brain Heart Infusion
CD	Corona Discharge
CT	Concentration · Time
CVCCs	Current Voltage Characteristic Curves
DBD	Dielectric Barrier Discharge
DC	Direct Current
DMEM	Dulbecco's Modified Eagle Medium
EHD	Electro-Hidro-Dynamic
FBS	Fetal Bovine Serum
NCD	Negative Corona Discharge
OES	Optical Emission Spectroscopy
PBS	Phosphate-Buffered Saline
PCD	Positive Corona Discharge
RNS	Reactive Nitrogen Species
RONs	Reactive Oxygen and Nitrogen Species
ROS	Reactive Oxygen Species
SPS	Second Positive System

Chapter 1

Introduction

1.1 Background

The transmission of microorganisms through airborne particles and contaminated surfaces represents a significant public health challenge, particularly in settings where individuals are in close proximity, such as hospitals, schools, and public transport systems. Microbial pathogens, including bacteria, viruses, and fungi, can persist in the air or on surfaces for extended periods, posing risks for both direct and indirect transmission of diseases. Understanding and mitigating these risks is crucial to preventing outbreaks and reducing the burden of infectious diseases globally and within Europe, where studies indicate that 6% of patients in acute care hospitals experience at least one healthcare-associated infection (HAI) [1].

The health impacts of airborne and surface transmission of microorganisms are profound, contributing to both acute and chronic illnesses [2]. Respiratory infections, including influenza, tuberculosis, and COVID-19, are among the most well-documented consequences of airborne pathogen transmission. These diseases impose a significant global burden: the World Health Organization (WHO) estimates that seasonal influenza alone causes up to 650,000 respiratory deaths annually. Tuberculosis, another airborne disease, is responsible for approximately 1.6 million deaths each year [2]. The recent COVID-19 pandemic has further highlighted the devastating effects of airborne transmission, with millions of deaths worldwide and long-term health complications for many survivors, including respiratory, cardiovascular, and neurological issues [3]. Additionally, surface-mediated transmission contributes to outbreaks of healthcare-associated infections, such as those caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile*, which are associated with high morbidity, mortality, and healthcare costs.

Nosocomial infections, also known as healthcare-associated infections, represent a critical subset of diseases linked to both airborne and surface transmission [4]. These infections often occur in hospital and clinical settings, where immunocompromised patients are particularly vulnerable. According to the Centers for Disease Control and Prevention (CDC), approximately 1 in 31 hospital patients in the United States acquires at least one HAI during their stay. The consequences of nosocomial infections extend beyond individual health, placing significant

economic burdens on healthcare systems. For instance, the annual cost of managing HAIs in the United States is estimated to exceed \$28 billion, encompassing direct medical expenses and associated loss of productivity [5]. In Europe, HAIs affect approximately 8.9 million patients annually, according to a report by the European Centre for Disease Prevention and Control [5]. These infections result in thousands of deaths and impose significant financial strain on healthcare systems, with estimates reaching billions of euros in associated costs.

The mechanisms by which microorganisms are transported in the air and on surfaces are varied and complex. In airborne transmission, pathogens are typically expelled from an infected individual through respiratory activities such as coughing, sneezing, talking, or even breathing. These activities generate droplets of different sizes, ranging from larger respiratory droplets that settle quickly to smaller aerosols that can remain suspended in the air for extended periods. Environmental factors such as temperature, humidity, and air circulation influence the persistence and spread of these particles. For example, studies have shown that low humidity and stagnant air conditions can enhance the survival and transport of airborne pathogens. On surfaces, microorganisms can adhere to a wide range of materials, including plastics, metals, and textiles, and their persistence depends on factors such as surface properties, temperature, and the presence of organic material [6]. For example, *Staphylococcus aureus* and *Escherichia coli* can survive for several days on materials like plastic and stainless, while viruses such as influenza can remain infectious on nonporous surfaces for 24-48 hours [7],

The COVID-19 pandemic has underscored the importance of addressing airborne and surface transmission of pathogens. Although SARS-CoV-2, the virus responsible for COVID-19, is primarily transmitted through respiratory droplets and aerosols, evidence has also highlighted the role of fomites—inanimate objects or surfaces—in contributing to the spread of the virus [8]. This dual mode of transmission brought global attention to the critical need for effective systems to reduce microbial loads both in the air and on surfaces. However, the pandemic should not be viewed in isolation. Many other pathogens, including those causing influenza, tuberculosis, and MRSA, also rely on similar transmission mechanisms. Thus, the broader scientific and technological community must focus on developing and implementing comprehensive strategies to mitigate these risks.

Various systems and technologies have been developed to address airborne and surface contamination. Among them, air filtration and purification systems, such as high-efficiency particulate air (HEPA) filters and ultraviolet germicidal irradiation (UVGI) systems, have been widely employed to reduce airborne microbial loads. HEPA filters physically trap airborne particles, including microorganisms, while UVGI systems utilize ultraviolet light to inactivate pathogens by disrupting their genetic material [9]. In addition, newer technologies, such as photocatalytic oxidation and bipolar ionization, are being explored for their potential to enhance air disinfection. The effectiveness of these systems is supported by evidence; for instance, studies have demonstrated that HEPA filtration can remove up to 99.97% of airborne particles as small as 0.3 microns, while UVGI systems have been shown to inactivate a broad spectrum of microorganisms, including SARS-CoV-2 [10].

The described technologies present several disadvantages when implemented in real-world environments. For example, HEPA filters only capture microorganisms without inactivating

them, which poses a risk of recontamination, either through the release of retained particles or during system maintenance. Some systems, such as UV or photocatalysis, require prolonged exposure times to achieve an adequate level of inactivation for air treatment.

Additionally, these systems often depend on external mechanisms to propel air, which can generate noise and vibrations. This is a significant drawback for installation in sensitive areas, such as hospitals or medical clinics. Furthermore, UV lamps can emit heat, causing discomfort, and their direct exposure is harmful to health.

Atmospheric non-thermal plasma (ANTP) has emerged as a promising alternative for microbial decontamination in both air and surfaces. This technology uses highly reactive ionized gases, including ions, radicals, and electrons, to inactivate microorganisms by disrupting their cell membranes and damaging their genetic material. Additionally, ionic wind is utilized to generate the ionic wind, eliminating the need for external mechanisms and avoiding noise generation. ANTP operates at room temperature, making it suitable for use on temperature-sensitive surfaces and in enclosed spaces where conventional methods may be less effective. Recent studies have demonstrated that ANTP can rapidly reduce microbial loads, including antibiotic-resistant bacteria and enveloped viruses, without generating harmful residues or byproducts [11, 12]. Additionally, its energy efficiency and scalability make it a compelling option for integration into existing infection control frameworks.

Surface decontamination strategies also play a vital role in reducing the transmission of microorganisms. Traditional cleaning agents, including alcohol-based disinfectants and quaternary ammonium compounds, remain a cornerstone of surface hygiene. These disinfectants work by disrupting microbial cell membranes or denaturing proteins, effectively killing or inactivating pathogens. Advancements in materials science have led to the development of antimicrobial coatings that provide long-lasting protection by continuously inactivating pathogens upon contact. Furthermore, automated disinfection systems, such as ultraviolet-C (UV-C) robots, have gained traction in healthcare and high-traffic environments for their ability to efficiently disinfect large surface areas. Studies have demonstrated that UV-C disinfection can reduce surface contamination by up to 99.9%, significantly lowering the risk of fomite-mediated transmission [13].

Despite these advancements, challenges remain in optimizing these systems for real-world applications. Factors such as cost, energy efficiency, ease of implementation, and potential side effects, such as the generation of harmful byproducts, must be carefully considered.

1.2 Review of technologies for air and surface disinfection

The persistence of pathogenic microorganisms in indoor air and on environmental surfaces poses a major challenge to infection control in hospital, public transport, schools and industrial facilities. Numerous studies have confirmed the role of contaminated environments in the transmission of pathogens, including bacteria (e.g., *Staphylococcus aureus*, *Mycobacterium tuberculosis*, etc), viruses (e.g., influenza, SARS-CoV-2), and fungal spores [14, 15].

Conventional disinfection methods - such as chemical biocides, ultraviolet radiation, and thermal treatment - have been historically employed to reduce microbial loads [7]. However, these methods often face limitations in terms of material compatibility, human safety, and efficacy under real-world conditions. This has driven the development of alternative technologies that operate under milder conditions, with lower chemical consumption, and can be deployed in real-time within occupied spaces.

This section aims to provide a brief overview of current and emerging air and surface disinfection strategies.

1.2.1 Conventional disinfection technologies

This section presents the principal conventional disinfection methods, highlighting their mechanisms of action, operational performance, and context-specific limitations. These technologies are widely employed in healthcare, transportation, and industrial settings to reduce microbial contamination on surfaces and in air, but each presents technical and practical constraints that can limit their effectiveness under real-world conditions.

Chemical disinfectants are among the most widely applied strategies for microbial control on surfaces and, to a lesser extent, in air. Compounds such as alcohols, chlorine derivatives, hydrogen peroxide, and quaternary ammonium compounds (QACs) function primarily by disrupting microbial membranes, denaturing proteins, and inducing oxidative damage. Ethanol and isopropanol, for example, are effective against a broad range of pathogens and are commonly used in hand sanitizers and surface sprays due to their rapid action and low cost [16]. Chlorine-based agents, such as sodium hypochlorite, are potent oxidants with established efficacy even against resistant viruses and spores [17]. Despite their broad-spectrum capabilities, chemical disinfectants may leave toxic residues, contribute to antimicrobial resistance, and degrade certain materials. Furthermore, when used as aerosols for air disinfection, they pose health risks related to inhalation and environmental exposure [15].

Ultraviolet germicidal irradiation (UVGI), particularly in the UV-C range (typically 254 nm), is a non-chemical disinfection method that inactivates microorganisms by inducing DNA and RNA damage through the formation of pyrimidine dimers. UVGI systems are applied in surface disinfection, water purification, and increasingly for air treatment in HVAC systems and healthcare environments. Their broad-spectrum efficacy against bacteria, fungi, and viruses—including SARS-CoV-2—has been widely demonstrated [18]. However, UVGI effectiveness depends on direct line-of-sight exposure, and any surface shadowed or covered by particulate matter may receive insufficient irradiation. Furthermore, the method requires a minimum exposure time to achieve effective microbial reduction, particularly in flowing air, where residence time may be limited. This temporal dependency reduces UVGI’s efficiency for dynamic air disinfection compared to technologies capable of real-time or continuous treatment. Additionally, UV-C light poses phototoxic risks to human tissues, restricting its use in occupied environments unless low-dose or far-UVC systems (e.g., 222 nm) are employed under controlled conditions [19].

Thermal disinfection is based on elevated temperatures to denature proteins and destroy microbial structures. Common methods include steam sterilization (autoclave) and dry

heat. These techniques are highly effective and can inactivate even heat-resistant bacterial spores. They are widely used for the sterilization of medical equipment and laboratory instruments. However, their energy requirements are substantial and their incompatibility with heat-sensitive materials precludes broader environmental use. Additionally, thermal methods are not suitable for air disinfection in dynamic or occupied settings.

Filtration, particularly using high-efficiency particulate air (HEPA) filters, constitutes a physical approach to air decontamination. HEPA filters operate through mechanisms such as interception, impaction, and diffusion to capture airborne particles as small as $0.3\ \mu\text{m}$ with an efficiency of 99.97%. These systems are commonly implemented in clean rooms, operating theaters, and commercial aircraft cabins. While HEPA filters effectively remove pathogens from the air, they do not inactivate them, which may pose a risk during filter replacement or failure. Furthermore, regular maintenance is required to ensure consistent performance, and the filters do not address gaseous contaminants or ultrafine particles outside their rated capture range.

Overall, while these conventional technologies provide a critical foundation for environmental disinfection, their limitations—whether due to safety concerns, application constraints, or lack of real-time inactivation—have prompted growing interest in complementary or alternative technologies capable of overcoming these shortcomings.

1.2.2 Emerging disinfection technologies

In response to the limitations of conventional disinfection methods, several emerging technologies have been developed that offer novel mechanisms of action and improved adaptability in dynamic or sensitive environments. These include photocatalytic oxidation (PCO), bipolar ionization, and atmospheric non-thermal plasma (ANTP), among others, each with distinct characteristics in terms of mechanism, efficacy, and safety.

Photocatalytic oxidation (PCO) uses semiconductor materials—most commonly titanium dioxide (TiO_2)—that are activated by ultraviolet or visible light to generate reactive oxygen species (ROS) such as hydroxyl radicals ($\cdot\text{OH}$) and superoxide anions ($\text{O}_2^- \cdot$). These species oxidize and decompose organic molecules and microbial structures, leading to effective inactivation [20, 21]. PCO has demonstrated broad-spectrum antimicrobial activity against viruses, bacteria, and fungi, and has been deployed in both surface and air disinfection systems [22]. Its main advantages include continuous operation, minimal chemical input, and potential for long-term application in indoor environments. However, the process is sensitive to environmental conditions such as humidity and light intensity, and incomplete oxidation of VOCs may result in the formation of toxic intermediates [23]. Recent developments include doping TiO_2 with metals or non-metals to extend photocatalytic activity into the visible light range, enhancing practical deployment [24].

Bipolar ionization technology generates both positively and negatively charged ions that interact with air contaminants. These ions can neutralize pathogens through the formation of ROS or by disrupting cell membranes, and promote the agglomeration of fine particles, aiding in their capture by filters [25]. Bipolar ionization is increasingly incorporated into HVAC systems due to its compatibility with occupied spaces and potential for continuous air

treatment. Nevertheless, the performance of bipolar ionization systems can be highly variable depending on environmental parameters and device design [26]. Additionally, some systems may generate low levels of ozone, prompting regulatory scrutiny and the need for careful monitoring.

Among the most promising emerging technologies is atmospheric non-thermal plasma (ANTP), a partially ionized gas generated at ambient temperature and pressure. ANTP produces a complex mixture of reactive oxygen and nitrogen species (RONS), UV photons, electrons, and ions. These components act synergistically to induce structural and oxidative damage in microbial cells, including lipid peroxidation, protein denaturation, and DNA fragmentation [27, 28]. Due to its non-thermal nature, ANTP can be safely applied to heat-sensitive materials such as textiles, polymers, or food packaging. Studies have shown that ANTP effectively inactivates a broad range of pathogens, including bacterial spores and enveloped viruses such as SARS-CoV-2 [29, 30]. ANTP has been employed using devices such as dielectric barrier discharges (DBD) and plasma jets, both in surface treatment and direct air disinfection. Its advantages include high efficacy, fast response, and chemical-free operation. However, ensuring homogeneous plasma distribution and controlling RONS concentrations remain engineering challenges that affect scalability [31, 32]. Therefore, ongoing research and development in the modeling and mechanistic understanding of ANTP systems is essential to tailor the production of reactive species to the specific microbial inactivation requirements. Additionally, advances in system design should aim at achieving modular and adaptable configurations capable of functioning effectively in complex, real-world environments. The potential formation of undesirable by-products, such as ozone, could be mitigated through the integration of catalytic post-treatment stages, which selectively neutralize these species without compromising antimicrobial efficacy.

Hybrid systems that combine ANTP with other disinfection methods, such as ultraviolet-C (UV-C) light or hydrogen peroxide vapor, are currently being investigated for their synergistic antimicrobial effects. These multi-modal systems show promise in critical environments like healthcare settings and high-containment laboratories, where conventional single-action methods may be insufficient [33, 34].

Overall, these emerging technologies represent a significant evolution in environmental disinfection strategies. They offer new capabilities for sustainable, chemical-free, and in-situ pathogen control, particularly in applications where conventional technologies are either insufficient or impractical. Further research is needed to optimize system configurations, standardize efficacy metrics, and confirm long-term safety before widespread commercial deployment.

1.3 Motivation and objectives of the thesis

In this context described in the background section, the development of systems that combine a high degree of microbial inactivation with the generation of airflow—without the need for external elements or moving parts emerges as a promising alternative to currently available inactivation systems. Furthermore, the creation of robust, optimized systems with a high level of development offers significant versatility, enabling them to adapt effectively to diverse needs in real-world environments.

The objective of this thesis is **to develop, model, and optimize atmospheric non-thermal systems that combine high efficiency in airflow generation with a significant degree of microbial inactivation, both for air treatment and surface disinfection**. Achieving this requires a thorough understanding, precise control, and accurate modeling of both the ionic wind generated by atmospheric plasma devices and the production of chemical species, as well as the synergy between these phenomena.

The modeling is based on the geometric and electrical parameters of the plasma systems, aiming to provide robustness and versatility, enabling their implementation across various industrial sectors.

- Electrical, fluid dynamic, and chemical modeling of plasma systems in an **open system** (ambient air) based on electrical, geometric, and operational mode parameters, to understand the dependencies among these factors. Identify the mechanisms that optimize chemical production and fluid dynamics, as well as the synergy between them.
- Electrical, fluid dynamic, and chemical modeling of plasma systems in air within a **closed system** or chamber, based on electrical, geometric, and operational mode parameters. Determine potential chemical transitions to define operational ranges according to real-world needs.
- Evaluation of the efficacy of plasma systems against airborne microorganisms based on geometric and electrical parameters in an **open system**. Correlation and modeling between the neutral chemical species generated in the discharge zone and the degree of microbial inactivation.
- Evaluation of the efficacy of plasma systems against microorganisms in a **closed system** (hermetic chamber), based on the same parameters. Determination of the effective chemical composition required to achieve a specific degree of microbial reduction. Adjustment of microbial kinetic models and evaluation of treatment effectiveness on surfaces made of various materials to assess deviations from theoretical behavior.
- Determination of effective doses (chemical concentration by exposure time) required to achieve a specific degree of microbial reduction, and how to achieve such concentrations based on the analyzed parameters. This enables the proposal of different plasma system alternatives to achieve a specific degree of inactivation. With this knowledge, flexibility and control are gained for implementing systems that can be adjusted to the diverse requirements of industrial environments.
- Analysis, implementation, and adjustment of requirements to ensure that the systems

match the conditions for real-world application (e.g., control of generated species using catalysts for safe use in the presence of people) while maintaining efficacy levels. Conduct testing in real-world environments.

- Disseminate and communicate the findings of proposed research and promote the long lasting impact of the initiated research line and the collaboration between Universidad Politénica de Madrid and Cedrión S.L.

1.3.1 Context of the PhD thesis

The doctoral thesis of Ms. Sonia Ruiz Trujillo is conducted within the framework of the industrial doctorate project: “Investigation of chemical species generated by atmospheric corona discharge and application to the development of plasma-based sterilization devices for environments and surfaces” (ref. IND2020/IND-17525). This project is funded by the Regional Government of Madrid through its "2020 Call for Industrial Doctorate Projects." The industrial doctorate project and the doctoral thesis are developed in collaboration between the Polytechnic University of Madrid (hereinafter UPM) and Cedrión: Ingeniería Técnica y Consultoría S.L. (hereinafter Cedrión). Prof. Andrés Díaz Lantada serves as the academic supervisor and co-director of the thesis on behalf of UPM, while Dr. Patricia Vega Martínez acts as the industrial supervisor and co-director on behalf of Cedrión. The project arises from a long-standing and fruitful collaboration between Cedrión and UPM since the company's foundation.

Additionally, the doctoral candidate conducted a research stay at the Korean Advanced Institute of Science and Technology (KAIST) under the supervision of Prof. Wonho Choe, where she gained expertise in advanced experimental techniques. Below is an overview of Cedrión's trajectory and its collaboration with UPM, which led to the development of this doctoral thesis.

Cedrión: Ingeniería Técnica y Consultoría S.L. is a start-up established in February 2017 under the Business Creation Program of the Polytechnic University of Madrid (Actúa UPM). Its activities focus on researching, developing, and manufacturing cooling devices for embedded electronic systems in the aerospace sector. Cedrión also participates in the ESA-BIC business incubation program, a joint initiative by the European Space Agency and the Regional Government of Madrid. This program provides economic, technical, and commercial assistance to entrepreneurs to help them advance their businesses using space technology.

Cedrión's primary focus is the development of cold plasma-based cooling devices using corona discharge technology (also referred to as non-thermal atmospheric plasmas). These devices are 2 to 7 times lighter than conventional finned heat sinks or fan-assisted coolers. They are also completely silent and contain no moving parts.

The scientific foundation of Cedrión's advanced cooling elements lies in Electro-Hydro-Dynamics (EHD). When an electric field is applied between two conductors, ions are transported in the direction of the field—a phenomenon known as ionic wind. During this process, these ions push fluid particles, such as air, between the conductors. This enables the movement of fluid between desired points while generating a cloud of ions and electrons referred to

as plasma. While there are multiple methods to generate EHD forces, Cedrión uses corona discharge due to its superior stability and ease of control.

The cold plasma generated triggers a series of chemical reactions in the air, resulting in the formation of various chemical species such as ozone, nitrogen oxides, hydrogen peroxide, and free radicals. The synergistic effects of these species make this technology highly promising for applications such as bacterial and viral inactivation, deodorization of refrigeration chambers and food storage spaces, and air and surface purification. Given that the generated species can be harmful to health, they can be removed using catalysts such as activated carbon or manganese dioxide, ensuring the technology is safe for use in the presence of people [35–37].

The Product Development Laboratory (LDP) of the Department of Mechanical Engineering at the Polytechnic University of Madrid was established in 1997 by Prof. Pilar Lafont Morgado to support students in materializing their projects, assist companies in the design and validation of new products, and research innovative materials and processes. Originally conceived as a digital design and fabrication laboratory, it served as a precursor to the “fablabs” proposed by Gershenfeld at MIT in 2003. Since 2015, the laboratory has been directed by Prof. Andrés Díaz Lantada, who has focused its research on developing healthcare devices by leveraging the geometric complexity, multifunctionality, and functional integration enabled by additive manufacturing technologies and the processing of active or intelligent materials.

In line with the LDP-UPM’s mission to support industrial innovation, it has collaborated with Cedrión since 1997 on the design, modeling, fabrication, and experimental testing of prototypes for their cold plasma generation systems. A precursor to this thesis is the doctoral dissertation of Dr. María del Mar Cogollo de Cádiz, titled “Research and development of electrodes for electro-hydro-dynamic technology applied to aerospace cooling,” conducted under another industrial doctorate project from the 2018 call by the Regional Government of Madrid.

The new doctoral thesis builds upon Cedrión’s previous developments, exploring for the first time the use of these technologies for sterilization and their introduction into the medical sector. Throughout the doctoral research, additional applications in the food industry have emerged, expanding Cedrión’s scope of action and ensuring the sustainability of the collaboration between both institutions.

1.4 Research methodology and PhD thesis outline

The PhD thesis is divided into two distinct parts, each further subdivided into two sections:

The first part focuses on the characterization, modeling, and optimization of the fluidic and chemical behavior of plasma devices in ambient air, based on geometric and electrical parameters. This is divided into open-system modeling and closed-system modeling, where the mechanisms of generation are fundamentally different yet complementary.

The second part addresses modeling in relation to microbial inactivation, also based on the previously mentioned parameters. Similarly, this analysis is conducted in both open and confined systems, with each scenario exhibiting markedly distinct mechanisms.

The correlation between these two parts provides the foundation for establishing the rules necessary for the subsequent scaling, adaptation, and implementation of the technology in real-world environments.

The developments have been carried out in the Division of Machine Engineering (DIM) at the School of Industrial Engineering and the company Cedrión. Additionally, the Department of Hygiene and Food Technology at the University of León contributed to the experiments with bacteria, while the National Center for Biotechnology of the Spanish National Research Council (CSIC) supported the virus testing.

Chapter 1 contextualizes the thesis by presenting the issues related to infections transmitted via air and surfaces, the limitations of conventional solutions, and their associated challenges. The objectives of the thesis and its content are outlined in detail.

Chapter 2 reviews the state of the art in non-thermal atmospheric plasma, focusing on corona discharge to understand the processes involved in the development of prototypes and the control and modeling of the generated systems. It summarizes the chemical processes involved in plasma generation and its applications.

Chapter 3 details the materials and methods used and developed during the experimental research process. It also describes the methodology followed for the design and fabrication of prototypes.

Chapter 4 presents the parametric analysis and electrochemical and fluidic modeling of corona discharge plasma systems in an open system. It analyzes both the neutral species generated (with ozone and nitrogen dioxide as references) in the ionic wind and the active species produced within the discharge using Optical Emission Spectroscopy. This analysis supports the design and development of adaptable devices for various applications and identifies optimal configurations for later microbial inactivation studies.

A similar analysis is conducted in **Chapter 5**, this time focusing on the electrochemical study within a closed chamber. A model is developed and adjusted for the observed kinetic behavior using nonlinear adjustment methods. The most efficient designs for achieving higher concentrations of oxidizing species are evaluated for their subsequent application in the inactivation of microorganisms on surfaces.

Chapter 6 applies the findings from **Chapter 4** to the inactivation of airborne microorganisms. The efficacy of viral inactivation is evaluated based on various parameters of atmospheric plasma systems.

Similarly, **Chapter 7** extends the results from **Chapter 5** to explore the inactivation of microorganisms in a closed chamber using a parametric approach. The obtained chemical concentration data is correlated with the degree of inactivation. Microbial kinetics are modeled, and the relationship between the dose delivered and the reduction achieved is analyzed. This provides a foundation for scaling and implementing the technology in real-world environments.

Finally, the conclusions drawn from the research conducted throughout the PhD thesis are summarized in **Chapter 8**. Additionally, new and innovative research directions and initiatives emerging from the work developed in the thesis are proposed.

Chapter 2

State of the art

Chapter 2 presents a review of the development of non-thermal atmospheric plasma devices, focusing on discharges in air. It outlines the definitions and general characteristics of corona discharge systems, highlighting their production mechanisms and the factors that have the greatest influence on the studied phenomenon. Special attention is given to understanding the chemistry generated within these systems under ambient temperature, pressure, and humidity conditions. Additionally, the chapter explores potential applications of atmospheric plasma, with a focus on the inactivation of airborne microorganisms and surface sterilization.

2.1 Context of atmospheric plasma and electrohydrodynamics

The scientific exploration of electrical phenomena in fluids and gases forms the foundation of our understanding of non-thermal atmospheric plasma, ionic wind, and electrohydrodynamics (EHD). Early records of such phenomena date back to ancient Greek philosophers, who noted the existence of electrical effects. However, systematic investigations into these processes began in earnest in the 17th century. A pivotal moment occurred in 1629 when Niccolò Cabeo documented the electrostatic repulsion of dust particles near an electrified body, providing one of the earliest observations of EHD phenomena [38].

The progression of plasma science gained momentum with the invention of high-voltage generators, such as the device created by Otto von Guericke in 1672. His experiments, including observations of faint flashes and clicking sounds, marked the first recorded instances of corona discharge. Shortly after, Francis Hauksbee coined the term "ionic wind" in 1719, describing the sensation of airflow generated by an electrified tube, as noted in his *Physico-Mechanical Experiments on Various Subjects*. This work inspired prominent scientists such as Isaac Newton, who confirmed Hauksbee's findings in the second edition of *Opticks*, naming the phenomenon "electric wind" [39].

The first practical applications of ionic wind soon followed. In 1750, Wilson harnessed the phenomenon to rotate a pinwheel, an early demonstration of electrohydrodynamic motion. Shortly after, Hamilton developed the "electric fly", a wire-based device that mimicked the

flapping of wings under high voltage. These devices, initially explained through principles of electrical repulsion and Newton’s third law, laid the groundwork for understanding the forces driving EHD phenomena. Later refinements by Tiberius Cavallo and Michael Faraday clarified the role of charged particles in generating such effects, with Faraday identifying ionic wind as a process of momentum transfer between charged and neutral particles [40, 41].

The 19th century brought further advancements in understanding plasma and EHD, culminating in foundational works by James Clerk Maxwell. In his seminal *Treatise on Electricity and Magnetism* (1873) [42], Maxwell provided the most comprehensive analysis of electric wind at the time, describing how air becomes conductive near a high electric field. These insights were further substantiated by discoveries such as Svante Arrhenius’s dissociation of sodium chloride into ions and J.J. Thomson’s identification of the electron in 1897, revolutionizing the study of gaseous conductivity and charged particles.

Plasma itself, as a distinct state of matter, was formally introduced in 1928 by Irving Langmuir, though its non-thermal variant—where ambient temperatures coexist with high-energy electrons—was not extensively studied until the mid-20th century. This type of plasma has since gained prominence for its ability to drive chemical reactions efficiently without global temperature increases, making it invaluable in environmental and medical applications [43].

Concurrently, advancements in electrohydrodynamics gained momentum. In 1929, Peek published groundbreaking work on corona discharge, deriving equations to calculate electric field current near the discharge point [44]. Further studies by Stuetzer and Robinson expanded our understanding of gaseous ionization, while Maxwell’s earlier qualitative models remained relevant, forming the basis of modern EHD theories. As computational capabilities grew, researchers turned to numerical methods and experiments to address the complexity of EHD phenomena. Kalman et al. and Rashkovan et al. explored electrode configurations for efficient heat transfer, demonstrating the practical viability of EHD in cooling electronic components.

In recent decades, the convergence of numerical simulations and experimental research has solidified the role of EHD and non-thermal plasma in advanced technologies. Applications range from air purification and medical sterilization to cutting-edge cooling systems for electronics and propulsion systems for spacecraft. These applications demand robust, modelable, and reproducible knowledge to address emerging challenges effectively. The interdisciplinary nature of these fields continues to drive innovation, bridging physics, engineering, and materials science.

2.2 Plasma: The fourth state of matter

Plasma, often referred to as the fourth state of matter, is an ionized medium composed of a mixture of free electrons, positive and negative ions, neutral atoms, and molecules in various excitation states. This state of matter forms when sufficient energy is supplied to a gas to overcome the binding forces that keep electrons attached to their atoms or molecules, generating a mixture of charged and neutral species. This ionization process gives plasma unique properties that distinguish it from conventional states of matter, such as solids, liquids, and gases (2.1) [45].

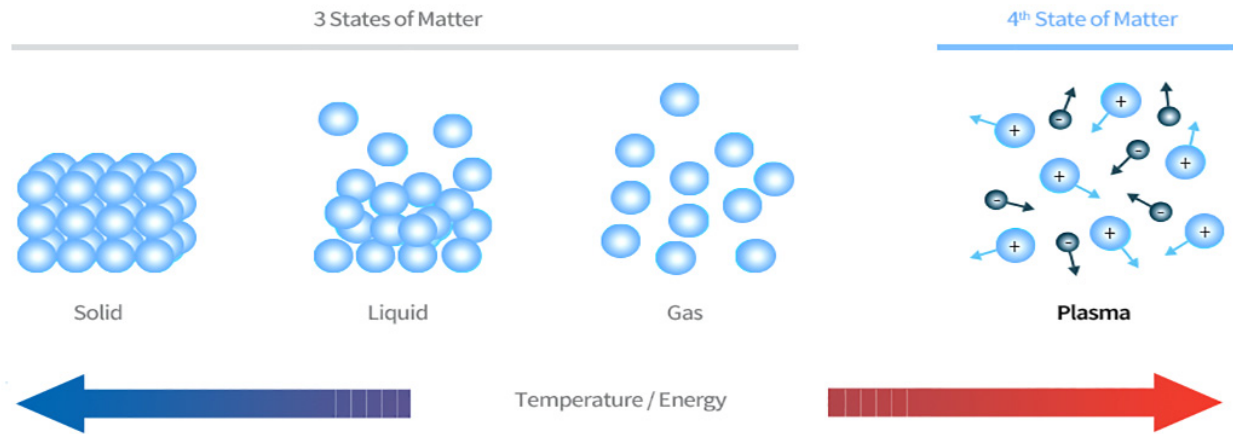


Figure 2.1: States of matter.

From a macroscopic perspective, plasma is characterized by its quasi-electrical neutrality, meaning the density of positive charges is, on average, equal to the density of negative charges. However, at microscopic scales, there may be regions with local charge imbalances that generate internal electric fields. Distinctive properties of plasma include[45]:

- High electrical conductivity: Due to the presence of free electrons and ions, plasma can efficiently conduct electric currents.
- Sensitivity to electromagnetic fields: Plasmas respond to external electric and magnetic fields, allowing for manipulation in various technological applications.
- Radiation emission: Transitions between energy states of atoms and molecules in plasma generate radiation emission across a wide spectrum, from infrared to ultraviolet.

Plasmas can be classified based on various criteria, including their local thermodynamic equilibrium (LTE) and particle density [45] (see Table 2.1).

Table 2.1: Main characteristics of LTE and non-LTE plasma.

Characteristic	LTE Plasmas	Non-LTE Plasmas
Current name	Thermal plasmas	Cold plasmas
Properties	• $T_e = T_h$ • High electron density: 10^{21}–10^{26} m^{-3} • Inelastic collisions between electrons and heavy particles create plasma reactive species, whereas elastic collisions heat the heavy particles (the electrons' energy is consumed).	• $T_e \gg T_h$ • Lower electron density: $< 10^{19}$ m^{-3} • Inelastic collisions between electrons and heavy particles induce plasma chemistry. Heavy particles are slightly heated by a few elastic collisions (hence electrons retain very high energy).
Examples	Arc plasma (core): $T_e = T_h \approx 10,000$ K	Glow discharges: $T_e \approx 10,000$ – $100,000$ K, $T_h \approx 300$ – 1000 K

T_e : electron temperature; T_h : heavy particle (ions and neutrals) temperature.

- Thermal plasmas: In these systems, electrons and heavy particles (ions and neutrals) are in thermodynamic equilibrium ($T_e = T_h$), sharing the same temperature. This type of plasma is found in industrial applications such as arc welding and material cutting processes.
- Non-thermal plasmas: Here, electrons have significantly higher energies than heavy particles ($T_e \gg T_h$), creating a temperature disparity. These plasmas are ideal for applications requiring high chemical reactivity without significantly raising the temperature of the surrounding gas, such as in surface treatments and gas purification [46].

Plasma is a versatile tool used in a wide variety of industrial and scientific fields. Some of the most relevant applications include [45]:

- Aerospace industry: Control of aerodynamic flows using plasma actuators.
- Electronics and microfabrication: Etching and deposition of materials in semiconductor manufacturing processes.
- Energy: Plasma confinement for controlled nuclear fusion research.
- Environment: Gas treatment and surface decontamination.

The study and development of plasma-based systems continue to grow due to their ability to provide innovative solutions to complex problems in science and engineering. A deep understanding of its properties and mechanisms enables the exploration of emerging applications and the improvement of existing ones.

2.3 Atmospheric non-thermal plasmas

Atmospheric non-thermal plasmas, also known as cold plasmas, operate at atmospheric pressure while maintaining a low temperature in the bulk gas phase. These plasmas are characterized by a significant temperature disparity between the electrons, which achieve high kinetic energies, and the heavier particles (ions and neutral species), which remain close to ambient temperatures. This unique feature enables high reactivity without causing substantial thermal damage to surrounding materials, making non-thermal plasmas particularly suitable for delicate applications.

Non-thermal plasmas are typically generated by applying an electric field to a neutral gas, creating ionization through electron collisions. The high-energy electrons are responsible for exciting and ionizing neutral species, producing a chemically reactive environment rich in radicals, excited atoms, and molecules [45].

- **Electron impact ionization:** Free electrons in the plasma gain energy from the electric field and collide with neutral gas molecules, leading to ionization and the production of additional electrons and ions. This cascade process sustains the plasma.
- **Excitation and de-excitation Processes:** High-energy electrons excite neutral gas molecules to higher energy states. These molecules then return to lower energy states, emitting photons and contributing to plasma luminosity.
- **Collisional processes:** Elastic and inelastic collisions between electrons, ions, and neutrals govern energy transfer within the plasma. These processes determine the overall reactivity and stability of the plasma.

Different configurations are employed to create and sustain non-thermal plasmas [45]:

1. **Dielectric Barrier Discharges (DBD):** In this setup, a dielectric material is placed between the electrodes to prevent arc formation and ensure a stable discharge.
2. **Corona Discharges:** These are initiated by applying a high voltage to a pointed or sharp-edged electrode, generating strong localized electric fields that produce plasma.
3. **Plasma Jets:** These devices expel plasma into the surrounding atmosphere, enabling applications in targeted areas.
4. **Microwave-Induced Plasmas (MIP):** Microwaves are used to ionize gases without electrodes, creating uniform and stable plasmas.

Non-thermal plasmas exhibit several advantageous properties, including:

- **High chemical reactivity:** The presence of reactive species such as ozone, hydroxyl radicals, and nitrogen oxides allows for efficient chemical processes.
- **Low thermal impact:** Minimal heating of the surrounding gas enables the treatment of heat-sensitive materials.
- **Versatility:** Non-thermal plasmas can be applied to a wide range of fields, including medicine, environmental science, and materials processing.

The unique properties of non-thermal plasmas have led to their adoption in various industries:

- Medical applications: Plasma is used for sterilization, wound healing, and cancer therapy due to its antimicrobial properties and ability to promote tissue regeneration.
- Environmental applications: Non-thermal plasmas are employed for air and water purification, as well as the removal of pollutants such as volatile organic compounds (VOCs).
- Surface modification: Plasma treatments improve adhesion, wettability, and coating deposition on various materials

2.3.1 Corona discharge and ionic wind generation

Corona discharges are a type of non-thermal plasma generated when a high electric field is applied to a sharp-edged or pointed electrode. This results in the ionization of the surrounding gas, producing a visible corona and a chemically active plasma region. Corona discharges are particularly significant due to their role in generating ionic wind, a phenomenon characterized by the movement of ions in the gas, which transfers momentum to neutral particles and induces a bulk flow [47].

Corona discharges occur when the electric field near a sharp electrode exceeds the breakdown strength of the surrounding gas, initiating ionization processes that sustain the discharge. High-energy electrons are accelerated by the electric field and collide with neutral gas molecules, creating positive ions and additional free electrons in a cascade process. This avalanche effect leads to the continuous production of charged particles near the electrode, sustaining the discharge. Additionally, photons emitted by excited species contribute to the photoionization of nearby neutral molecules, extending the plasma region. The accumulation of ions near the electrode generates space charge effects, which modify the local electric field and influence the stability and extent of the corona discharge. Importantly, the forces at play include Coulomb forces, which arise from the interactions between charged particles. These forces act to repel like charges and attract opposite charges, driving the motion of ions and electrons within the plasma. Coulomb forces, combined with the electric field, play a central role in shaping the behavior and dynamics of the corona discharge [48, 49].

Ionic wind is generated through the transfer of momentum from charged particles in a corona discharge to neutral gas molecules during collisions. When a high electric field is applied near a sharp electrode, it accelerates ions and electrons within the ionization region. These charged particles, driven by the electric field, collide with neutral gas molecules, imparting momentum and creating a net flow of the gas, referred to as ionic wind [50].

The strength of the electric field plays a critical role in determining the efficiency of momentum transfer and the resulting airflow. A stronger electric field increases the density and energy of the ions, leading to more frequent and energetic collisions with neutral molecules. Similarly, the geometry of the electrodes significantly affects the distribution of the electric field. For instance, pointed or sharp-edged electrodes create highly localized fields that enhance ionization and facilitate efficient ionic wind generation. The type of gas used also influences the process, as different gases have varying ionization energies and mobilities. Air is commonly employed

due to its availability and favorable ionization properties.

Corona discharges can be classified into positive and negative types, each characterized by distinct regions and dynamics within the discharge zone. These regions include the ionization region, drift region, and outer region, which collectively govern the behavior and stability of the discharge.

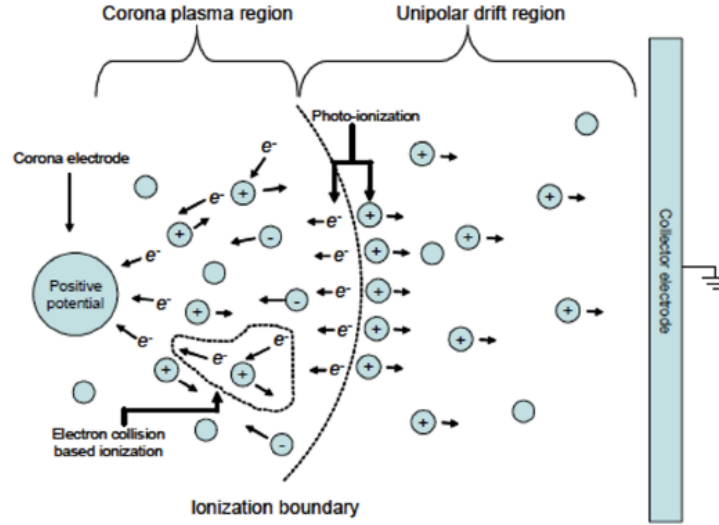


Figure 2.2: Scheme of mechanism of positive corona discharge process with direct current applied for a wire-to-plane electrode configuration [51].

- In positive discharge, the phenomenon occurs when the corona electrode, characterized by its greater curvature, functions as the positively charged component and is subjected to a very high voltage. In contrast, the collector electrode, which is negatively charged, is grounded or maintained at a lower potential. Ionization initiates in the region nearest to the corona electrode once the threshold voltage is exceeded.

Due to the positive charge of the corona electrode, electrons are drawn towards it under the influence of the applied electric field. During this process, these electrons collide with surrounding air molecules, leading to the dissociation of the molecules into free electrons and positive ions. Within the plasma region, this cascade effect results in an electron avalanche directed toward the corona electrode, alongside the formation of positive ion-electron pairs (Figure 2.2).

At the ionization boundary, a photo-ionization process generates additional electrons and ions. Notably, this process is non-local and can occur at locations distant from the corona electrode. In the drift zone, cations formed through collisions in the plasma region are accelerated toward the grounded collector electrode due to Coulomb forces. Simultaneously, any remaining anions in the drift zone are attracted back toward the corona electrode, returning to the plasma region.

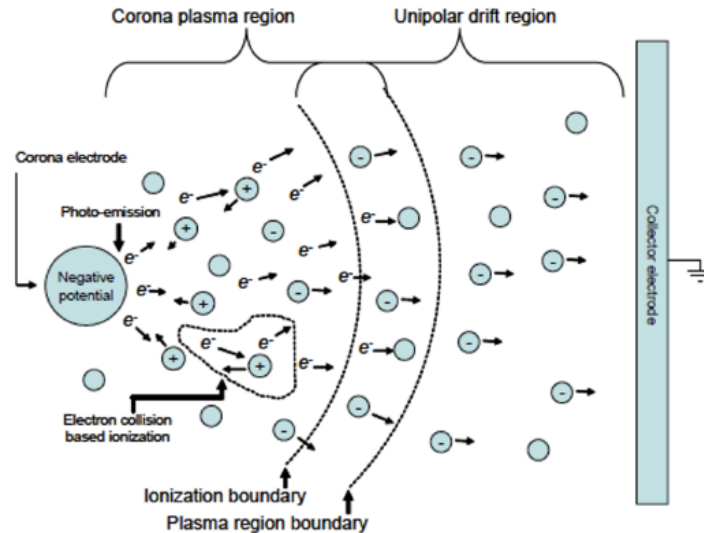


Figure 2.3: Scheme of mechanism of negative corona discharge process with direct current applied for a wire-to-plane electrode configuration [51].

- In negative discharge, the process begins when the high-voltage electrode, characterized by its greater curvature, is subjected to a negative potential, while the collector electrode is positively charged. Unlike positive discharge, the negative discharge is non-uniform, although it quickly distributes along the surface of the ground electrode. Ionization near the corona electrode generates an electron avalanche. In this scenario, free electrons are repelled by the negatively charged high-voltage electrode and move towards the positively charged collector electrode.

Additionally, secondary electrons are produced through photo-emission processes. Electrons are expelled from the plasma zone and directed toward the ground electrode.

Within the drift region, this electron movement results in an expansion of the drift zone, facilitating additional chemical reactions and the production of charged compounds. Electrons with sufficient energy may continue ionizing and surpass the ionization barrier, extending their influence into the drift zone (Figure 2.3).

To address this behavior, many researchers propose the existence of a distinct plasma region boundary specific to negative corona discharge. This boundary begins at the ionization front and extends several millimeters into the drift region. Its presence is essential to initiate certain chemical reactions and achieve discharge stability.

Figure 2.4 displays a schematic of corona discharge regimens in both positive and negative discharge. The progression of both positive and negative discharges can be observed as the applied voltage increases.

The dynamics of the particles differ significantly between the two types of discharges. In positive corona discharges, the motion is dominated by the relatively slow drift of positive ions toward the electrode, resulting in a localized and stable ionization region. Negative corona discharges, however, are characterized by rapid electron avalanches and the generation

of negative ions, creating a more expansive and energetic ionization region [52].

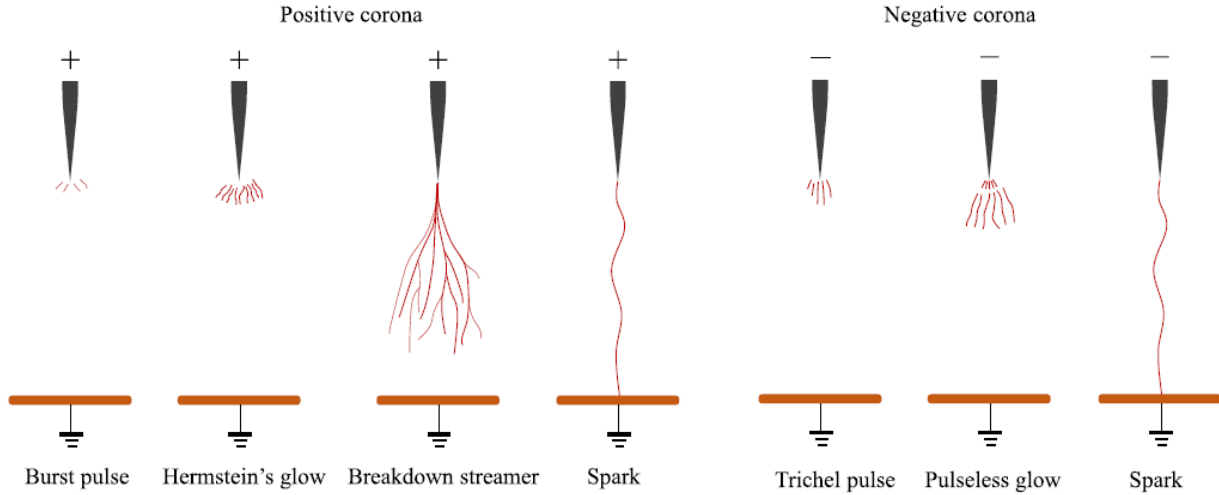


Figure 2.4: Discharge regimes of positive and negative coronas [46].

The distinct characteristics of positive and negative corona discharges make them suitable for different applications. Positive discharges are often used where stability and precision are required, such as in electrostatic precipitation and air purification. Negative discharges, with their higher electron energies, are preferred for processes like ozone generation and surface modification, where intense ionization is beneficial.

Positive corona discharge demonstrates greater efficiency in generating ionic wind and produces fewer chemical compounds. In contrast, the behavior in negative corona discharge is the opposite. The production of free electrons in negative discharge is higher than in positive discharge. This is due to the denser ionization zone in negative discharge, even though the number of electrons generated near the corona electrode is lower. Considering atmospheric air, positive corona discharge generates less ozone and is more energy efficient in its production [53].

The physicochemical processes occurring in the discharge, using air as the ionization medium, are summarized in the Table 2.2.

Regarding the electrode configuration for this type of discharge, two electrodes are required: one that is thin with a highly curved shape, referred to as the corona electrode, and another that is broader, known as the ground or collector electrode. When a high potential difference is applied between these electrodes—typically in the kilovolt range—a strong electric field is established, initiating the discharge process.

Table 2.2: Physico-chemical mechanisms of cold plasma.

Physico-chemical mechanisms of cold plasma	
Mechanism	Description
Photo-ionization	$\text{Photon} + A \rightarrow A^+$
	Ionization of gas molecules due to photonic bombardment by ultraviolet light, radiation, among others.
Excitation	$e^- + A \rightarrow A^* + e^- \rightarrow A + e^- + \text{Photon}$
	The atom promotes to a state of higher internal energy by orbital displacement of its electrons.
Ionization	$e^- + A \rightarrow A^+ + 2e^-$
	If the energy of the electron is even higher, the collision results in the detachment of an electron from the impacted atom.
Recombination	$e^- + A^+ \rightarrow A + \text{Photon}$
	Shock between a positive ion and an electron that produces the release of a photon due to insufficient energy in the process.
Bond with neutral molecule	$A + e^- \rightarrow A^-$
	Low energy free electron collides with a neutral atom and generates a negatively charged compound.

The electrical behavior of the discharge is influenced by several design factors, particularly the geometry and spacing of the electrodes. The separation distance and the shape of the electrodes directly affect the discharge's electrical characteristics, including the voltage at which it occurs. While the dielectric breakdown voltage in air is theoretically about 3 kV/mm under standard conditions, the curvature and configuration of the electrodes can modify this threshold. A critical geometric factor is the aspect ratio, which defines the relationship between the curvature radii of the electrodes or, in the case of pointed tips (such as needles), the ratio of the tip's surface area to its height.

The configuration of the electrodes also plays a pivotal role in shaping the discharge behavior. Figure 2.5 shows the most common corona configuration used for ionic wind generation. The most common are wire-to-plane [54–56], wire-to-cylinder [47], needle-to-plane [57], point-to-grid [58], needle-to-ring [59], blade-to-plane [1]. The wire-to-plane configuration is one of the most commonly employed electrode arrangements. In this design, the discharge occurs within the region between the electrodes, where the wire remains effective along its entire length, provided it maintains a consistent distance from the ground electrode (plane). A significant advantage of this configuration is the creation of a large effective volume for corona discharge, even with a single wire.

However, when multiple wires are used, interference in the electric field between them can occur. To address this issue, each wire must be individually insulated to ensure proper discharge functionality. While this approach effectively prevents interference, it also increases the overall size of the device and may impact air movement within the system.

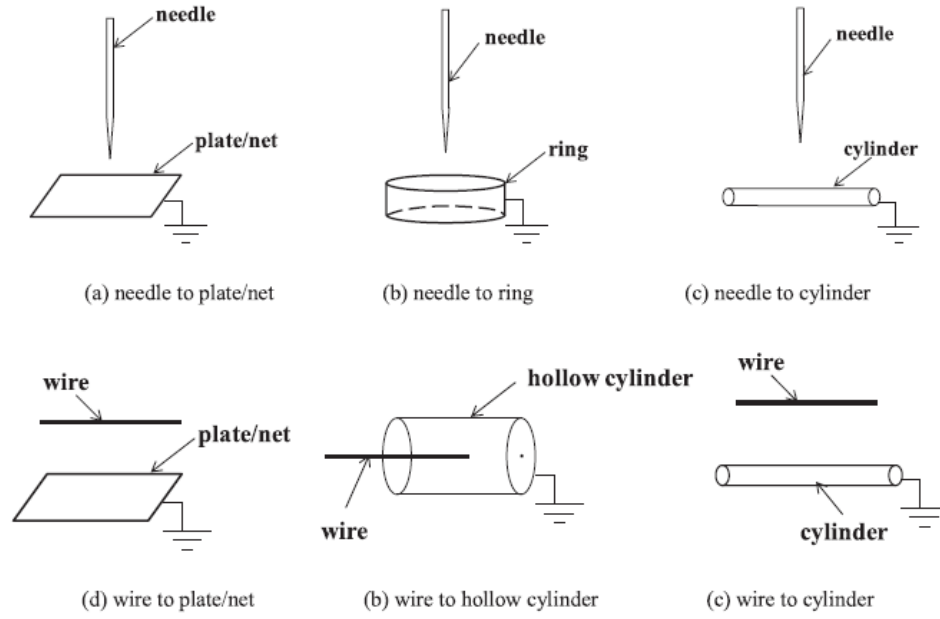


Figure 2.5: Different corona prototypes with several electrode configuration [46].

2.3.2 Chemical reactions

Corona discharge plasmas are complex, non-thermal environments where energetic electrons drive the formation of reactive species, including atomic oxygen (O), ozone (O₃), nitric oxide (NO), nitrogen dioxide (NO₂), hydroxyl radicals (OH), and hydrogen peroxide (H₂O₂). These reactive species result from a combination of dissociation, ionization, excitation, and recombination processes in atmospheric air [60–62]. Additionally, the role of nitrogen molecules in excited states (N₂(A³Σ_u⁺), N₂(C³Π_u)) and ionized nitrogen (N₂⁺) is critical in understanding the chemical dynamics [63, 64].

In plasma systems, it is possible to define two distinct phases based on the characteristics and behavior of the species present: a neutral phase and an active phase. The neutral phase primarily consists of chemically stable and electrically neutral species, while the active phase comprises highly reactive, short-lived, and often charged components. These phases coexist and interact dynamically, shaping the overall chemical and physical properties of the plasma [62, 65].

The neutral phase is predominantly formed by long-lived species that exhibit relatively low reactivity and stability over time. These include molecular gases such as oxygen (O₂) and nitrogen (N₂), which constitute the bulk of atmospheric plasma. Additionally, stable products of plasma-driven reactions, such as ozone (O₃) and nitrogen dioxide (NO₂), are key components of the neutral phase [65–67]. In humid environments, water vapor (H₂O) and its stable derivatives, such as hydrogen peroxide (H₂O₂), also contribute to the neutral phase. These species act as reservoirs, storing the chemical products of the plasma and reflecting its long-term stability and energy distribution [68].

On the other hand, the active phase is characterized by short-lived species with high reactivity. These include free electrons, positive and negative ions (e.g., O₂⁺, O[−]), and neutral radicals such as atomic oxygen (O) and hydroxyl radicals (OH) [66, 69]. These species play a critical role in initiating and propagating chemical reactions within the plasma. Their transient nature means that their concentrations are typically much lower than those of long-lived species, yet their high energy and reactivity enable them to drive rapid chemical transformations, such as the dissociation of molecular gases and the formation of reactive species.

The distinction between long-lived and short-lived species also highlights the interplay between the neutral and active phases. Long-lived species—such as O₃, NO₂, and H₂O₂—accumulate over time and can persist even after the plasma is turned off, allowing them to interact with surfaces or diffuse into the surrounding environment [65]. In contrast, short-lived species, such as electrons, radicals, and ions, exist only within the active plasma region and decay rapidly once the plasma ceases.

This dynamic equilibrium between the neutral and active phases governs the plasma’s overall behavior. For example, long-lived species in the neutral phase often serve as precursors or stabilizers for reactions initiated by short-lived species. In turn, the active phase drives energy transfer and chemical activation, enabling the formation of stable products that accumulate in the neutral phase. Together, these phases define the plasma’s chemical complexity, its ability to modify surfaces, and its effectiveness in applications such as environmental remediation, sterilization, and material processing.

Due to its experimental simplicity, optical emission spectroscopy is the most commonly used diagnostic technique for characterization of the plasma sources. Optical emission spectroscopy probes only the excited levels because it measures the radiation emitted by the excited atoms or molecules in the plasma. At low pressures or in plasmas in thermal equilibrium, interpretation of optical emission spectra is feasible. Due to the plasma conditions, the population density of the probed energy state is related to lower states via the Saha equation and the Boltzmann distribution [69–71]. For non-equilibrium atmospheric pressure plasma, the situation is different. The strong non-equilibrium makes it difficult to interpret the measured spectra and to receive the absolute values of the ground-state densities. Nevertheless, a lot of information can be gained from OES even at atmospheric pressure. The information can be derived from the absolute and relative line intensities, the line widths, the appearance of certain lines attributed to forbidden states or the emission continuum radiation.

OES is employed to capture the optical signature of the excited species within the discharge layer. The spectra reveal that in all cases the excited nitrogen second positive series is the dominant emission, appearing between 290 and 410 nm. No other significant emission lines are observed within the 280-600 nm wavelength range regardless of operating power [70]. Further analysis of the second positive system makes it possible to obtain an estimate of the gas temperature within the discharge layer. It is well-known that under atmospheric pressure conditions, the rotational temperature of excited nitrogen is in thermodynamic equilibrium with the background gas. By comparing the recorded emission spectra against a synthetic spectra calculated at a known temperature an approximation of the gas temperature is obtained.

Beyond UV and thermal effects, it is well-known that air plasma generated at atmospheric pressure creates highly reactive short-lived species, O, OH, N, and O_2^- , which diffuse away from the plasma region, rapidly reacting to form a variety of longer-lived RONS, including O_3 , H_2O_2 , nitric oxide (NO), and other NO_x [72, 73]. The density of some of these species can be quantified from FTIR absorption measurements obtained during plasma operation. Under low power conditions, it can be seen that O_3 is the primary long-lived product of the discharge. O_3 is primarily produced in the discharge layer through a three-body reaction involving atomic oxygen



Conversely, under high power conditions, the composition of the discharge effluent is dominated by NO_x including nitrous oxide (N_2O) and nitrogen dioxide (NO_2). The origins of the transition between O_3 dominated to NO_x dominated discharge chemistries is 2-fold: first, as discharge power increases, the rate of thermal decomposition of O_3 rapidly increases. Second, an increased power yields more NO which acts to poison O_3 production by simultaneously reducing O_3 through direct decomposition to NO_2 and through the consumption of O, a key precursor for O_3 formation [66, 70].



Time-resolved FTIR measurements reveal the evolution of O_3 , NO_2 , and N_2O un the discharge effluent and clearly demonstrate the poisoning effect [65, 72]. Under high power operation, O_3 formation is rapidly inhibited as the discharge reactor reaches thermal equilibrium and the nitric oxide concentration increases. As O_3 poisoning occurs, a significant increase in NO_2 is observed.

A summary of the main reactions involved in the process is described in Table 2.3.

Table 2.3: Summarize key reactions governing the system in positive discharges.

Category	Reaction Description
Formation of O_3	Dissociation of molecular oxygen: $e^- + O_2 \rightarrow O + O + e^-$ Recombination of atomic oxygen with O_2 : $O + O_2 + M \rightarrow O_3 + M$ (where M is N_2 or O_2)
Formation of NO_2	Production of NO via interaction of $N_2(A)$ with O_2 : $N_2(A) + O_2 \rightarrow NO + N$ Reaction of NO with O_3 : $NO + O_3 \rightarrow NO_2 + O_2$ Reaction of NO with atomic oxygen and a third body: $NO + O + M \rightarrow NO_2 + M$ Destruction of NO_2 by atomic oxygen: $O + NO_2 \rightarrow NO + O_2$ Dynamic equilibrium: Reactions $NO + O + M \rightarrow NO_2 + M$ and $O + NO_2 \rightarrow NO + O_2$ stabilize NO_2 .
Metastable Nitrogen Species ($N_2(A)$, $N_2(C)$)	Excitation of $N_2(A)$ by electron impact: $e^- + N_2 \rightarrow N_2(A) + e^-$ Formation of $N_2(C)$ and subsequent emission from the second positive system: $e^- + N_2 \rightarrow N_2(C) + e^-$ $N_2(C) \rightarrow N_2(B) + h\nu$ (Second positive system emission) Quenching of $N_2(A)$ by O_2 : $N_2(A) + O_2 \rightarrow N_2(X) + O + O$

2.3.3 Main mechanisms of microbial inactivation by ANTP

The biological effects of RONS are well-known. O_3 is a powerful oxidant; it can oxidize organic matter either directly or through OH produced during the decomposition of O_3 [74]. First, it interacts with the double bonds of unsaturated lipid groups of membrane enzymes. This damage causes disruption of cell permeability, leading to leakage and oxidation of intercellular contents and biomolecules such as nucleic acids and eventually the lysis of the cell. Another potent oxidant produced by CAP is H_2O_2 , which has inactivation mechanisms similar to those of O_3 [75]. Generally, cytotoxicity caused by H_2O_2 begins with its penetration into the cell and then the production of OH [76]. OH is an extremely potent oxidizing agent that can be created from the decomposition of both O_3 and H_2O_2 . Its primary targets are cell

walls and membranes, therefore all the compounds composing of these two structures. The mechanism of OH reaction with lipids refers to its H-abstraction from the unsaturated carbon bonds of the fatty acids, ending in lipid peroxidation. Simultaneously, OH also reacts with membrane proteins. In comparison with the direct activity of O_3 , the mechanism of OH inactivation is less selective [75–77]. Since the low power CAP treatment accomplished a high inactivation level within a short time frame, it is reasonable to conclude that O_3 alone is not solely responsible. Therefore, the simultaneous exposure to multiple RONS, UV and elevated gas temperatures are likely to result in synergistic effects that accelerate spore inactivation in the presence of plasma [78].

ANTP inactivates viruses through physical and chemical mechanisms driven by the plasma’s active agents.

Physical Mechanisms

- Electric fields. Plasma’s electric field stresses viral components, causing electroporation, rupture, or deformation of key structures like the envelope or spike proteins, impairing infectivity [79].
- UV Radiation: Damages viral DNA/RNA by creating pyrimidine dimers and disrupts proteins through oxidation and disulfide bond breaking. UV also generates reactive oxygen species (ROS), amplifying the damage [80, 81].
- Charged Particles: Ions and electrons disrupt viral structures, cause ionization, and alter environmental conditions (e.g., pH), further stressing the virus [82].

Chemical Mechanisms

- Reactive Oxygen Species: ROS oxidize the viral lipid envelope, proteins, and nucleic acids, leading to structural damage and loss of function [12, 83].
- Reactive Nitrogen Species. RNS modify proteins and nucleic acids via nitration and oxidation, weakening the virus and inhibiting replication. They also generate highly reactive compounds like peroxynitrite [83, 84].

Combined Effects

- Synergy of Agents: ROS, RNS, UV radiation, and electric fields work together to enhance viral inactivation.
- Virus Dependency: Enveloped viruses are particularly vulnerable due to their lipid content, making them more susceptible to oxidative damage.

In summary, ANTP employs a combination of physical and chemical actions to effectively deactivate viruses, making it a powerful tool for disinfection.

2.4 Applications of atmospheric non-thermal plasma

Atmospheric non-thermal plasma (ANTP) has garnered significant attention across various scientific and industrial domains due to its unique ability to generate chemically active species

at low temperatures. The distinct properties of ANTP, including high reactivity, minimal thermal impact, and operational flexibility, have enabled a wide range of applications in fields such as medicine, environmental science, material processing, and even refrigeration systems. Some of application areas of this type of plasma are described below.

Medical Applications

ANTP has emerged as a transformative tool in healthcare, providing innovative solutions for sterilization, tissue regeneration, and cancer therapy:

- **Sterilization and disinfection:** The reactive oxygen and nitrogen species (RONS) generated in ANTP are highly effective against a broad spectrum of pathogens, including bacteria, viruses, and fungi. This property makes ANTP an essential tool for sterilizing medical instruments, surgical surfaces, and air in hospital environments [85–87].
- **Wound healing:** ANTP has shown significant promise in accelerating wound healing by promoting angiogenesis, stimulating cell proliferation, and reducing microbial load. Its non-invasive nature ensures patient comfort while delivering therapeutic effects [88, 89].
- **Cancer therapy:** Research into ANTP-assisted oncology focuses on its ability to induce selective apoptosis in cancer cells through oxidative stress mechanisms. The precise control of plasma exposure enables targeted treatment, minimizing damage to surrounding healthy tissues [90–92].

Surface Disinfection and air treatment

ANTP excels in microbial inactivation, making it a powerful tool for ensuring hygiene and safety in various settings

- **Surface disinfection:** Plasma treatments are highly effective in decontaminating surfaces by inactivating bacteria, viruses, and fungal spores. The reactive species generated in ANTP disrupt microbial membranes and DNA, ensuring complete sterilization without the need for harsh chemicals. Applications include food processing facilities, hospital environments, and public spaces [30, 86, 93].
- **Airborne Microbial Inactivation:** ANTP systems have been employed to treat indoor air by removing pathogens, allergens, and volatile organic compounds. These systems provide a chemical-free and energy-efficient alternative for maintaining air quality in hospitals, laboratories, and commercial buildings. Plasma-driven air treatment is particularly useful in pandemic scenarios, as it can inactivate airborne viruses such as influenza and coronaviruses [94, 95].

Environmental Applications

The capability of ANTP to interact with complex chemical compounds has led to its adoption in environmental remediation and pollution control:

- **Air and water purification:** ANTP efficiently removes volatile organic compounds (VOCs), nitrogen oxides (NO_x), and sulfur oxides (SO_x) from industrial emissions. It is also used to decontaminate water by degrading organic pollutants, neutralizing pathogens, and breaking down persistent microplastics [96–98].

- Waste treatment: Plasma-based systems can convert hazardous waste into less harmful or inert compounds, facilitating safer disposal and reducing environmental impact [99, 100].
- Greenhouse gas mitigation: ANTP has potential in mitigating the effects of greenhouse gases by decomposing carbon dioxide (CO_2) and methane (CH_4) into less harmful or reusable forms [101, 102].

Surface Modification and Material Processing

ANTP offers a highly controllable method for altering surface properties without affecting the bulk material, making it invaluable in material science and industrial manufacturing:

- Adhesion and coating: Plasma treatments enhance the adhesion of paints, adhesives, and coatings by modifying surface energy and cleaning contaminants at the molecular level [103].
- Etching and deposition: ANTP enables precise etching for microelectronics fabrication and the deposition of thin films for protective or functional coatings in various industries [104].
- Textile and polymer treatment: Plasma technology improves the dye uptake, water repellency, and antimicrobial properties of textiles and polymers, enhancing their performance and durability [105].

Cooling and Refrigeration Applications

In the field of refrigeration, ANTP has begun to demonstrate its potential for enhancing cooling systems through innovative approaches:

- Electrohydrodynamic cooling (EHD): ANTP-generated ionic wind has been successfully utilized to enhance heat transfer in refrigeration and electronic cooling systems. By directing plasma-induced airflow across heat exchangers or surfaces, it is possible to dissipate heat more efficiently, reducing the thermal load on refrigeration units [106].
- Energy efficiency: ANTP cooling systems reduce the need for mechanical fans or compressors, offering quieter and more energy-efficient alternatives to traditional refrigeration technologies [46, 107].
- Surface decontamination in refrigeration units: ANTP can be applied to sterilize refrigeration compartments, preventing the buildup of biofilms and reducing microbial contamination in food storage areas. This improves hygiene and extends the shelf life of perishable items [108].

Agricultural and Food Applications

The use of ANTP in agriculture and food industries is an emerging area of interest, focusing on sustainability and food safety:

- Food preservation: Plasma treatments inactivate spoilage microorganisms and pathogens on food surfaces without compromising quality or taste, extending shelf life and ensuring safety [109, 110].

- Seed germination and plant growth: Studies have demonstrated that plasma exposure can enhance seed germination rates, stimulate plant growth, and improve resistance to diseases [111, 112].
- Decontamination: ANTP effectively removes pesticide residues and other contaminants from fresh produce, providing a chemical-free alternative for food cleaning [107].

The versatility of atmospheric non-thermal plasma lies in its ability to address challenges across diverse sectors while maintaining efficiency and environmental sustainability. The integration of ANTP into refrigeration systems exemplifies how this technology can innovate even well-established fields, enhancing performance while reducing environmental impact. Ongoing advancements in plasma generation, diagnostics, and application design continue to expand its potential, driving innovation in both fundamental science and practical applications. Future research and interdisciplinary collaboration will play a pivotal role in unlocking new possibilities for ANTP, solidifying its status as a transformative technology.

Chapter 3

Materials and methods

Chapter 3 provides a detailed description of the fundamental prototype configurations, the design process, and the fabrication and assembly of the atmospheric plasma systems required to optimize diverse parameters. This chapter also details the materials and methods used in the development of the case studies presented in the following chapters, along with the experimental procedures for various analyses. These include in-depth descriptions of the electrical, fluidic and chemical evaluations aimed at enhancing the generation of reactive species and ionic wind, as well as the protocols employed for the microbial inactivation tests.

3.1 Research methodology

This PhD thesis has been conducted through a collaboration between *CEDRION C.T.I. S.L.* and the *Division of Machine Engineering at the School of Industrial Engineering, Universidad Politécnica de Madrid (UPM)*. Founded in 2017, CEDRION C.T.I. S.L. is a start-up specializing in the development of electrohydrodynamic (EHD) systems for applications across diverse industrial sectors, mainly in air purification, object and material disinfection, and lightweight cooling. Moreover, the thesis is carried out in collaboration with the *Department of Hygiene and Food Technology at the Faculty of Veterinary Medicine, Universidad de León (ULE)*, to conduct bacterial inactivation tests, and with the *National Center for Biotechnology of Scientific Research Council* for virus inactivation experiments. The microbiological assays are performed by these institutions, while the PhD student is responsible for designing and optimizing the systems employed in the experiments, in addition to analyzing and modeling the resulting data.

This research centers on the investigation of patented electrode configurations by CEDRION [113]. The investigation specifically focuses on modeling and optimizing the fluidic and chemical behavior of the system based on its electrical performance and geometric parameters for application in microbial inactivation. Two types of scenarios are analyzed. The first involves industrial air treatment applications, where the behavior of plasma systems in an "open" configuration is studied and optimized. In this case, the input composition of the device is consistently air, which is converted into reactive species, generating a specific airflow to maximize the inactivation of airborne microorganisms.

The second scenario simulates an industrial application for the disinfection of objects within a cabin. This setup employs a "closed" system in which plasma devices are placed to recirculate the air inside the chamber, generating a specific concentration of reactive species for the viral and bacterial inactivation of surfaces of medical interest.

The ultimate goal is to achieve the highest possible reduction in both airborne and on surfaces microorganisms confined within a disinfection chamber, using air under ambient temperature, pressure, and humidity conditions.

Corona discharge systems are modeled to understand their fluidic and chemical behavior, aiming to enhance their versatility in meeting diverse industrial requirements. These requirements may include constraints related to energy consumption, degrees of inactivation, and the physical size of the systems, depending on the spatial limitations of their intended installation environments.

The methodology employed in this research is based on rapid prototyping, which involves the efficient creation of prototypes to facilitate iterative testing and refinement. In this study, 3D printing is utilized to produce a large number of precise geometries quickly, enabling systematic variations in design and configuration to evaluate their effects on fluidic and chemical behavior.

This approach allows for consistent and repeatable experimentation, reducing variability in the results. Once the fluidic and chemical modeling is completed, microbial inactivation tests are performed using the most effective configurations identified. These tests focus on assessing the interaction between microorganisms and the reactive species generated by the systems, as well as the impact of the plasma environment on microbial membrane surfaces. If the results obtained do not match specific acceptance criteria, the process is iterated by modifying the design and repeating the entire workflow.

This modeling is essential due to the variability of the results reported in the literature for this type of system. Minor geometrical modifications can lead to significant changes in the plasma and ionic wind properties. In addition, industrial scale application requires a thorough understanding of these systems.

In this PhD thesis, two types of operation modes for corona discharge, positive and negative, have been selected and evaluated. This approach is adopted because positive corona discharge demonstrates greater efficiency in generating ionic wind while producing fewer reactive species. In contrast, negative corona discharge exhibits the opposite behavior [53]. Understanding and controlling both the composition and flow enables adaptation to various applications. Moreover, the system operates under direct current (DC).

The positive DC corona discharge has a significantly higher capacity to move a significantly larger volume of air, achieving an ion flux approximately four times higher than the negative discharge. This is attributed to the nature of the particles involved: negative polarity primarily mobilises highly electronegative molecules, such as oxygen, while positive polarity drives the movement of particles with lower electronic affinity, such as nitrogen [52]. However, negative discharge produces concentrations of reactive species an order of magnitude higher, promoting ozone generation due to a higher concentration of reactive species [68]. The ozone production

rate and the spatial distribution of the reactive plasma region differ significantly due to the impact of the discharge polarity on the electron density and the extent of the plasma region.

In addition, direct current (DC) has been chosen instead of alternating current (AC) to achieve higher operational stability and better geometrical optimisation.

Regarding microbial inactivation, experiments are conducted with various viruses and bacteria to evaluate the effectiveness of plasma treatments and the resistance of these microorganisms to different device configurations. Two coronaviruses are selected for testing due to their significant impact during the development of this thesis, particularly in the context of the SARS-CoV-2 pandemic in 2020 [3], and their rapid spread, which resulted in a high number of infections [114]. Specifically, tests are carried out with SARS-CoV-2 and HCoV-229E, the virus responsible for the common cold. The germicidal effect is also evaluated using MS2 bacteriophage virus. This virus is highly resistant, as it infects bacteria and is adapted to hostile environments, making it an excellent indicator of the lethality of the corona discharge plasma systems analyzed.

Both Gram-positive and Gram-negative bacteria are used in the experiments to identify potential differences in their resistance to atmospheric plasma treatment, which could be attributed to structural differences in their cell walls. Gram-positive bacteria possess a thick cell wall, whereas Gram-negative bacteria have a thin cell wall covered by an outer lipid membrane. *Listeria monocytogenes* is chosen as the Gram-positive bacterium, and *Escherichia coli* as the Gram-negative representative.

The following sections present the different devices studied, classified according to their configuration, mode of operation, and type of current generated. Additionally, the setups and experimental procedures employed are described in detail.

3.2 Electrode design, characteristics and manufacturing

3.2.1 Electrode configuration and design

Corona discharge systems consist of two electrodes: a corona electrode (or emitter electrode) with a small radius of curvature, and a collector electrode with a larger radius, resulting in a high aspect ratio between them.

The design of non-thermal atmospheric plasma devices generated by corona discharge, studied and developed in this research, is based on the wire-to-two-parallel-planes geometry. This geometry was selected to achieve maximum efficiency in both the generation of ionic wind and its correlation with the production of chemical species. Additionally, the aim was to model and optimize the devices developed and patented by Cedrión [113], focusing on the production of chemical species that were optimal for microbial inactivation. Previous studies have focused on fluidic optimization and heat transfer for their application in cooling systems [115, 116], but these do not thoroughly explore chemical modeling or its correlation with fluid dynamics.

In the geometry studied, the corona electrode was centrally positioned between the two grounded electrodes. In this configuration, ionic species generated in the ionization zone were

propelled toward the collector electrodes. The airflow produced moves along the surfaces of the grounded electrodes without directly colliding with any walls. The direction of the airflow moves from the corona electrode to the collector electrode, regardless of whether the corona was positive or negative. The placement of both the corona and collector electrodes is critical, as it significantly affects key factors such as the breakdown voltage, the distribution of the electric field, the entry and exit points of the airflow, the velocity of the ionized fluid upon exit, and the chemical species produced.

Figure 3.1 shows the base channel used in the devices analyzed in this study. It consists of a wire as the corona electrode and two parallel planes with rounded ends as the collector electrodes. The minimum distance between the electrodes is called gap.

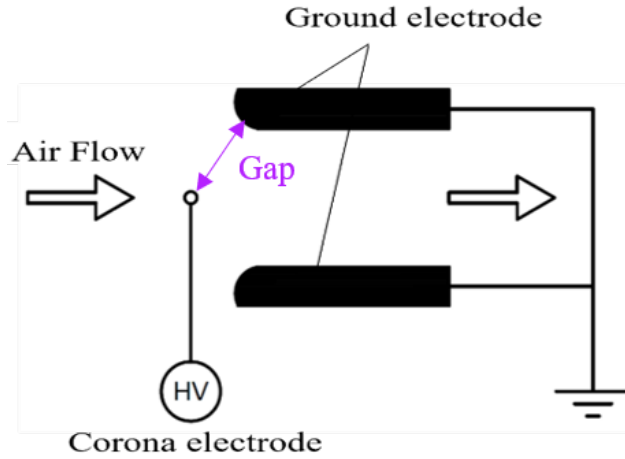


Figure 3.1: The positive corona discharge geometry (corona electrode connected to high voltage) with a wire-to-two-planes configuration has been developed and optimized in the present study.

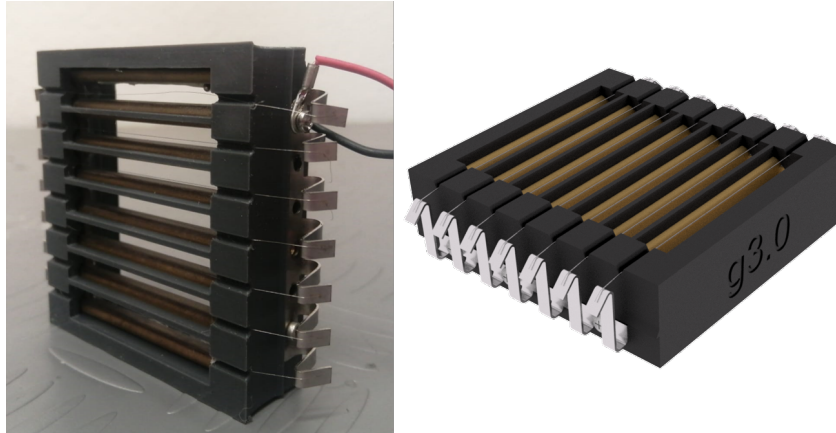


Figure 3.2: Render of the N7H50 device (7 wires, each 50 mm in length) used as a reference in several executed experimental campaigns.

The geometry formed by a determined number of these parallel channels in this configuration is referred to using the nomenclature NOTUSXHY, which is simplified to NXHY. N denotes the word "NOTUS", the name internally assigned to this type of configuration. Here, X

represents the number of wires, and Y indicates their length in millimeters. One of the key devices analyzed in this research was the N7H50, meaning a device composed of 7 wires, each 50 mm in length. Based on this arrangement, multiple geometries and configurations have been designed and evaluated. Subsequently, it was used as a scaling reference for various experimental and real-world applications.



Figure 3.3: Various designs of atmospheric plasma devices based on the described geometry.

The geometric parameters evaluated are:

- Wire diameter.
- Distance between electrodes (gap),
- Number of wires.
- Device arrangement (in series or parallel).
- Operating modes or discharge types (positive and negative corona discharge)

In the positive corona discharge mode, the corona electrode (wire) was connected to high voltage while the collector was grounded. In the negative discharge mode, the connections were reversed, with the corona electrode at low voltage and the collector electrode at high voltage.

3.2.2 Electrode manufacturing

As previously mentioned, the experimental development was carried out using the rapid prototyping methodology, which involves designing and prototyping through 3D printing. This methodology was used both to generate ANTP prototypes and to design and manufacture experimental setups, ensuring complete adaptability to experimental requirements.

The materials used in plasma devices include:

1. Tungsten wires as corona electrodes.
2. Brass collector electrodes, selected based on preliminary studies.
3. Dielectric supports, fabricated from plastic, which serve as structural supports and separators between electrodes while also defining and maintaining the specific geometries to be evaluated.

The dielectric supports, which are critical to the configuration of the devices, were manufactured via 3D printing using materials such as PLA (polylactic acid) or ABS (acrylonitrile butadiene styrene). The choice of material depends on the operational conditions: for tests conducted under ambient temperature, pressure, and humidity, PLA was suitable. For analyses requiring higher temperatures, other 3D-printable plastics were employed to meet the thermal requirements.

An additional advantage of using 3D printing is the ability to recycle the plastic material, contributing to sustainability and efficient material usage.

The combination of carefully selected materials and the flexibility of 3D printing enables the optimization of experimental designs, providing a highly efficient and responsive approach to addressing technical challenges.

The 3D printing process used in this work was Fused Deposition Modeling (FDM). Figure 3.4 shows some of the 3D printers utilized during the development of this research. Additionally, in certain parallel studies, Selective Laser Sintering (SLS) printers were employed when specific resins were needed for the experiments.

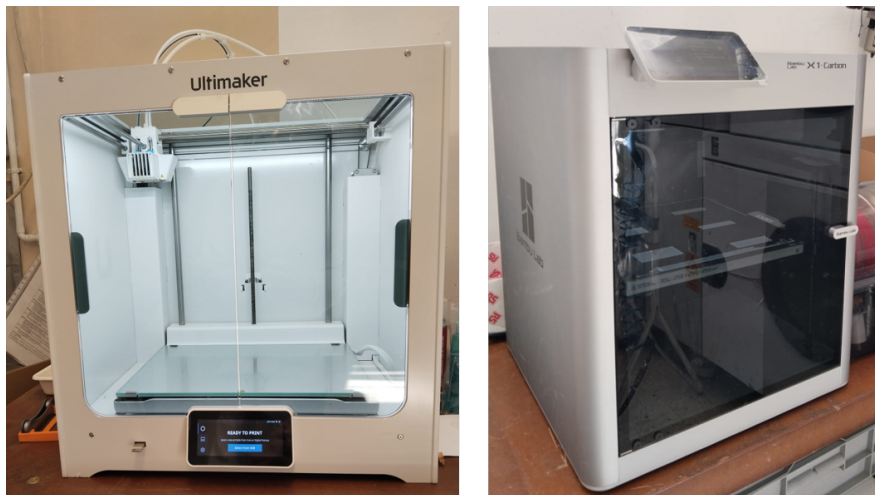


Figure 3.4: 3D printers used in this study include models such as Ultimaker S3 and Creality K1 Max.

The corona electrode consists of a micrometric wire that must be securely fixed in a central and stable position relative to the grounded electrode. It was crucial to ensure that the insulating support structure remained intact and did not deform under mechanical stress or exposure to elevated temperatures. To secure the components, M2 brass screws were used, which were soft-soldered to a cable. Folded steel sheets were utilized as a base for micro-welding the filament in place (see Figure 3.5).

The spot microwelding process was carried out using a ZoneRay Dental Lab Spot Welding Machine (SKU: HL-WDI) 3.5, which allows for adjustable power settings tailored to the specific material being welded. A custom-designed tool was generated and manufactured to create a tab-shaped structure that holds the corona filament securely (Figure 3.6).

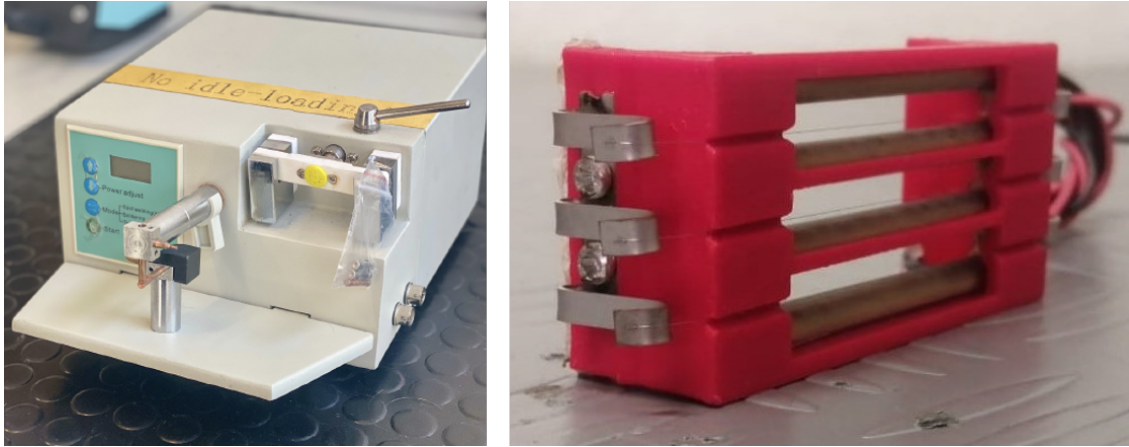


Figure 3.5: Spot microwelding machine (left) and wire microwelded to the folded steel sheet (right).

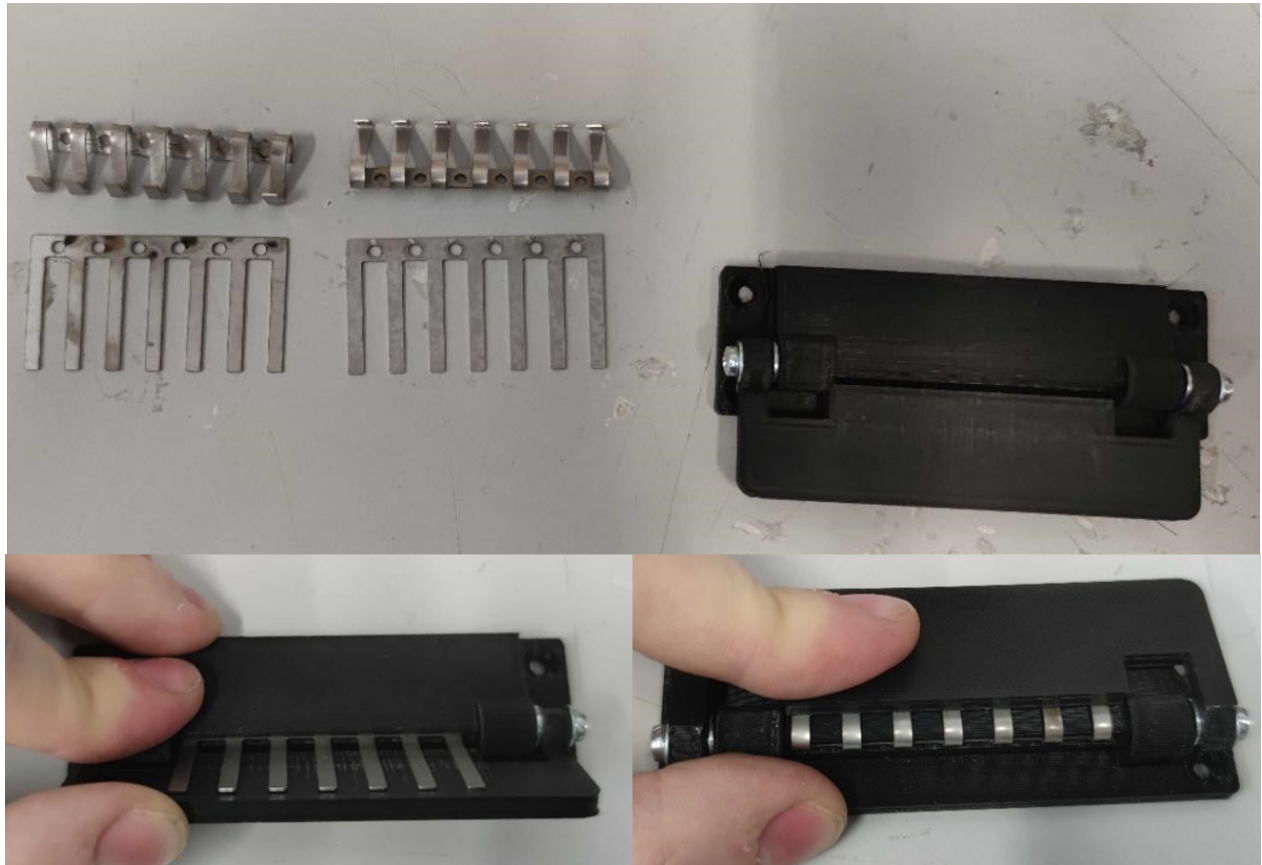


Figure 3.6: Tool and process for bending the steel sheet.

The tab assembly was attached to the polymer support using M2 threaded inserts embedded in the structure, along with 4 mm M2 screws (see Figure 3.7). The procedure involves aligning the wire centrally within the curved sheet and using the microwelding device to apply weld points at specified locations, as shown in Figure 3.8.

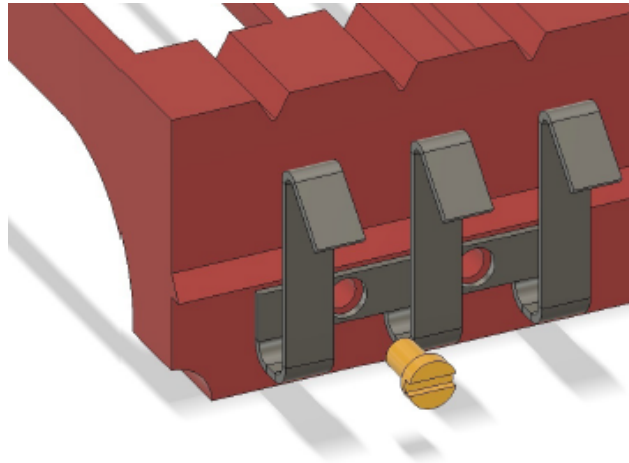


Figure 3.7: Mechanical attachment of the steel sheet to the 3D-printed dielectric support.



Figure 3.8: Microwelding process using a plasma device.

3.3 Experimental setup and empirical process

3.3.1 Electrical tests

Following the design and fabrication of the devices, their characterization was carried out. The operating range and stability of the device were determined by the characteristic voltage - current curves (CVCCs) that represented the electric current versus voltage provided. The electrical behavior directly impacted the fluidic and chemical generation of the system. The analysis included studying various voltages, power consumption levels, and design parameters that influenced the electrostatic behavior of the device.

High-voltage power supplies were used to operate the various plasma devices. Specifically, two Heinzinger models were utilized: the LNC 6000-10, with a range of 0 to 6 kV and 0 to 10

mA, and the LNC 10000-5, with a maximum range of 10 kV and 5 mA (Figure 3.9). Both power supplies operate in direct current (DC).



Figure 3.9: High voltage power supplies Heinzeinger LNC 6000-10 and LNC 10000-5 models

Electrical characterization involved grounding the collector electrode and applying high voltage to the corona electrode. The CVCCs were analyzed to determine the operating range of the devices. For each variable and operation mode studied, five experimental tests were performed to assess the measurement's experimental error.

The procedure consisted of gradually increasing the voltage until discharge begins, a point at which the dielectric breakdown of the air was reached, and it was marked by an initial voltage (V_0). From this point on, the air between the electrodes became a conductive medium, and the current indicated by the power supply stabilized at 0.01 mA.

This initial voltage depended on various design parameters, operation mode and ambient conditions, among others. The test ranged from 2.5 to 10 kV under atmospheric air conditions.

The efficiency of the electrical discharge was influenced by various geometric and performance parameters. From the curves, the following parameters were determined: the ignition voltage (V_0), the final or operating voltage (V), which defined the system's operational range within which it functioned stably, and the power consumed by the corona discharge.

The curves were influenced by design parameters such as the diameter of the corona electrode, the distance between electrodes (gap), the number of wires and the operation mode (positive and negative corona discharge).

3.3.2 Ionic fluid velocity tests

One of the most relevant aspects of EHD-plasma technology is its ability to provide air flow without the need for external systems. In previous studies, efforts were focused on optimizing the velocities produced according to the system design parameters and its electrostatic behavior, to obtain scalable and adaptable devices for different applications [116].

This research also aimed to evaluate the generation of ionic wind and to correlate and model it in relation to the production of chemical species.

For fluid characterization, static, total and dynamic pressures at the outlet of the EHD plasma generators were evaluated. A custom-designed 50 mm diameter wind tunnel was integrated with the device output. The design is developed using Autodesk Fusion 360 and fabricated using 3D printing technology.



Figure 3.10: Differential pressure sensor, Sensirion, model SDP810.

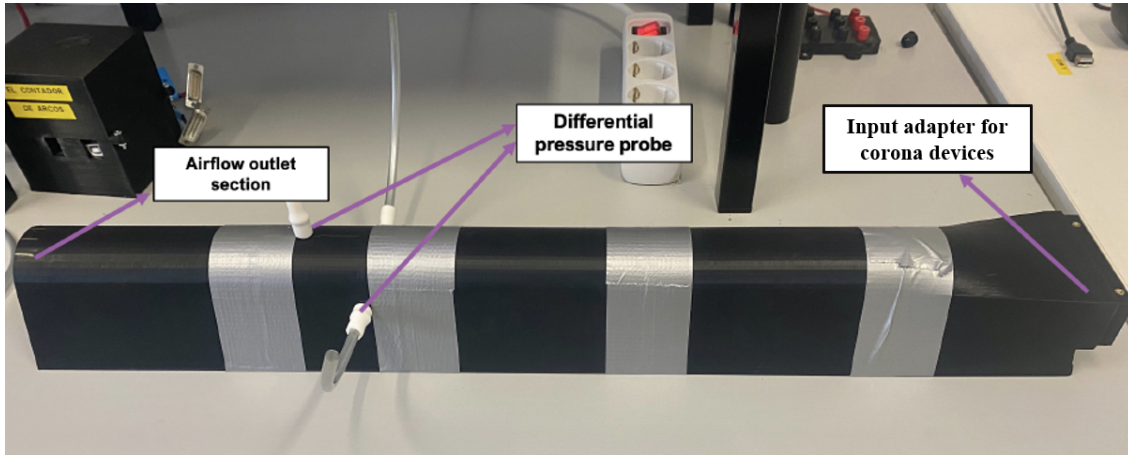


Figure 3.11: Custom-designed wind tunnel used in the open system tests.

The tunnel was equipped with a pressure measurement section, with a central point for total pressure and a wall point for static pressure. The difference between the total and static pressure gave the dynamic pressure. A differential pressure sensor (Sensirion, model SDP810, Figure 3.10) was used to acquire the pressure data. The dynamic pressure values were used to determine the flow velocities for each experimental condition. The velocity values were obtained through equation $p = \frac{1}{2}\rho v^2$, which is a simplification for the incompressible fluid dynamics of Bernoulli's equation. Velocity data were recorded as a function of the electrical, geometric, design, and/or operational mode variables analyzed in each case. Similarly, five replicates of each test were performed to determine the experimental error.

Custom adapters for the tunnel were prepared for each specific device dimension. Figure 3.12 provides a schematic of the tunnel section used, detailing its different parts.

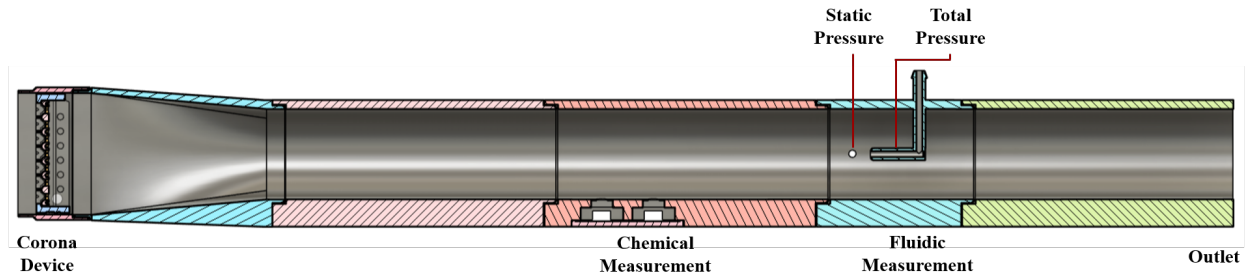


Figure 3.12: Schematic of the 3D printed tunnel used for the fluidic and chemical characterization tests of the neutral phase generated by the corona discharge plasma devices.

3.3.3 Neutral compounds test

The neutral phase of plasma referred to the neutral species generated as part of the ionic wind, which emerged from the core of the discharge. These species predominantly consisted of long-lived compounds that remained more stable over time.

Characterization of the neutral phase generated by corona discharge plasma devices was performed by fluidic and chemical analysis of the produced flow. The chemical composition was analyzed by focusing on two long-lived neutral compounds, ozone and nitrogen dioxide, which were predominantly generated in atmospheric discharges. These compounds were quantified using specific electrochemical sensors (Ec Sense, sensor module, TB200B-ES1-O3-5 and TB200B-ES1-NO2-5 for positive discharge; TB200B-ES1-O3-50 and TB600B-NO2-50 for negative discharge, Figure 3.13). This analysis was conducted in parallel with the fluidic tests performed in the previously described wind tunnel. To facilitate monitoring without disturbing airflow, a sensor housing area was created in the wall of one of the tunnel sections. Measurements were taken both at the wall and at the center of the tunnel exit to compare concentrations. Since the data at the wall were slightly lower, multiple calibration curves were generated, and the data were corrected to reference the central region, where the maximum concentration was expected. Additionally, the influence of the sensor placement on pressure was evaluated by taking measurements with and without the electrochemical sensors.



Figure 3.13: Electrochemical sensors for ozone and nitrogen dioxide. Fuente: Ec Sense.

The mass flow rates of the chemical compounds evaluated can be calculated from the measured

velocities at the outlet of the system and the concentration of these compounds. Knowing the area at the outlet of the device and the velocity, the ionic wind flow rate of the system can be determined (Eq. 3.1).

$$Q = v \cdot A \quad (3.1)$$

$$m_{C_i} = Q \cdot [C_i] \quad (3.2)$$

where Q is the ionic wind flow rate [m^3/s], v is the velocity [m/s], and A is the area [m^2] at the outlet of the device. The mass flow rate of each compound is m_{C_i} [mg/s], and $[C_i]$ is the concentration of the compounds [mg/m^3]. The modeling and correlation of the previously exposed parameters allowed for a better understanding and scaling of the proposed EHD-plasma devices.

3.3.4 Active compounds test - OES

Optical emission spectroscopy (OES) represents an effective technique for analyzing the emitting electrode in the corona discharge under investigation since it allows the evaluation of discharge emissions with very small dimensions (in the order of microns). In addition, OES is a non-invasive method, which makes it particularly suitable for this type of application. This technique offers significant advantages for determining various characteristics parameters of the plasma, depending on the specific emission band observed.

The objective was to determine the influence of different electrical and geometrical parameters of corona discharge plasma devices on photon emission characteristics and spectral changes, as well as the influence of the mode of operation (positive or negative discharge).

The OES experiments were performed using an Optical Spectrometer (HR4Pro, High-resolution User configurable Spectrometer) in the 200 - 400 nm range, with optical resolution of 0.28 nm, a fiber (QP600-2-UV-BX 600 μm Premium Fiber, UV/VIS, 2 m, BX Jacket) and a collimating lens (74-UV UV/VIS Collimating Lens, 200 - 2500 nm).



Figure 3.14: HR4Pro, high-resolution user configurable spectrometer

Data were collected with an integration time of 100 ms and 10 scans to average and boxcar width. Custom 3D-printed holders were used to position the collimating lens and perform measurements centered on the emitting electrode. Calibration experiments determined the optimal measurement position. Similar emission values were recorded at both the center and ends of the wires, denoting symmetrical behavior along the wire. Therefore, all measurements were taken at the center of the wire (see Figure 3.15). The collimating lens was placed in front of the wire at 5 mm distance. Dielectric barriers were removed to allow detection of the emitted light.

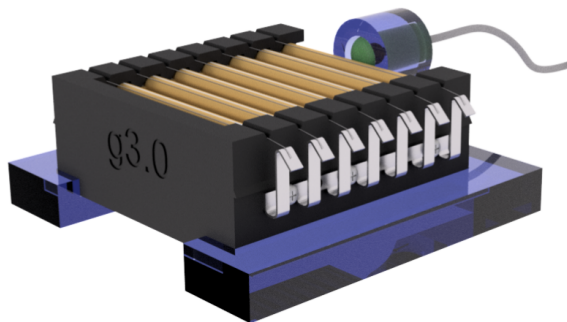
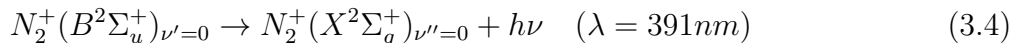
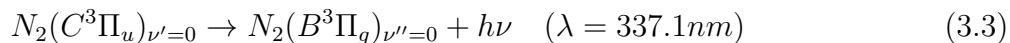


Figure 3.15: Emission spectrum measurement probe arrangement in a 7-wire corona discharge device. The collimating lens was placed in the center of the wire.

The molecular emission spectra observed were attributed to the second positive ($C^3\Pi_u - B^3\Pi_g$) systems of the molecular nitrogen. The first negative ($B^2\Sigma_u^+ - X^2\Sigma_g^+$) nitrogen system was detected but with very weak light intensity and was nearly undetectable in the case of positive discharge.

The reaction of the second positive and first negative system of nitrogen molecules are primarily described in



In the gas phase, the molecular rotation was quantified, since a vibrational transition could be a change in the rotational modes.

The emissions obtained for each operating mode, as well as the analyzed geometric and electrical variables, were evaluated. Conclusions were derived from these analyses to define the design, modeling, and scaling of the systems.

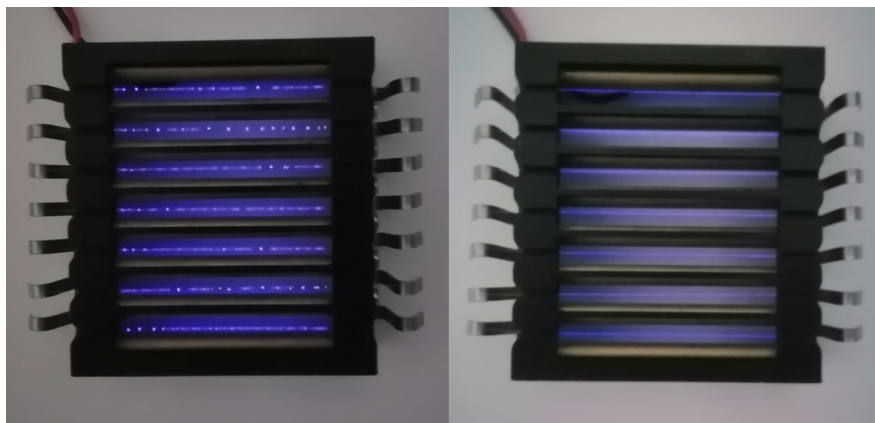


Figure 3.16: Plasma generated using the N7H50 device, consisting of 7 wires each 50 mm in length, in negative corona discharge (left) and positive corona discharge (right).

Figure 3.16 shows the light emitted within the plasma, located around the corona electrode, for both positive and negative corona discharges for the N7H50, 7-wires and 50 mm length corona device. It can be observed that in the positive discharge, the distribution of light was uniform along the length of the wire, whereas in the negative discharge, small "clusters" of plasma were formed.

3.3.5 Virus inactivation tests in an open system

Positive-strand RNA viruses were selected to determine and optimize the effect of atmospheric plasma on both aerosols and surfaces. The evaluation was based on the electrical and geometric parameters of the system, correlating and optimizing the process through the adjustment of the generated chemical composition and the plasma properties in the discharge zone. The selected viruses include SARS-CoV-2, HCoV-229E, and the MS2 bacteriophage. These viruses were chosen for the study, rather than bacteria, as their primary mode of transmission is airborne.

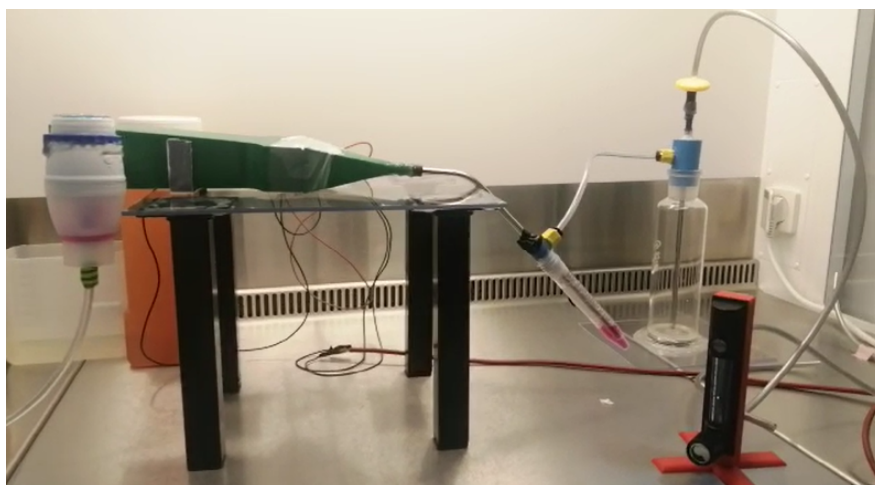


Figure 3.17: Experimental setup designed and manufactured for viral inactivation experiments, installed within a biosafety cabinet at at CNB-CSIC.

The experimental setup for the microbial inactivation tests in an open system was designed and built to prove the effectiveness of the atmospheric plasma device against viruses present in the airflow. Figure 3.17 shows an image of the setup installed in a biosafety cabinet at the National Center of Biotechnology (CNB-CSIC) for inactivation virus testing. Figure 3.18 presents a schematic diagram that describes the components that constitute the installation. The installation had three different parts: first a viral aerosol generator, then a zone where the contaminated flow was treated by the plasma device, and finally a sample collector[27], [36]. The nebulizer (OMRON A3) was charged with the viral suspension and the air needed to generate the aerosol was provided by the compressor. The air stream was controlled by a valve and a flowmeter (Key Instruments, MR3000, 1-10 L/min), with the flowrate ranging from 5-6 L/min. A mixture of air and droplets, that transport the virus particles, was poured into the 3D printed conduct where the plasma device was located. Afterwards, a rectangular section of the conduct was adapted along the channel to house the plasma device. The contaminated airflow passed through the device and was treated by the reactive species generated. Downstream of the device, the channel was changed to a conical shape, and finally, the samples were collected in a reservoir. Although there are several methods of virus recovery from air, a liquid impactor was chosen for this study[37]. A glass reservoir was filled up with 3 mL of collection liquid (Dulbecco's Modified Eagle Medium, DMEM or lysogeny broth (LB)). The aerosol particles present in the airflow went through a 4 mm diameter tube immersed in the collection liquid. Then a security container and a filter (20 μ m) were placed at the collector outlet to prevent viral particles from escaping from the system. Finally, a vacuum pump (FisherbrandTM, Diaphragm Pump) ensured that the airflow was constant during the experiment.

All viral inactivation trials using EHD-plasma devices conducted in the closed study had two phases. A control phase, in which the viral solution was passed through the system with the plasma device switched off, and a treatment phase, in which the device was turned on. The conditions were the same in both cases. The time set for each assay was 20 minutes, determined by control tests to ensure adequate viral titer collection.

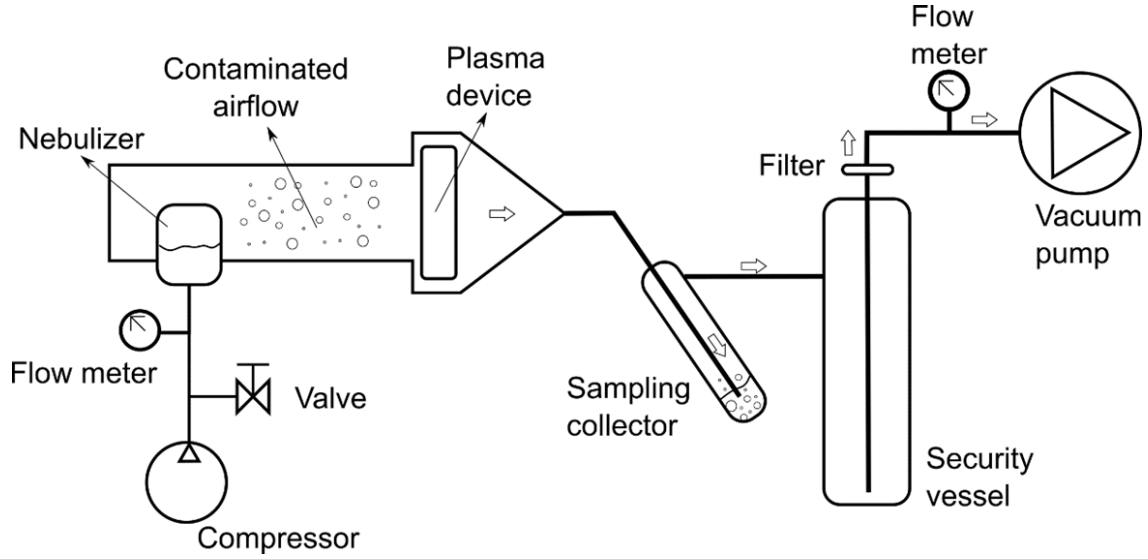


Figure 3.18: Schematic diagram of the experimental setup for virus inactivation assays using plasma devices.

The logarithmic reductions in microbial inactivation measure the effectiveness of a treatment in reducing microorganisms, expressing how many times the initial population has been decreased on a logarithmic scale. Each "log" reduction corresponds to eliminating 90% of the remaining microorganisms. This method standardizes the evaluation of processes like pasteurization or sterilization, making comparisons easier and ensuring adequate safety levels.

The control phase allows obtaining the losses of viral material due to the transport of the aerosols through the system. These losses were considered in the calculation of treatment efficacy for each virus and condition tested. Eq. 3.5 was used for the determination of the logarithmic reductions:

$$RD = \log \left(\frac{C_{ti}}{C_{tf}} \right) - \log \left(\frac{C_{ci}}{C_{cf}} \right) \quad (3.5)$$

where C_{ti} and C_{tf} are the initial and final virus concentrations after treatment, and C_{ci} and C_{cf} are the initial and final concentrations in the control, respectively. All of them are expressed in [pfu/mL] (plaque-forming unit). The second term of the equation corresponds to the system losses.

The determination of the percentage reduction (PR) was calculated based on logarithmic reductions using the following formula:

$$PR = (1 - 10^{-RD}) \times 100 \quad (3.6)$$

where PR represents the percentage reduction [%], and RD denotes the logarithmic reductions.

The assays were performed as follows: 3 mL of viral sample (HCoV-229E or SARS-CoV-2 suspended in DMEM+0.2% FBS; or bacteriophage MS2 in LB) were placed in the reservoir of

the nebulizer. The vacuum pump located at the end of the system was turned on. Then, the EHD-plasma generator was switched on (in the case of the control assays, it was off), and kept on for 2 minutes to homogenize the air flow and the species generated. The compressor that transmitted the viral aerosols through the system was then activated. After the 20-minute test, two aliquots were collected from the nebulizer (100 μ L) and another two from the sample collector.

3.3.6 Virus titration and quantification in an open system inactivation tests

The analysis and processing of viral samples were carried out by the staff at the National Center for Biotechnology, part of the Spanish National Research Council (CSIC). The procedure is detailed below.

SARS-CoV-2 and HCoV-229E

Virus strains

SARS-CoV-2 was isolated from a nasopharyngeal swab of a 69-year-old male COVID-19 patient in Madrid, Spain. HCoV-229E was obtained from Volker Thiel at the Institute of Virology and Immunology, University of Bern, Switzerland, and virus stocks were prepared as previously described [117]. All experiments with SARS-CoV-2 were conducted in biosafety level 3 (BSL-3) facilities at CNB-CSIC, following institutional guidelines.

Cell lines

Human liver-derived Huh-7 cells, susceptible to HCoV-229E infection, were provided by Dr. L Carrasco at the Centro de Biología Molecular Severo Ochoa in Madrid, Spain. Vero E6 cell lines, derived from African green monkey kidney epithelial cells and used to grow SARS-CoV-2, were obtained from Dr. E Snijder at Leiden University Medical Center in the Netherlands. All cell lines were cultured in DMEM supplemented with 2 mM L-glutamine, 1% Non-essential amino acids, 10% fetal bovine serum, and antibiotics. Virus titration was performed using a plaque assay method, where cells infected with serial dilutions of virus inoculum were overlaid with DMEM containing 0.6% low-melting agarose or 0.7% melting agar for SARS-CoV-2 and HCoV-229E respectively, and 2% FBS. After 96 hours of infection, cells were fixed with 10% formaldehyde and stained with 0.1% crystal violet to visualize and count viral plaques.

Bacteriophage MS2

Like coronavirus, bacteriophage MS2 is a single-stranded RNA virus, but with a protein instead of a membrane envelope. It infects *Escherichia coli* bacteria through the pilus F structure. Therefore, the *E. coli* Hfr Hayes strain was used as a production and titer strain, as it contains the genes necessary for pili production integrated into its genome [118]. A culture of *Escherichia coli* Hfr in 25 mL liquid LB (10 g/L tryptone, 5 g/L yeast extract, 10 g/L sodium chloride) is allowed to grow to the exponential phase of bacterial growth (optical density of around 0.4 when measured at 600 nm) and then infected with bacteriophage MS2 at a multiplicity of infection of 0.025. After a period of four hours, the phage has infected the bacteria, carrying out its reproduction cycle and generating more copies of the virion.

An additional bacterial lysis process was then applied, by the addition of chloroform (1:10 ratio, 4°C, overnight) and followed by the action of lysozyme (1 mg/mL) and DNase (1 µg/mL), at room temperature, for one hour. Bacterial was removed by centrifugation for 15 min at 4 °C. The resulting supernatant suspension was titred to determine the concentration of MS2 in pfu (plaque-forming units) per mL. For this purpose, serial dilutions of the phage culture were performed. 0.1 mL of each of these dilutions were used to infect 0.3 mL of a culture of *E. coli* Hfr, which were mixed into 4.5 mL warm top agar (0.7% (w/v) agar-agar in LB) and plated onto solid LB-agar plates (1.5% (w/v) agar-agar in LB medium). These were incubated at 37°C overnight.

3.3.7 Microbial inactivation and electrochemical characterization in a closed system tests

The inactivation of microorganisms present on surfaces was carried out for both viruses and bacteria. For viruses, the study focuses on positive-sense RNA viruses, including HCoV-229E (common cold virus) and SARS-CoV-2. For bacteria, both Gram-positive and Gram-negative species were analyzed to assess potential differences in inactivation kinetics. These differences may arise due to the distinct morphological structures of these bacteria Figure 3.19, which could lead to varying inactivation mechanisms.

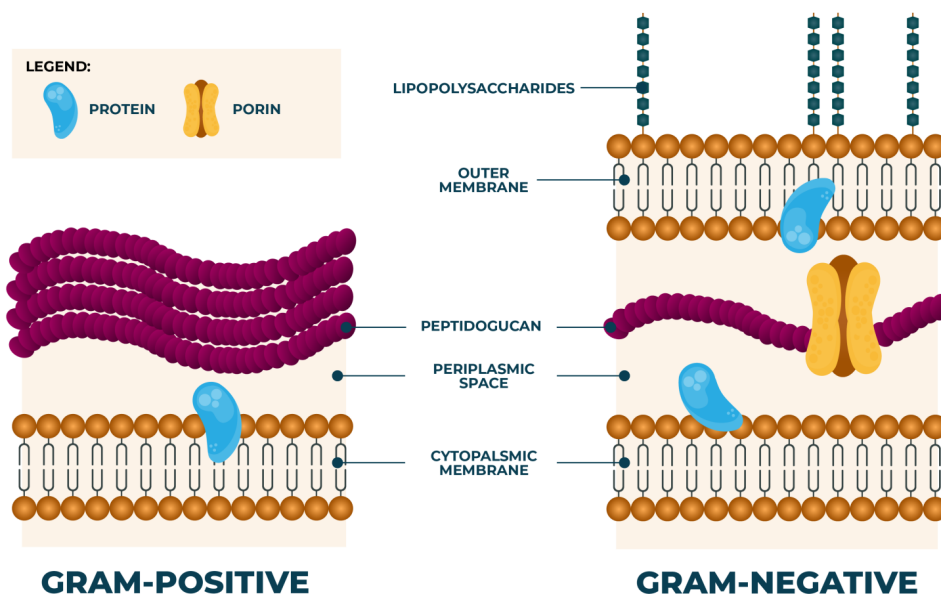


Figure 3.19: Structure of Gram-positive and Gram-negative bacteria.

Gram-positive bacteria have a thick cell wall, while Gram-negative bacteria possess a thin cell wall layer covered by an outer lipid membrane. *Listeria monocytogenes* was selected as the Gram-positive bacterium, and *Escherichia coli* as the Gram-negative counterpart. Experiments were conducted on both agar plates and various material surfaces to evaluate the effects comprehensively.

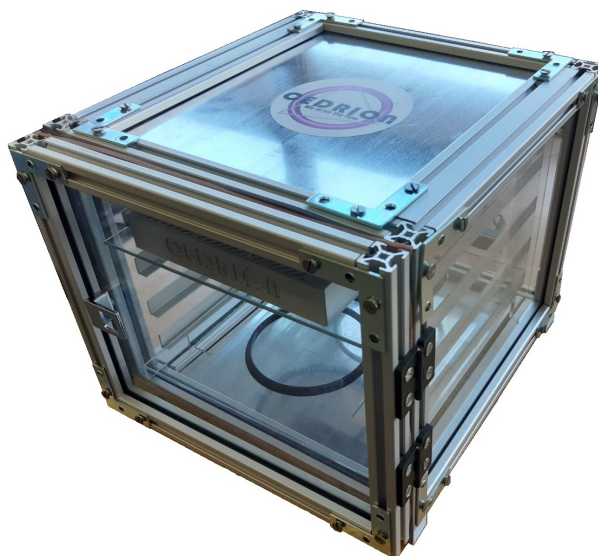


Figure 3.20: Hermetic chamber used in closed system experiments.

The non-thermal atmospheric plasma device was contained within a sealed chamber with an interior volume of 17 liters. This chamber has a double-walled structure that facilitates the recirculation of the air inside (Figure 3.21). The air movement was generated by the plasma systems, which combine the generation of a highly oxidizing atmosphere with the production of airflow, eliminating the need for external propulsion systems. The plasma devices were positioned at the top of the chamber to capture air from the upper zone, which was then recirculated through the double walls. The samples were placed in the middle section of the chamber, supported by a tray that allows air flow. Ionic wind passes through the device impacts with the samples and returns through the double wall.

The chamber was equipped with ozone and nitrogen dioxide sensors (Ec Sense, sensor module, TB600B-O3-100, TB200B-ES1-NO2-1000, Figure 3.13) located at the bottom. These sensors measure the concentration of each species, as well as temperature and humidity. Thus, the species generated inside were monitored, using these compounds as reliable indicators due to their significant production ratios.

A hermetic door was used to effectively contain the atmospheric conditions within the chamber. The system allowed for reaching a specific degree of species concentration for each condition. Samples were introduced into the chamber, positioned on a tray at mid-height, subjected to specific operation modes and treatment durations, and removed upon completion of the assay. The temperature inside the chamber was maintained constant at 20°C, with temperature differentials kept below 5°C. The relative humidity was maintained around 40%.

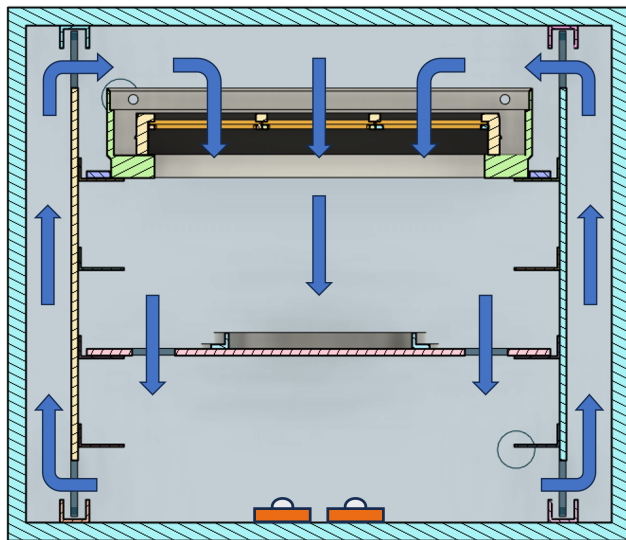


Figure 3.21: View of the interior and airflow within the sealed chamber for the generation and accumulation of chemical species, and its application in microbial inactivation. The upper zone houses the atmospheric plasma device, the intermediate zone contains microbiological samples, and the lower zone hosts electrochemical sensors (O_2 and NO_2) for gas measurement.

The chamber was used in closed-system experiments for both chemical characterization and modeling, as well as for viral and bacterial inactivation tests. The devices were positioned at the top of the chamber and, through the generation of ionic wind, drove the fluid through the double walls, allowing chemical species to diffuse throughout the interior of the chamber.

For microbiological inactivation tests, samples were placed inside the chamber and subjected to a specific treatment configuration. Additionally, the concentration of chemical species were evaluated using the sensors described.

3.3.8 Virus sample preparation, titration, and quantification in an close system inactivation tests

As the same way that it mentioned before, the analysis and processing of viral samples were carried out by the staff at the National Center for Biotechnology, part of the Spanish National Research Council (CSIC).

Samples preparation

Amplification in HuH7 cells (human hepaotocarcinoma) of HCoV 229E and in VeroE9 cells (African green monkey kidney) of SARS-CoV-2

Cells were inoculated at multiplicity of infection 0.01 in a 20 mL volume of medium in closed F175 vials (175 cm² surface area). Cells were incubated at 32°C. At day 2 and 3 post-inoculation, 10 mL of culture supernatant was collected and 10 mL of fresh medium was added. At day 4 post-infection, the entire volume of supernatant (20 mL) was collected. Virus stocks were stored at -80°C in the NCB2 laboratory.

Titration of virus stock in cell cultures

To determine the virus titer of day 2, 3 and 4 post-infection stocks, cells grown in 12-well plates were used. Serial factor 10 dilutions of each of the stocks were prepared and 100 μ L of the 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} y 10^{-6} dilutions were inoculated into each well. After 2h of adsorption at 32°C, the virus inoculum was removed and replaced with 1 mL of semisolid medium (1:1 mixture of 1.4% agar and DMEM supplemented with 4% fetal bovine serum – FBS-) for 1h and the semi-solid medium was removed. Cells were then stained with crystal violet (0.1% in methanol:water 20:80) for 30 min to observe the lysis plaques. A viral titer of $1 \cdot 10^6$ pfu/mL was obtained.

Biosafety experimental conditions

For the necessary preliminary adjustments in the preparation of the different materials to be treated contaminated with virus, the human alpha coronavirus 229E was used, belonging to risk group 2 of human pathogens, very similar in structure, but less dangerous than SARS-CoV-2 since it produces the typical picture of the common cold. In this phase, all tests were performed in a biological containment level 2 (NCB2) laboratory. In the final phase of this study, a wild isolate of SARS-CoV-2 native to the Madrid area and belonging to human pathogen risk group 3 was used, so all tests had to be performed in the NCB3 laboratory. The type and number of tests to be performed in this final phase were determined by the experimental results obtained in the first phase of the study with HCoV-229E. In all cases, the samples were evaluated in duplicate using the corresponding controls at each point.

Sample preparation. Study of the drying time

One of the most important aspects of sample preparation is to determine how drying time affected viral titer since it is expected that germicidal efficacy assays would be performed on surfaces contaminated with viral suspensions subsequently subjected to a drying process at room temperature before exposure to the effect of atmospheric plasma. For this purpose, 300 μ L of HCoV-229E suspension of known titer was applied to the center of each p-100 petri plate and spread with a sterile glass seeding loop. Subsequently, the plate surfaces were exposed to the sterile laminar flow of the biosafety cabinet to facilitate drying of the samples, performing a drying time curve with 15 min increments. After that, viruses were collected from each plate by washing the surface with 300 μ L of PBS and titration of each sample was performed.

Exposure of materials to atmospheric plasma in the airtight chamber

- Preparation of samples of different materials, obtaining squares of 3x3 cm. Materials tested:
 - External filtration layer of FFP2 antiparticulate mask.
 - Protective suit fabric type 3B.
 - Polypropylene surface.
 - Steel surface.
 - Glass surface.
- Clamping of the samples of each type of material on the base of p-60 petri plates

using customized adapters obtained by 3D printing to ensure proper positioning of the materials to be tested.

- Impregnation of the samples with 300 μ L of viral suspension of known titer (range 10^5 - 10^6 pfu/mL). The viral suspension was in DMEM medium plus 2% FBS, or in such medium supplemented and previously diluted 1/10 with PBS.
- Drying for 45 minutes as explained in the previous section or treatment of the sample without drying.
- Introduction of the samples in the airtight chamber. The chamber has a capacity for two plates with 8 adapters for p-60 plates, but for greater reliability of the results, guaranteeing that the samples had identical treatment, only one of the plates was used in each exposure. Therefore, the number of samples per exposure was eight.
- Generation of atmospheric plasma, power, and time curves under controlled conditions at room temperature.
- Extraction of the plates and recovery of the viruses impregnated in the different materials by lishing and resuspension in 300 μ L of PBS.

During the tests, the temperature in the chamber was continuously monitored to verify that it remains constant, not detecting in any case a difference greater than 2–5°C between the points inside and outside. In addition, the possible exposure to the ozone generated was also continuously monitored to verify that the threshold limit value of 0.01 ppm was not exceeded at any time, especially when the chamber door was opened.

Titration of the collected viral suspensions

After performing the atmospheric plasma exposure assays, the HCoV-229E and SARS-CoV-2 control and treated viral suspensions were titrated by assessing their infectivity in susceptible Huh7 (HCoV-229E) and Vero E6 (SARS-CoV-2) cells using titration assays as indicated above. In these assays, two replicates for each dilution and a minimum of 8 dilutions of each viral suspension were performed for each titration following a 1/10 dilutional series in order to obtain the most suitable dilution for reliable lysis plate counting. The results were then analyzed by comparing the viral titers in control and treated samples and determining the reduction in viral viability that exposure to atmospheric plasma could have produced in the two types of viruses.

3.3.9 Bacterial sample preparation and recovery in a closed system

Bacteria and culture conditions

The bacteria used in this study were *Escherichia coli* CECT 515 and *Listeria monocytogenes* CECT 911. For each strain, a colony was inoculated in 2 mL of brain heart infusion (BHI) broth and incubated at 37°C for 24 hours until reaching the stationary growth phase (approximately 10^9 cfu/mL) (colony-forming unit).

Validation of Agar-BHI petri dish

Validation was carried out on agar-BHI petri dishes where the test bacteria were treated

in the culture medium. This evaluates the effectiveness of ionic wind inactivating bacteria directly. Tests were conducted with positive discharge at 50 W and negative discharge at 30 W. 100 μ L of the dilution considered was plated on agar-BHI plates, and tests were conducted in EIRA, introducing two plates for each treatment time of 1, 2, 4, 6, 10, 15, 20, and 30 minutes for positive discharge, and 1, 2, 4, 6, and 10 minutes for negative discharge. After the tests, the plates were incubated at 37°C for 24 hours, followed by colony counting. Each test was conducted in triplicate.

Material surfaces

Sample surfaces used in the assay were made of stainless steel and glass, with a size of 50x50x0.5 mm. Before the test, samples were sterilized by immersion in 70% ethanol for 1 day, dried, wrapped in aluminum foil, and autoclaved at 121°C and 1.2 atmospheres pressure for 20 minutes. Inoculation of material surfaces The surfaces were categorized into control and treated samples. Control samples include negative controls (3 replicates) without inoculation and positive controls (3 replicates) inoculated with 100 μ L. Treated probes (2 replicates for each treatment time) were also inoculated with 100 μ L. To ensure homogeneous treatment across the probe surface, the inoculum was spread using a Digralsky loop. Some probes were treated in the chamber (wet droplet condition), while others were allowed to dry (dry droplet condition) under aseptic conditions before treatment.

Treatment and bacterial recovery

Ionic air treatments in the chamber were conducted for 1, 2, 4, 10, 20, and 30 minutes. Between each treatment, the chamber was ventilated for a few minutes to reduce the concentration of reactive species to zero. Bacterial recovery from controls and treated surfaces employs Ringer's solution and swabs. For each surface, a 15 mL Falcon tube was filled with 5 mL of Ringer's solution, and the following steps were followed:

1. Swab moistened in Ringer's solution.
2. Swabbing the sample surface twice, horizontally and vertically.
3. Optionally, remoisten the swab lightly if necessary.
4. Swabbing the sample surface diagonally in both directions. Each tube was vortexed for 1 minute, with a 5 second pause at the 30 second mark. If necessary, serial dilutions of 1/10 were made in Eppendorf tubes with Ringer's solution. Finally, 100 μ L of the dilution was plated on agar-BHI plates and incubated at 37°C for 24 hours, followed by colony counting. Each assay was performed in triplicate.

The logarithmic reductions for each studied were calculated:

$$RD = \log \left(\frac{C_{ti}}{C_{tf}} \right) \quad (3.7)$$

where C_{ti} and C_{tf} are the initial and final bacterail concentrations after treatment, are expressed in cfu/mL (colony-forming unit).

The percentage of microbial inactivation obtained (PR) was determined, as in the case of

virus assays, through logarithmic reductions using the following formula:

$$PR = (1 - 10^{-RD}) \times 100 \quad (3.8)$$

where PR represents the percentage reduction [%], and RD denotes the logarithmic reductions.

3.3.10 Microbial inactivation models

The theoretical microbial inactivation models, which will later be used to fit the data obtained in the case of surface studies on petri dishes, are described.

Logarithmic or Chick-Watson model

The logarithmic model, or Chick-Watson model, is the simplest one and assumes first-order kinetics. In this model, microbial inactivation is governed by the disinfectant concentration and time. It is described by an equation that relates the reduction in the microbial population to the inactivation constant (k), the disinfectant concentration (C), and time (t). This model is suitable for homogeneous populations but struggles to account for initial delays (shoulders) or prolonged resistances (tails). From this model, the decimal reduction time (D) can be derived, which indicates the time required to reduce the microbial population by 90%.

$$\log \left(\frac{N_t}{N_0} \right) = -k \cdot C \cdot t$$

- N_0 : Initial concentration of microorganisms.
- N_t : Microorganism concentration at time t .
- k : Inactivation constant.
- C : Disinfectant concentration.
- t : Exposure time.

Decimal reduction (D):

$$D = \frac{1}{k \cdot C}$$

Hom model

The Hom model extends the logarithmic model by introducing a non-linear relationship with both the disinfectant concentration and time. This allows it to capture more complex phenomena, such as shoulders at the start of inactivation or tails at the end. These effects arise when the microbial population is not homogeneous, meaning there are differences in resistance among the microorganisms.

$$\log \left(\frac{N_t}{N_0} \right) = -k \cdot C^n \cdot t^m$$

- n : Nonlinear dependence on concentration.

- m : Nonlinear dependence on time.
- $m > 1$: Shoulders (initial delays).
- $m < 1$: Tails (prolonged persistence).

Weibull model

The Weibull model is specifically used for heterogeneous populations. It describes inactivation as a process where microorganisms have a distribution of resistances. Its equation introduces a shape parameter (p) that controls the curvature of the inactivation: high p values produce convex curves, while low values generate concave curves. This model is especially useful for describing initial delays and prolonged resistances within the same population.

$$\log \left(\frac{N_t}{N_0} \right) = - \left(\frac{t}{\delta} \right)^p$$

- δ : Characteristic time for decimal reduction.
- p : Shape parameter:
- $p > 1$: Convex curve.
- $p < 1$: Concave curve.

Biphasic model

The biphasic model is an alternative to describe populations composed of two groups of microorganisms with clearly differentiated resistances. The equation considers two subpopulations: one more sensitive and the other more resistant. Each subpopulation has its own inactivation constant, and the model combines both to describe the overall curve. It is ideal for situations where inactivation begins rapidly due to the death of the more sensitive microorganisms but slows down as the more resistant ones persist.

$$\log\left(\frac{N_t}{N_0}\right) = \log\left[P \cdot e^{-k_1 \cdot t} + (1 - P) \cdot e^{-k_2 \cdot t}\right]$$

- P : Sensitive fraction.
- k_1, k_2 : Inactivation constants.

Biphasic model with shoulder

The biphasic model with shoulder adds a term representing an initial delay before significant inactivation begins. This delay may be due to defense mechanisms of the microorganisms or initial system conditions. This model combines the effects of initial delays, heterogeneous populations, and prolonged tails, making it suitable for more complex situations.

$$f(t) = \frac{e^{k_1 \cdot S}}{1 + (e^{k_1 \cdot S} - 1) \cdot e^{-k_1 \cdot t}}$$

In all these models, key parameters can be extracted, such as inactivation constants (k), decimal reduction times (D), and proportions between subpopulations. These parameters allow analyzing the effectiveness of the disinfectant and the resistance of microorganisms, providing a tool to optimize disinfection or sterilization processes.

The models subsequently used for fitting are the Weibull, biphasic and biphasic with shoulder.

Chapter 4

Electrochemical analysis in an open system

This chapter¹ presents a parametric analysis to investigate the mechanisms driving the generation of ionic wind and reactive oxygen species in non-thermal atmospheric plasma systems produced by the described corona discharge devices in an open system. Both positive and negative discharges are examined to investigate electrical, fluid dynamic, and chemical processes, focusing on the relationship between ion wind dynamics, oxidant species production, and the influence of electrical and geometrical characteristics of the systems. The analysis also involves the validation of previously developed models through experimental work with prototypes, allowing the optimization of electrode configurations. These results are then applied to improve the efficiency of air treatment systems, specifically for the removal of airborne microorganisms such as bacteria and viruses, ensuring that the designs are in accordance with the requirements of air purification applications.

¹Ruiz-Trujillo, S. Vega-Martínez, P., Herrera-Arteaga, M. I. and Díaz Lantada, A. (2025). Experimental research on atmospheric non-thermal plasma devices under positive and negative discharge operating conditions: effects on ionic wind and generated species. *Plasma Processes and Polymers*, e70024. This publication is totally included.

4.1 Plasma device and operation parameters

The corona discharge plasma system used as the basis for the experimental campaign described in this chapter consists of a wire-to-two-plane configuration with seven tungsten wires, each 50 mm in length, with a wire-to-two-plane configuration. Devices with different numbers of wires are studied to determine their impact on the efficiency of ionic wind generation and reactive species production as described in the materials and methods section and with previously presented test benches.

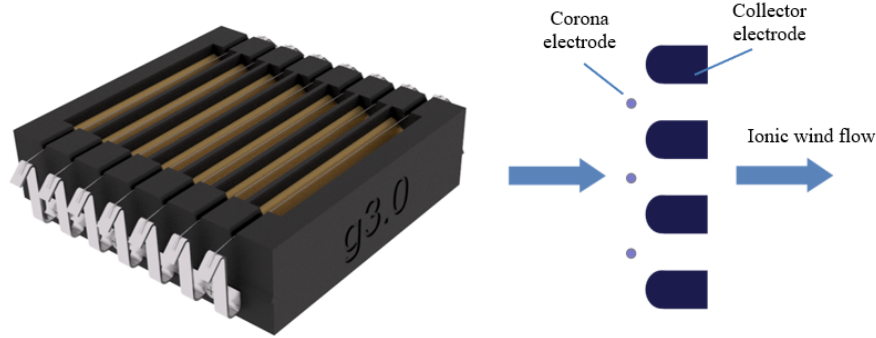


Figure 4.1: Rendering of the corona discharge device used in the experimental tests (left), with 7 tungsten wires confronted to two brass planes. On the right, a section of the plasma device is shown, where the wires facing the two planes can be observed.

Both positive and negative DC discharge modes are examined, operating within a voltage range of 0 to 10 kV.

4.2 Electrical characterization

This section focuses on the influence of the geometrical parameters of plasma devices on their electrical behavior. The characteristic voltage-current curves (CVCCs) are analyzed as a function of the distance between electrodes (gap), the diameter of the wire, and the effect of increasing the number of wires in parallel, evaluated both in positive and negative discharges.

CVCCs provide information about voltage threshold for breakdown, stable and unstable discharge regimes, and overall efficiency of plasma generation under different conditions can be identified. The observed CVCCs correlate with Townsend discharge theory [119], a well-established framework in plasma physics. Townsend's law describes the initiation and maintenance of gas discharges, emphasizing the role of ionization coefficients and secondary electron emissions for a coaxial cylindrical configuration.

$$I = K \cdot V(V - V_0) \quad (4.1)$$

where I is the current, V is the applied voltage, V_0 is the ignition voltage, and K is a constant that depends on geometrical, gas characteristics and environmental factors. This law is later adapted by various authors to suit different types of geometry. Meng et al. [120] proposed

a current-voltage relationship fit a larger number of configurations. The discharge ignition voltage is also taken into account. By a logarithmic fit of the equation, a linear trend of the data is observed (Figure 4.3).

$$I = K \cdot (V - V_0)^m \quad (4.2)$$

$$\log I = m \cdot \log(V - V_0) + a \quad (4.3)$$

where m , V_0 , and K are dependent on the disposition of the electrodes, the polarity of the discharge, the mobility of the species and the physical parameters of the gas. Figure 4.2 shows the electrical characterization curves (CVCCs) for the plasma devices under study in positive and negative corona discharge. The gap, defined as the minimum distance between electrodes (ground and corona), and the wire diameter have been systematically varied to analyze their impact on the electrical behavior of the plasma.

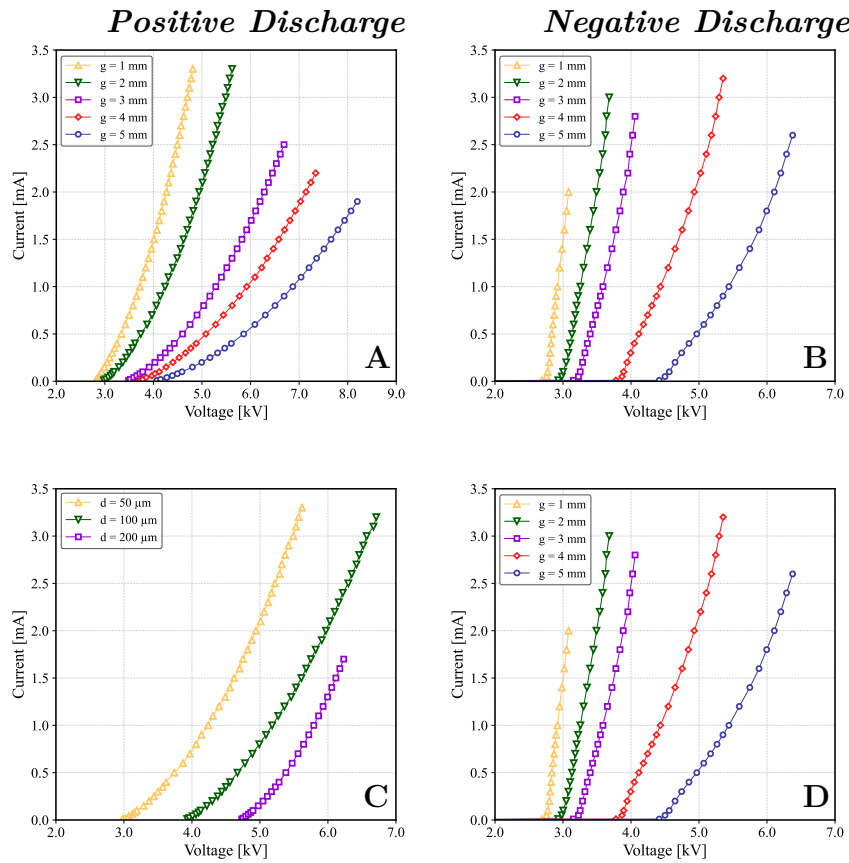


Figure 4.2: CVCCs for positive (A) and negative (B) corona discharge at different gaps, with a 50 μm wire diameter. Positive (C) and negative (D) CVCCs at different wire diameters, with a gap of 3 mm.

As can be seen in Figure 4.2, the ignition voltage depends on the geometrical parameters. It was observed that it increases with larger gaps. In the same way, an increase of the wire diameter results in higher ignition voltages. This trend is similar for both positive and negative discharge.

Figure 4.3 shows the fitting curves of CVCCs for different gaps and wire diameters, using Eq. (4.3) as proposed by Meng. A linear trend is observed in the positive discharge results, with the lines obtained in the case of gap variation being practically parallel, while those corresponding to diameter variation are overlapped. Similar behavior is noted in the case of negative discharge; however, the relationship between $\text{Log}(I)$ and $\text{Log}(V-V_0)$ (4.3) seems non-linear at the beginning of the curve, possibly due to processes such as secondary electron emission and ionization saturation. Electrons dominate the current, and their rapid multiplication through impact ionization may produce deviations from linearity.

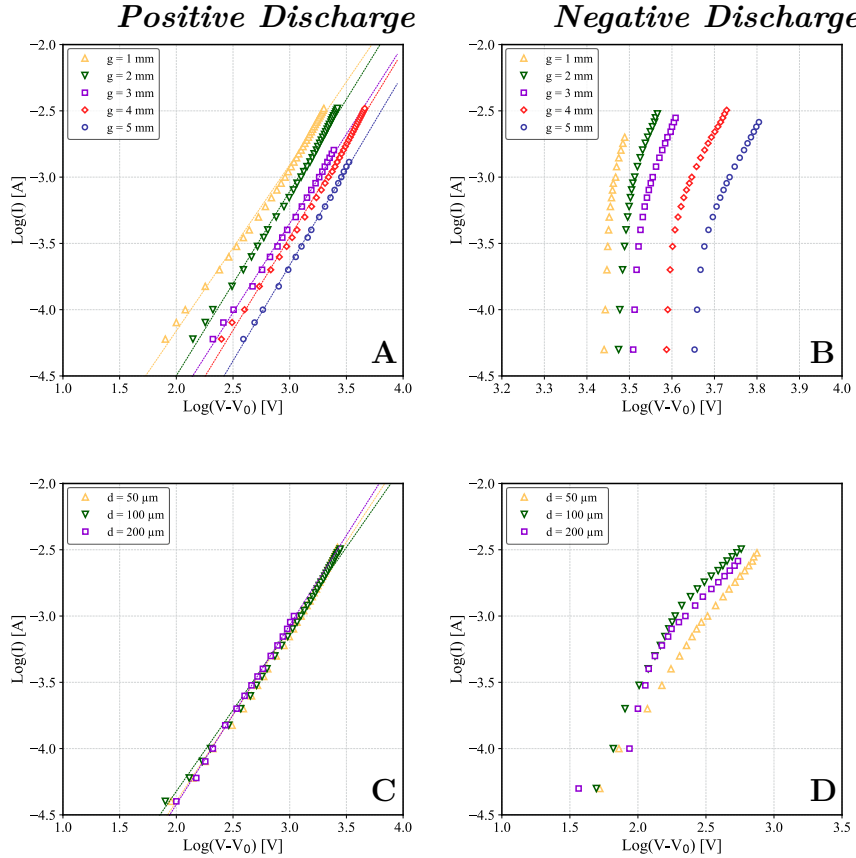


Figure 4.3: Relationship between $\text{Log}(I)$ and $\text{Log}(V - V_0)$ for different gaps in positive (A) and negative (B) discharge with $50 \mu\text{m}$ wire diameter and different wire diameters in positive (C) and negative (D) discharge with a gap of 3 mm.

Furthermore, at higher voltages, ionization saturates, and electron recombination and collisions are expected to become more significant, increasing the nonlinearity. In contrast, the positive corona discharge is dominated by positive ions, which flow more uniformly and produce a more stable and linear relationship between current and voltage, maintaining a straight line in the logarithmic plot. This behavior agrees with expectations according to previous studies by our team [115, 116].

4.3 Neutral phase analysis

Characterization of the neutral phase generated by corona discharge plasma devices is performed by fluidic and chemical analysis of the produced flow. This is carried out in the wind tunnel described in Chapter 2, Experimental set-up section. Pressure sensors and electrochemical cell sensors are used for the evaluation of the neutral phase as detailed in that chapter.

An analysis of the neutral phase of the gas generated by the EHD plasmas devices is presented. Two long-lived species, ozone and nitrogen dioxide, are taken as reference compounds because of their industrial relevance and eco-impacts. The aim is to study the relationship between the production of these compounds and the velocity generated by the system in both types of discharge, positive and negative. The dependence of the electrical and geometrical parameters is discussed.

4.3.1 Effect of geometrical parameters: gap and wire diameter

The relationship between fluid and chemical behavior of the neutral gas phase in plasma devices, depending on the electrode gap and wire diameter, under positive and negative discharge, is thoroughly analyzed to provide a comprehensive understanding of their impact on atmospheric non-thermal plasma (ANTP) generation by corona discharge.

Clearly, variations in the gap significantly influence the behavior of the neutral gas, when considering the two relevant compounds under study: ozone and nitrogen dioxide. As the gap increases, the electric field distribution within the discharge region is altered, which affects the ionization processes and, consequently, the plasma characteristics. A larger distance generally requires a higher breakdown voltage, which can lead to changes in the energy distribution between the plasma species and the chemical reactions involved. In contrast, a smaller separation increases the electric field strength, promoting more efficient ionization and potentially increasing the production of reactive species.

Likewise, the diameter of the wire electrode plays a crucial role in determining the fluidic and chemical properties of the plasma. The electrical plasma current increases as the diameter of the emitting electrode increases. This is because a larger emitting electrode provides a larger surface area for gas ionization. In addition, more surface area is available for electron generation.

Figure 4.4 shows the mass flow rates of ozone (O_3) and nitrogen dioxide (NO_2) and the velocity measurements as a function of the current of the device for different gaps. The velocity is determined by the dynamic pressure at the outlet of the device, determined by:

$$p = \frac{1}{2}\rho v^2 \quad (4.4)$$

where p is dynamic pressure measured [Pa], ρ is the density of the gas (air in this case) [mg/m^3], and v is the velocity [m/s]. The mass flow rate is obtained with the following expression:

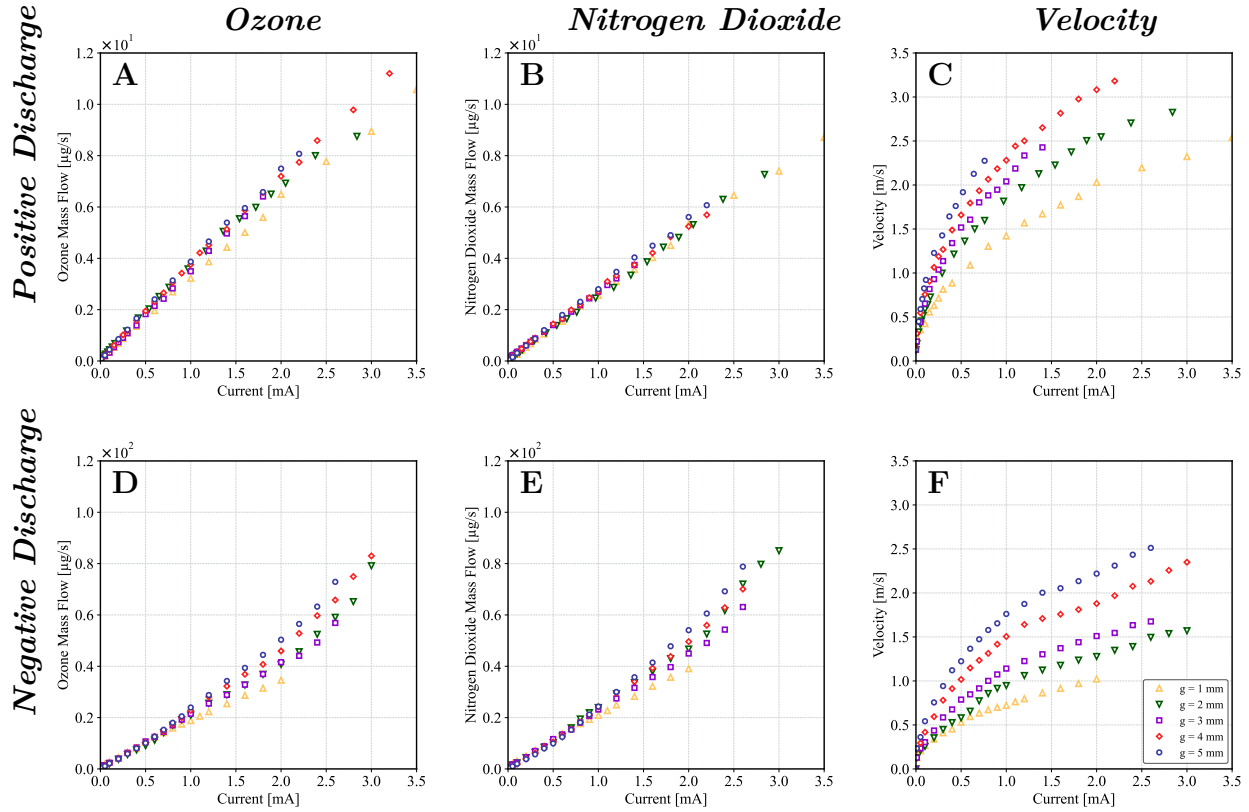


Figure 4.4: Ozone and nitrogen dioxide mass flow rate and velocity as a function of current supplied to the plasma devices for positive (A, B, C) and negative (D, E, F) discharge at different gaps and wire diameter = 50 μm .

$$Q = v \cdot A \quad (4.5)$$

$$\dot{m}_i = Q \cdot [C_i] \quad (4.6)$$

Q is the ionic wind flow rate; v is the velocity, and A is the area at the outlet of the device. The mass flow rate is $\dot{m}_i$ and $[C_i]$ is the concentration of each compound.

A linear dependence between mass flow rates and current is clearly observed for the case of positive discharge, as well as, and a linear trend is also seen in negative discharge, although the dispersion of the data grows as the current value increases. It can be noticed that the production rate of each compound depends on the current of the system, regardless of the gap. It is observed that the velocity decreases with smaller gaps for both positive and negative discharge.

The concentration behavior has opposite trend, at lower gaps, O_3 and NO_2 concentrations are higher for positive and negative discharge. Notice, the concentrations obtained in the case of negative discharge are one order of magnitude higher than those for positive discharge. Considering the velocities in negative discharge, these are lower than in positive. This is consistent with the generation mechanisms of each type of discharge.

According to the theoretical mechanism, the positive corona produces a lower density of

free electrons; however, these electrons have a high energy, since they are in the region of a high potential gradient. In contrast, the negative discharge produces a high electron density, mainly of lower energy, because they are placed in a region of reduced potential gradient. The higher electron density should increase the reaction rate for reactions that do not require a large amount of energy to happen. This is the case for ozone production, as the reactions that generate ozone are relatively low energy. Therefore, the higher number of electrons in a negative corona leads to greater production [65].

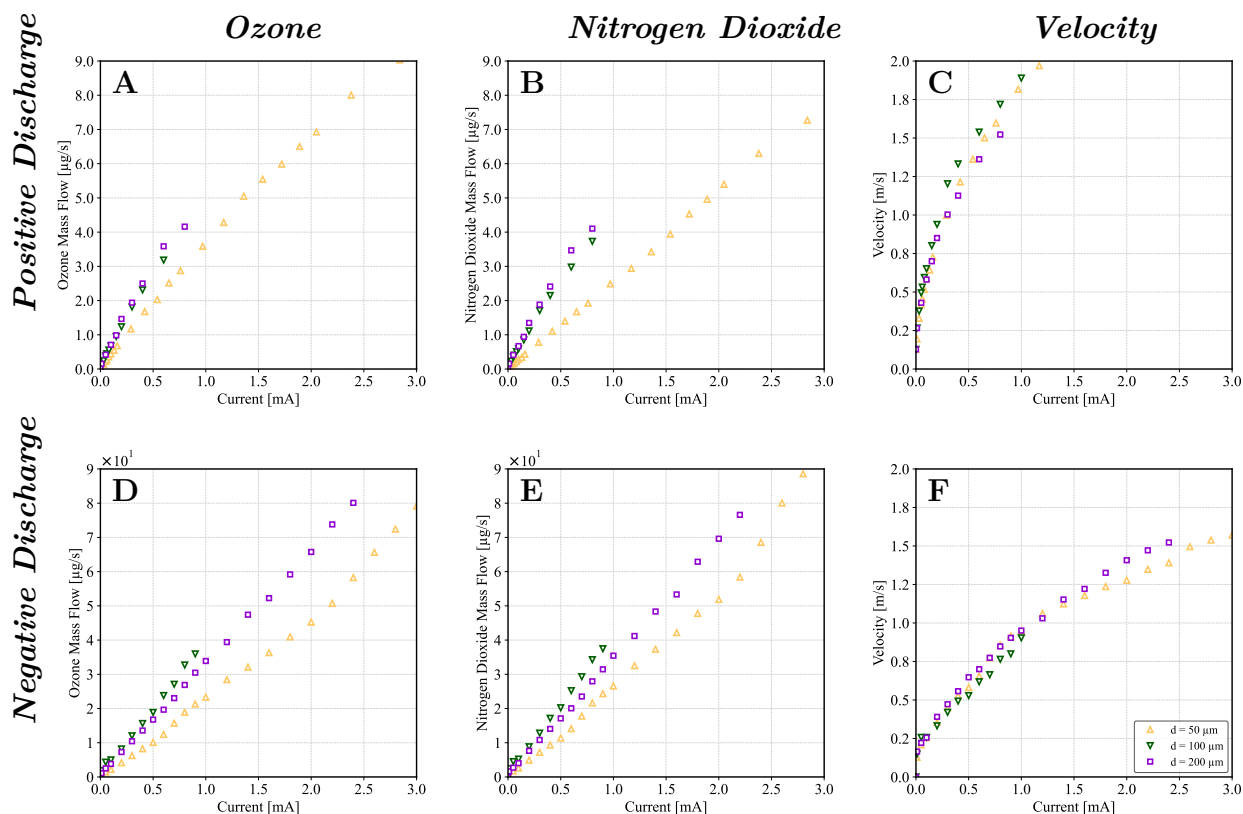


Figure 4.5: Ozone and nitrogen dioxide mass flow rate and velocity as a function of current supplied to the plasma devices for positive (A, B, C) and negative (D, E, F) discharge at different wire diameters and $g = 3 \text{ mm}$.

Furthermore, variations of the diameter of the emitting electrode are also analyzed. The Figure 4.5 presents the data obtained for the mass flow rates of O_3 and NO_2 and the velocity as functions of current, both for positive and negative discharge. It can be seen that the mass flow rate values vary as a function of the wire diameter, while the velocity remains constant independently of the diameter, that increases with the current value. Higher O_3 and NO_2 concentrations are detected with larger wire diameters. In these cases, a higher rate of chemical conversion is observed, which does not happen in the case of velocity generation. A linear trend is also observed between the mass flow rates of O_3 and NO_2 and the current supplied to the device in both types of discharges. A wider dispersion is observed for negative discharge at a diameter of $50 \mu\text{m}$. In corona discharge, the diameter of the emitting electrode significantly influences the concentration of reactive species due to its effect on the electric field strength and the rate of ionization. A larger wire diameter results in a lower electric

field current near the conductor surface for a given applied voltage, leading to a more stable discharge and enhanced chemical reactions over a large volume of gas. This increased volume contributes to higher concentrations of species like ozone and nitrogen dioxide. The formation of O_3 occurs primarily through the reaction of O_2 with atomic oxygen O , which is produced by electron-impact dissociation [121, 122]:



where M presents the third collision molecule to absorb excess energy and stabilize ozone formation. Additionally, the reaction between metastable molecular nitrogen and atomic oxygen can lead to the formation of nitric oxide (NO).



NO_2 is generated from nitrogen monoxide via the reaction with O_3 [122, 123]:



and as a result of the reaction between nitric oxide and atomic oxygen [122, 123]:



The lifetime of NO is relatively short because it oxidizes rapidly in the presence of O_3 , becoming NO_2 . This oxidation process significantly reduces the concentration of NO , preventing its growth in the neutral phase. As mentioned before, the current supplied to the corona device also influences the mass flow rate of these species. O_3 and NO_2 concentrations exhibit a linear relationship with the electrical current of the discharge.

$$\dot{m} \propto I$$

where $\dot{m}_i$ is the mass flow rate of the species, and I is the discharge current. In both positive and negative corona discharges, increasing the supplied current results in higher production rates of O_3 and NO_2 . However, the chemical reaction rates are more sensitive to the field distribution, which varies with the wire diameter.

4.3.2 Effect of wires configuration

The effect of increasing the number of wires in series for both types of corona discharge is analyzed, in connection with the scalability and industrial application of these systems. A “stage” refers to a single corona discharge device with 7 wires. By placing multiple stages in series, the total number of wires increases, multiplying the total electrical current. This configuration is evaluated to determine its impact on the parameters under study, including species production rates and fluid dynamics. Figure 4.6 shows an example of 3 stages of 7-wire corona discharge devices in series.

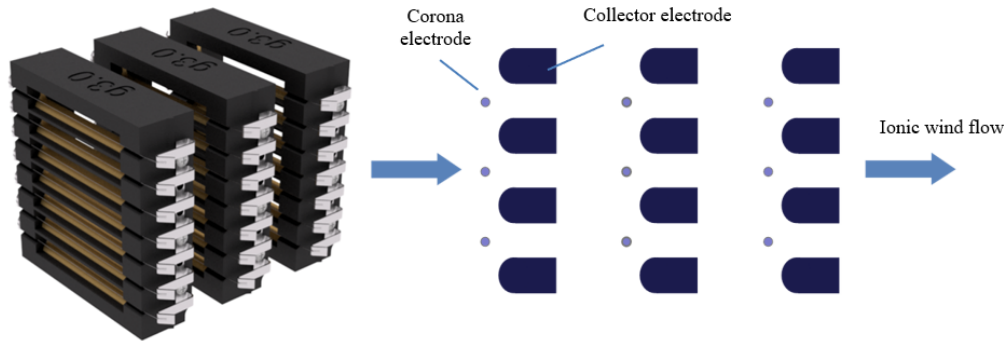


Figure 4.6: Rendered design of 3 stages of 7-wire corona discharge devices in series (left). On the right, a schematic of the discharge in the 3 stages configuration is shown, where the wires facing the two planes can be observed.

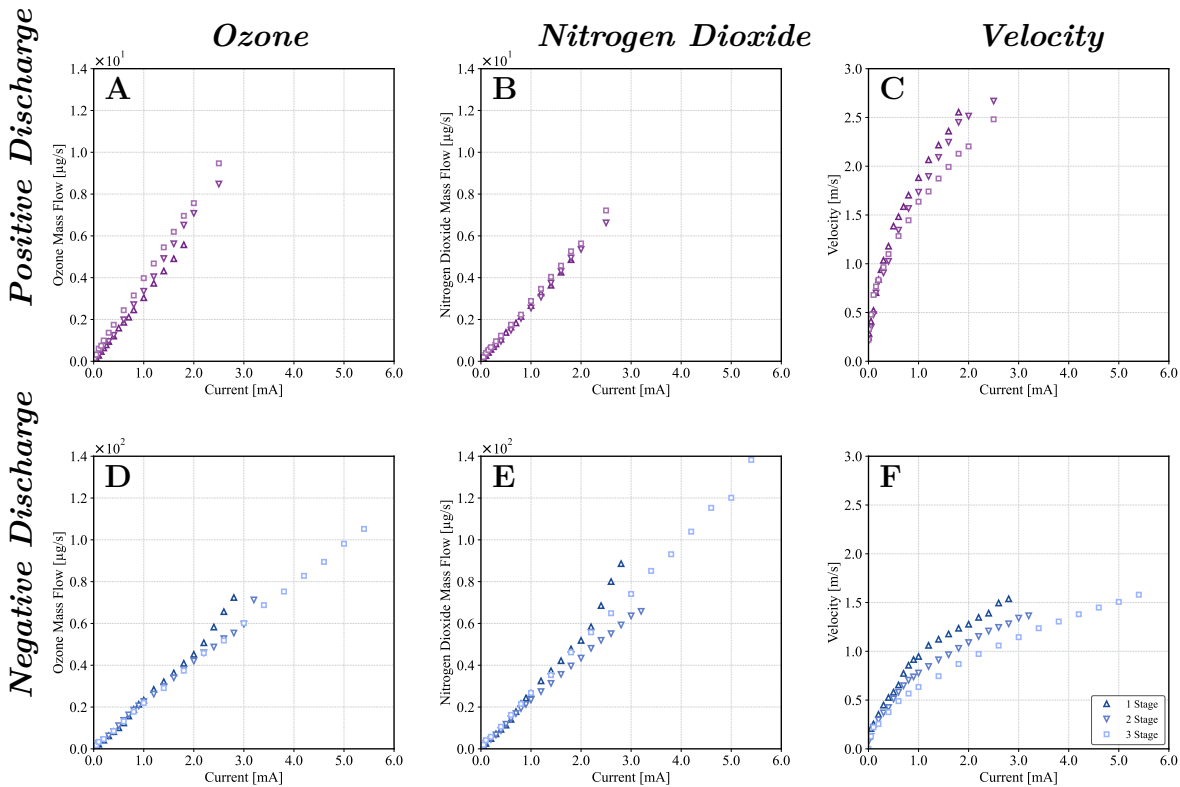


Figure 4.7: Ozone and nitrogen dioxide mass flow rate and velocity as a function of current supplied to the plasma devices for positive (A, B, C) and negative (D, E, F) discharge at different gaps and wire diameter = $50 \mu\text{m}$.

For this experimental campaign, a reference gap and wire diameter are used, specifically a gap 3 mm and wire diameter equal to 50 μm in all the devices. Figure 4.7 collects the data obtained for the case of series stage placement. A trend analogous to the previous case studies can be observed. Both ozone and nitrogen dioxide mass flow rates present linear dependencies with respect the current. This suggests that the rate of species production depends on the total current supplied to the plasma system. However, the velocity is not enhanced by adding more of wires or stages of the plasma device, although it becomes higher when raising the total current. Increasing the number of stages impacts the static pressure but has no effect on the dynamic pressure, resulting in a constant velocity at the same current level.

4.4 Active phase analysis

This section shows the results obtained from the study of the emission electrode (wire) and its variation as a function of the gap (distance between electrodes), wire diameter, wire number, and the type of discharge. The emission spectrum of the plasma corona is recorded between 200 and 450 nm, where the most intense emission is observed, using the Optical Emission Spectrometry equipment detailed in Chapter 3. The emission spectrum detected can be attributed to the N_2 second positive band system (SPS: $N_2[C^3\Pi_u]v' \rightarrow N_2[B^3\Pi_g]v''$), specifically at wavelengths 315.93 nm, 337.1 nm, 357.69 nm, and 380.49 nm, with a dominant emission ($v' = 0, v'' = 0$) band head at 337.1 nm [64, 124, 125].

The weak emission at 391.4 nm is attributed to the (0-0) transition of the N_2 first negative system (FNS: $N_2^+[B^3\Sigma_u^+]v' \rightarrow N_2^+[X^2\Sigma_g^+]v''$). Due to this, the analysis is focused on the study in the N_2 second positive system [64, 124, 125].

The emission spectra are measured in both types of discharge, positive and negative discharge, and the spectrum observed with greater intensity is analyzed. The wavelengths with the widest emission band, in relative intensity, are 337.1 nm and 357 nm. The data obtained for each case study are presented as a function of the current supplied to the devices.

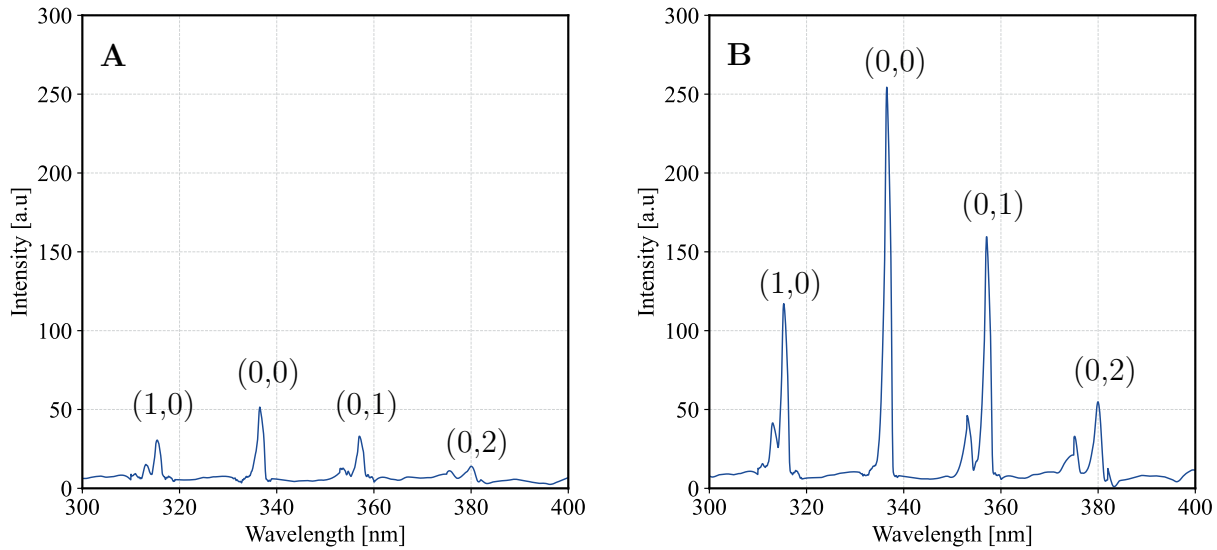


Figure 4.8: Emission spectrum of the $N_2(C^3\Pi_u - B^3\Pi_g)$ second positive band of N_2 obtained in the wavelength region 300–400 nm for positive (A) and negative (B) discharge for a corona plasma device of 7 wires, gap 3 mm and wire diameter of 50 μm at current of 3.0 mA. Vibrational transition (v', v'') is indicated on each spectrum.

The spectra for a 7-wire corona discharge plasma device, with a gap 3 mm and a wire diameter of 50 μm , in positive and negative discharge, supplied with a current of 3.0 mA are shown in Figure 4.8. This provides strong evidence of the second positive band of the nitrogen, identifying 4 vibrational states of nitrogen. It is assumed that the plasma particles in this band are in steady state thus excited states follow the Boltzmann distribution.

The band of highest emission intensity corresponds to the $\Delta\nu = 0$ transition at a wavelength of 337.1 nm. The emission in negative discharge is more intense than in positive discharge, about 5 times stronger. The negative discharge also has a greater difference between the different bands.

The spectrum band changes as a function of the electrical and geometrical parameters that are analyzed, taking as reference the transitions $\Delta\nu = 0$ (0,0), and $\Delta\nu = -1$ (0,1) with wavelengths of 337.1 nm and 357.69 nm, respectively.

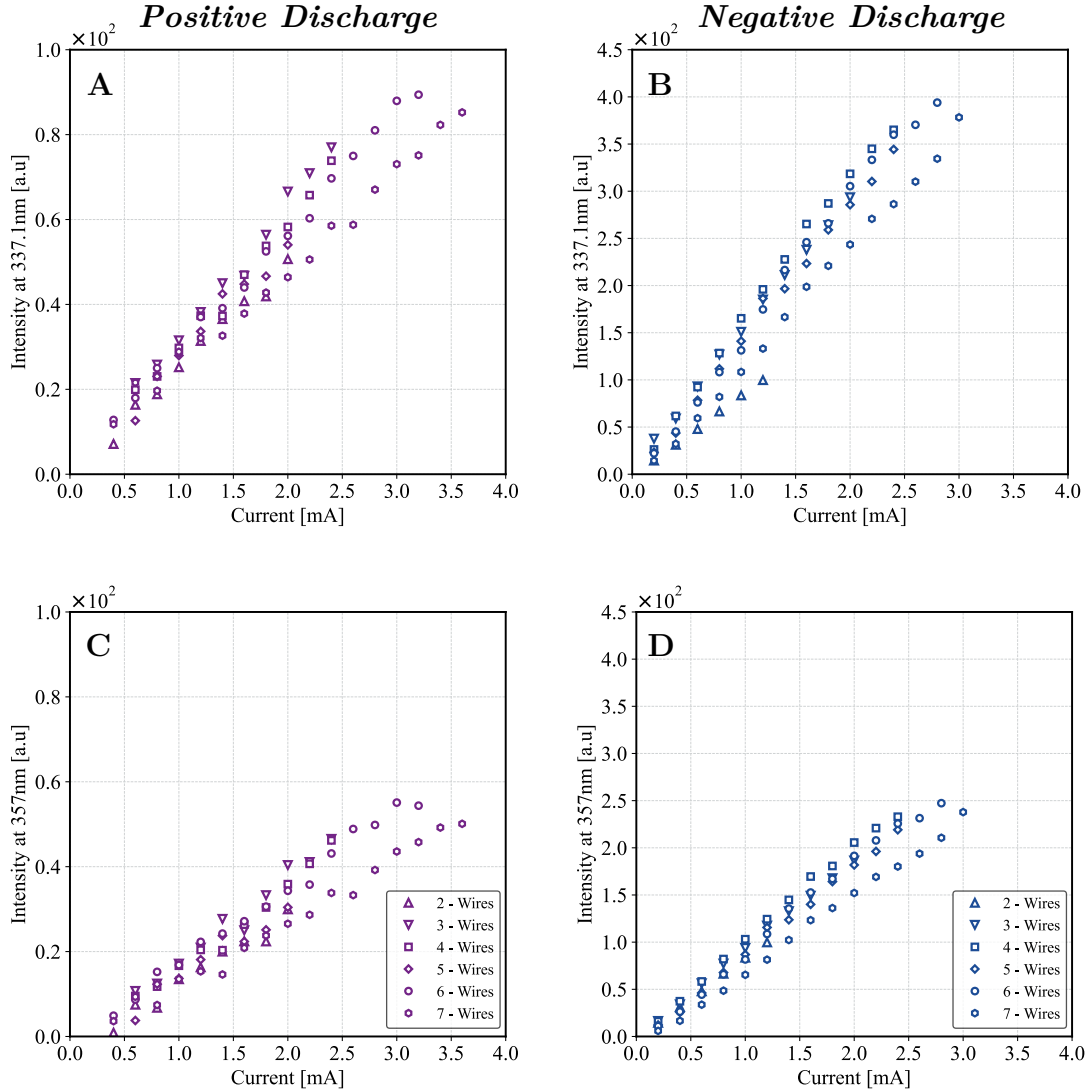


Figure 4.9: Emission intensity of positive (A, C) and negative (B, D) discharge at 337.1 and 357 nm for different wire numbers at a gap of 3 mm and a wire diameter of 50 μm .

Figure 4.9 shows the results of intensity against current supply for positive and negative discharge at 337.1 nm and 357 nm for different numbers of wires (gap = 3 mm and ϕ wire = 50 μm) in arbitrary units. Linear trends between the emission intensity and the electric current supplied by the plasma devices are detected, regardless of the number of wires, for

both wavelengths analyzed. The emission ranges for the case of negative discharge are 4–4.5 times higher than in positive discharge.

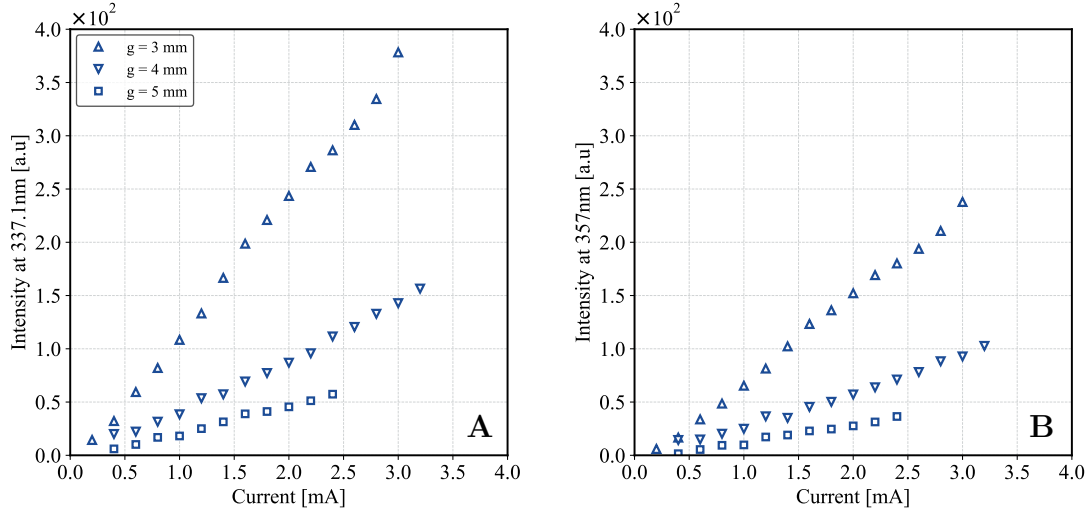


Figure 4.10: Emission intensity of negative discharge at 337.1 nm (A) and 357 nm (B) for different gaps with 7 wires and wire diameter of $50 \mu\text{m}$.

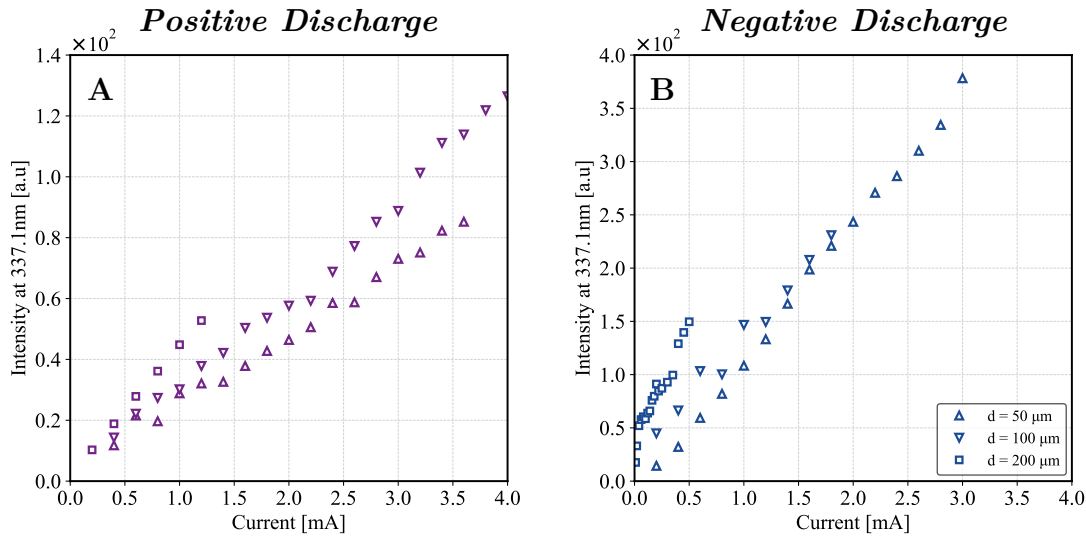


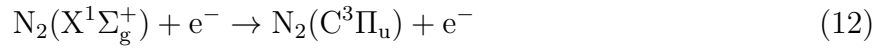
Figure 4.11: Emission intensity of positive (A) and negative (B) discharge at 337.1 nm for different diameter of wire, gap 3 mm, 7-wire devices.

Similar trends are observed for the gap variation (Figure 4.10) and wire diameter variation (Figure 4.11), showing a linear relationship between emitted intensity and supplied current. It can be appreciated that the slopes of the curves are greater with larger gaps and decrease as the gap increases (Figure 4.10). Increasing the diameter of the emitting electrode (wire) leads to a slight growth in emission, regardless of the type of discharge (Figure 4.11). Emission values about 4 times higher are observed in the case of negative discharge. The decrease in

emission intensity of the second positive nitrogen system with increasing electrode spacing can be attributed to several factors. As the gap grows, the electric field weakens, resulting in less energetic electrons and, consequently, fewer collisions to excite nitrogen molecules, leading to lower emission intensity. Additionally, electron and plasma densities decrease with greater gaps, further reducing excitation rates, while increased energy losses, such as collisional and radiative losses, contribute to the reduction in emission [67, 126].

This behavior aligns with the linear relationship observed between the supplied current and the emission intensity in the second positive nitrogen system and can be explained by energy transfer processes in the plasma during corona discharge. This system corresponds to the electronic transition from the $C^3\Pi_u$ to the $B^3\Pi_g$ state of nitrogen, where the emission intensity depends on the excitation of N_2 molecules [67, 126]. As current increases, more electrons are accelerated in the electric field, gaining kinetic energy and causing more frequent excitation of nitrogen molecules, thus enhancing the emission.

Electrons collide with N_2 molecules, exciting them to higher energy states such as $C^3\Pi_u$, described by the reaction:



These excited molecules then radiatively decay to the $B^3\Pi_g$ state, emitting photons. The intensity of this emission, $I(\lambda)$, is proportional to the number of excited N_2 molecules, which depends on the electron density n_e and the energy distribution among the electrons [67, 126]. The excitation rate can be described by:

$$I(\lambda) \propto n_e \cdot \sigma_{\text{exc}}(E_e) \cdot v_e$$

where $\sigma_{\text{exc}}(E_e)$ is the excitation cross-section of N_2 and v_e is the electron velocity [67]. Since the electron density and energy increase with the current, the emission intensity of the second positive system of N_2 is linearly related to the supplied current.

The linearity of this relationship is maintained regardless of the geometrical configuration (wire number, diameter, gap) and the type of discharge (positive or negative), since the main factor driving the excitation is the total energy delivered through the current. As verified in our experiments, geometrical parameters affect the electric field distribution, they do not alter the fundamental process of energy transfer between the electric field and the electrons. Similarly, although the type of discharge influences the charge carriers (electrons in negative corona, positive ions in positive corona), the overall excitation rate remains proportional to the supplied current.

The intensity of the second positive N_2 system is significantly higher in negative corona discharges compared to positive corona discharges due to the nature of electron dynamics. In negative corona discharges, there is a greater availability of energetic electrons, which are the primary agents for exciting nitrogen molecules. The electric field in the negative corona tends to accelerate electrons more efficiently than in the positive corona, where the dominant charge carriers are positive ions, which have much lower mobility and energy [65, 67]. This results in a higher excitation rate of N_2 negative discharges, leading to stronger emission intensities.

4.5 Conclusion

Several experimental campaigns are performed to understand the complex dependencies between geometrical parameters and their impact on the non-thermal plasma generation by corona discharge. Key geometrical parameters in plasma devices, such as the minimum distance between electrodes (gap) and the diameter of the electrode, are studied, and their effects on plasma from electrical, fluid, and chemical production species points of view. Also, the same plasma systems are evaluated in positive and negative discharge. This approach not only improve the understanding of plasma physics but also provides valuable information in the design and optimization of future plasma-based technologies for several applications in biomedical applications, air and surface treatment, and cooling systems, for example. The systematic characterization and the deeper understanding of these phenomena is crucial in validating performance models, facilitating precise predictions, and supporting the design of optimized systems.

This research confirms that the production of long-lived species in the neutral phase, taking ozone (O_3) and nitrogen dioxide (NO_2) as references, in non-thermal plasma corona discharge systems is strongly dependent on the electrical characteristics, with mass flow rates showing a linear correlation with the total discharge current. This behavior proved consistent in both positive and negative discharge types. It is observed that the electrode gap did not significantly influence the mass flow rates of these species. Larger gaps, however, led to increased ionic wind velocities but reduced concentrations of O_3 and NO_2 , suggesting that the expansion of the discharge region facilitates greater momentum transfer to neutral air particles but dilutes the chemical species generated. Greater emitting electrode diameters implied an increase in the mass flow rates of both species.

The increase in the number of wires leads to an increase in the total supplied current, which in turn proportionally raised the mass flow rates. This indicates that chemical production efficiency is primarily driven by the current rather than by geometric factors, such as the number of wires. This fact is particularly relevant for scaling up plasma systems, as it suggests that increasing the number of wires in plasma devices may lead to a proportional rise in the chemical production. Moreover, the addition of stages in series increases the static pressure but has no significant effect on the velocity.

Besides, a relevant result of this investigation is the significant difference between positive and negative discharge in chemical production. Negative discharge generated mass flow rates of O_3 and NO_2 approximately 10 times higher than observed in positive discharge, which can be attributed to differences in electron energy distributions and ionization efficiencies between the two modes. In spite of the lower chemical production, positive discharges showed higher fluid velocities, which contributed to greater energy transfer to the neutral gas phase and stronger ionic wind generation in these conditions. This particular fact is interesting for the scalability and real-environment applications of plasma devices, as the systems may be selected and adjusted according to specific requirements, with the possibility of combining both types of discharges.

Furthermore, optical emission spectroscopy (OES) analysis of the active phase of the plasma revealed dominant nitrogen emissions, specifically from the second positive system (SPS) of

nitrogen, with peak emission intensities at 337.1 nm and 357 nm. These emissions are directly related to electron excitation processes, and their intensity showed a linear relationship with the supplied current of the system. The intensity of the nitrogen bands is found to be 4 to 4.5 times greater in negative discharges, correlating with the higher electron energies and greater chemical activity in these conditions. Additionally, larger wire diameters are shown to increase emission intensity, likely due to enhanced local electric fields that raise electron acceleration and excitation rates.

The results of the study highlight the importance of considering both electrical and geometrical parameters in the optimization of corona discharge non-thermal plasma systems. The linear dependencies observed between current and both chemical production and emission intensity suggest that an accurate control of electrical inputs is critical for achieving desired plasma characteristics. In addition, a deeper knowledge about the differences between positive and negative discharge types, and their relation to geometrical parameters can guide the design of systems suited to specific applications, whether the goal is to enhance chemical species production, air purification, or maximize ionic wind generation for cooling or fluid dynamics applications. Authors consider that the presented experimental methods together with the described analytical models provide a systematic methodology for designing and optimizing these engineering systems.

Chapter 5

Electrochemical analysis in a closed system

Expanding on the knowledge presented in the previous chapter, Chapter 5 focuses on modeling the electrochemical behavior of plasma systems within a closed chamber. The analysis examines the influence of geometric and electrical parameters on the production of reactive species, utilizing ionic wind to achieve a homogeneous concentration within the analysis chamber. The goal is to develop and validate practical and easily implementable models that describe and optimize the chemical composition of the plasma, enabling its subsequent evaluation as a germicidal agent. These systems are designed to operate with ambient air at room temperature, atmospheric pressure, and typical humidity levels, without requiring additional chemical precursors.

The industrial application of these models is directed at the design and production of disinfection chambers for objects in various fields and applications, among others. These include environments where specific levels of disinfection or sterility are essential, such as hospitals, material pass-through cabinets in pharmaceutical or biosafety laboratories, dental labs, surgical theaters, and more. The chapter highlights the potential for these analyses to optimize plasma systems for customized disinfection solutions, addressing the specific demands of diverse sectors.

5.1 Plasma device and operation parameters

The analysis in this chapter focuses on prototypes of various configurations of corona discharge plasma devices, examining in detail the influence of geometric and electrical parameters on the generation of specific chemical compositions within a closed chamber. Key factors such as the number of wires and devices, their fluidic disposition (series or parallel), the gap (distance between electrodes), and their correlation with geometric parameters are thoroughly investigated.

The study begins with a comprehensive examination of positive corona discharge. Negative corona discharge is also analyzed, as it generates a higher concentration of reactive species, as

demonstrated in Chapter 4, and because the production of these species is found to be limited with positive discharge. The ultimate goal is to achieve a chemical composition capable of inactivating microorganisms inside the chamber. The focus is on identifying the configuration that maximizes chemical production and correlating it with microbial inactivation efficiency.

As described in Chapter 3, Materials and Methods, experiments are conducted in a 17L hermetic chamber. Selective measurements of ozone and nitrogen dioxide (neutral/long-lived species produced in higher proportions) are performed using electrochemical sensors. These species are used as reference points for modeling and their correlation with microbial inactivation. The sensors are shielded against potential electromagnetic interference to ensure accurate measurements.

The operational range for the experiments, as detailed in the relevant section, was maintained between 0 and 10 kV, using the resources described therein.

The N7H50 device (with the same basic configuration, wire-to-two-planes), consisting of 7 wires, each 50 mm in length, is again used as a reference for this study. Variations in the gap (distance between electrodes) and the number of devices were introduced. Another device used as a basis is the N7H150, which is three times the size of the N7H50, comprising 7 wires, each 150 mm in length.

The N7H50 devices are configured in series and parallel arrangements. In the series configuration, the current exiting the first device flows into the next one, and so on. In contrast, the parallel configuration places the devices side by side or combines them into a single, larger device, as is the case with the N7H150. The electrical characterization curves for these devices are presented in Chapter 4.

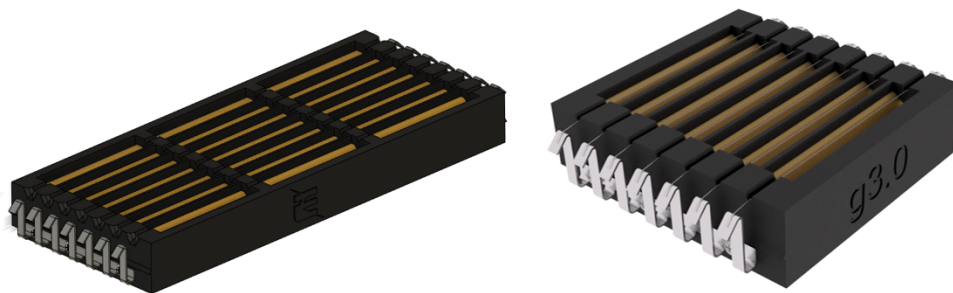


Figure 5.1: Devices with 7 wires, with length of 150 mm in length (right) and 50 mm (left), used as the basis for the study performed.

The power consumption of the device is determined per unit wire length. Since the wires are electrically connected in parallel, the current and power increase proportionally with the number of wires for the same configuration.

The following sections provide a summary of the main results obtained in the study, with a focus on identifying and extracting variables that play a key role in the generation of specific chemical compositions. Additionally, a modeling proposal is presented and experimentally validated.

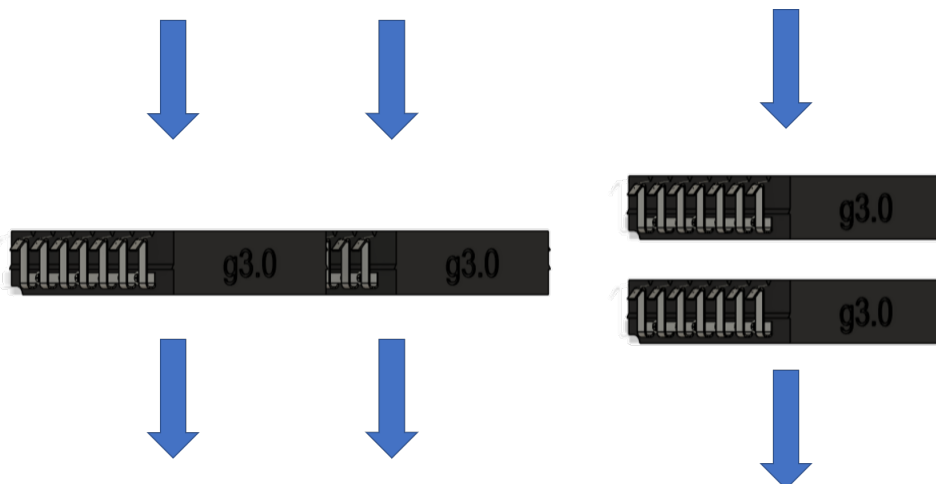


Figure 5.2: 7-wire device (N7H50) configured in parallel (right) and in series.

5.2 Analysis and results

This section presents the key results and conclusions derived from the analysis of EHD plasma systems generated by corona discharge within a 17L hermetic chamber. The study investigates various geometric parameters, including the gap (distance between electrodes), different numbers of wires and devices, and their configurations, as well as their correlation with electrical parameters. Both positive and negative corona discharge modes were evaluated, with the negative discharge demonstrating higher levels of reactive species concentration.

The analysis began with a detailed examination of the positive corona discharge mode to establish trends in species generation. Once these trends were identified, the negative corona discharge mode is analyzed, focusing on the most promising configurations selected based on the initial results.

As described in Chapter 3 (Materials and methods), concentration data for long-lived neutral species, such as O_3 and NO_2 , are collected using electrochemical sensors positioned at the bottom of the chamber. These species are used as references due to their higher concentrations relative to other generated compounds. The measurements are conducted under ambient conditions of temperature, pressure, and humidity, and data are recorded as concentration versus time for the various study cases.

These results will be used for kinetic modeling, providing insights into system behavior based on the fixed parameters and enabling a deeper understanding of the influence of geometric and electrical configurations on plasma performance.

5.2.1 Analysis of gap variation and arrangement

An analysis is conducted to determine the influence of geometric parameters, configuration, and the number of devices on the generation of chemical species over time within a closed chamber. Nitrogen dioxide (NO_2) is used as the reference for comparing species generation trends across these parameters, as ozone (O_3) shows an analogous behavior within the analyzed ranges. Additionally, NO_2 data shows lower variability, making it a more reliable indicator.

A comparison of the variations of the gaps between the positive and negative corona discharges is included. The study of negative discharge is initiated to address the need for significantly increasing the concentration of reactive species for microbial inactivation applications.

The device used to evaluate these parameters is the N7H50, consisting of 7 wires, each 50 mm in length and 50 micrometers in diameter and a fixed power of 10 W.

Figure 5.3 displays the concentration of NO_2 over time for N7H50 devices at different gaps and a power of 10 W. As observed, the initial slopes are very similar in all cases; however, the steady-state values differ significantly. The maximum concentration (steady state) is higher at smaller gaps and decreases as the gap increases. This behavior is analogous to the trends observed in the open-system analysis discussed in Chapter 4.

For a gap of 5 mm, steady-state NO_2 concentrations reach approximately 220 ppm, while for a gap of 2 mm, this value increases to around 270 ppm.

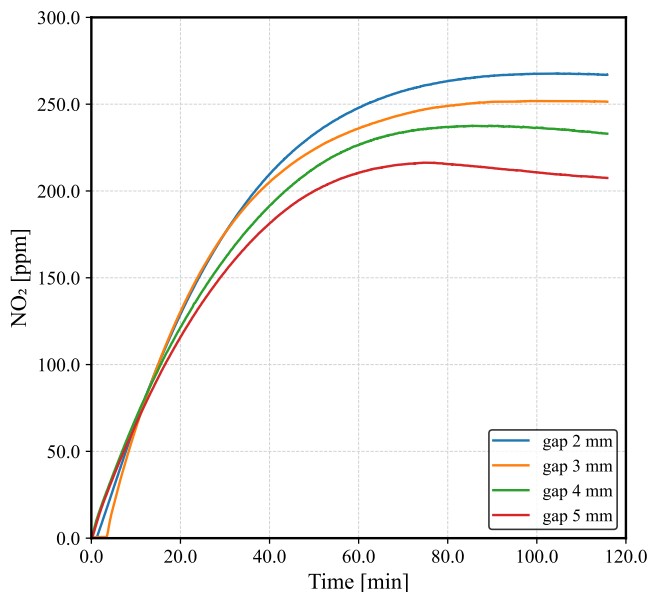


Figure 5.3: NO_2 concentration curves over time for different gaps (2, 3, 4, and 5 mm) in positive corona discharge using the device with 7-wire, 50 mm in length (N7H50) at 10 W power.

Similarly, in negative discharge, higher NO_2 concentrations are achieved as the gap decreases. Furthermore, the concentration in negative discharge is approximately 10 times greater than

in positive discharge, consistent with the trends observed in the open system analysis.

This is illustrated in Figure 5.4, which shows NO_2 concentration over time for both positive and negative corona discharges at gaps of 2 mm and 3 mm with a power of 10 W.

The arrangement of devices in series and parallel configurations is evaluated to determine whether it has any influence on performance. No significant differences were observed, as shown in Figure 5.5, which presents NO_2 concentration over time for the N7H50 system in both configurations. The curve for the parallel configuration is slightly lower, although this could be attributed to measurement deviations rather than a real difference in behavior.

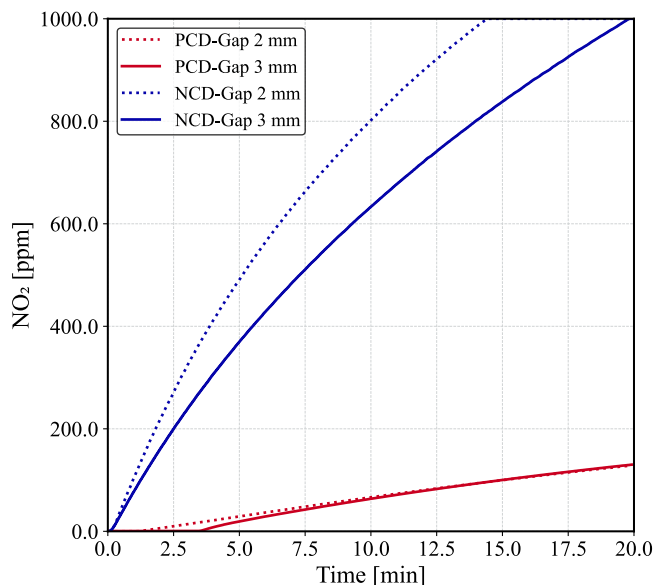


Figure 5.4: Comparison of NO_2 concentration curves over time at different gaps (2 and 3 mm) in positive and negative corona discharge using the for device with 7-wire, 50 mm in length (N7H50) at 10 W power.

Finally, the series arrangement of 1, 2, and 3 devices operating at the same total power is analyzed. Figure 5.6 compares NO_2 concentration data at 10 W of power for these configurations. As shown, the curves overlap, indicating that increasing the number of devices in series does not significantly affect the concentration of generated species.

However, increasing the number of devices in series and parallel allows for a higher total power/current in the system. This, in turn, enables the generation of higher concentrations of reactive species.

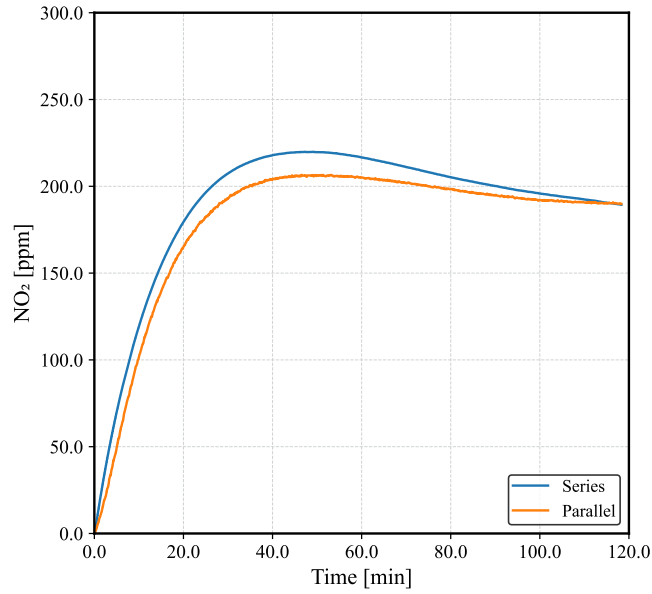


Figure 5.5: Comparison of NO₂ concentration over time in series and parallel configurations for devices with 7-wire, 50 mm in length (N7H50) and a 5 mm gap at 10 W power.

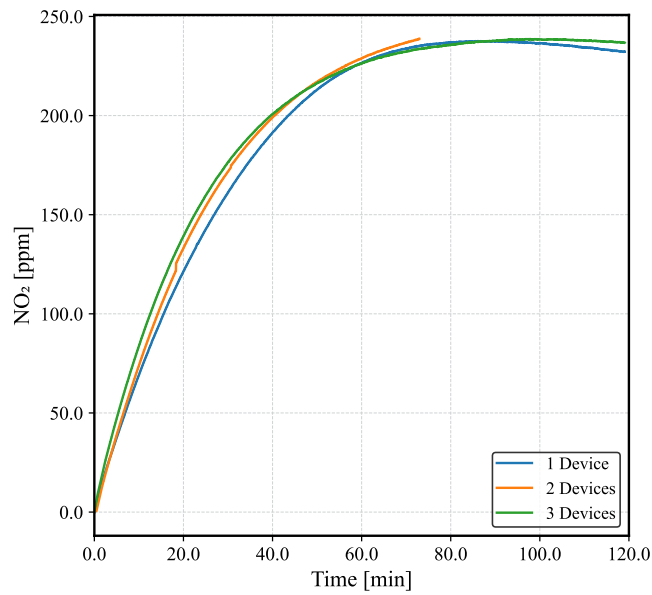


Figure 5.6: Comparison of NO₂ concentration over time with an increasing number of devices in series (1, 2, and 3) using device with 7-wire, 50 mm in length (N7H50) and a 5 mm gap at 10 W power.

5.2.2 Analysis of power variation and modeling

This section provides a summary of the power variation tests conducted with three N7H150 devices, each consisting of 7 wires and measuring 150 mm in length, within a closed 17L chamber under positive corona discharge. These prototypes were selected as they allow for an extended evaluation of power levels and reveal notable trends in the generation of ozone and nitrogen dioxide over time. Additional tests were conducted with other device configurations that exhibited behaviors similar to those observed here and in previously described setups.

Some of the results were also compared with those obtained from negative corona discharge, given its relevance in applications where significantly increasing the concentration of chemical compounds in the chamber is required.

Figures 5.7 and 5.8 show the concentrations of O_3 and NO_2 over time, respectively, at various power levels for the 7-wire, 150 mm device (N7H150) with a 3 mm gap. The slopes of the curves increase as the total power supplied to the system rises, as do their maximum values.

For ozone generation, higher power levels result in concentrations that rise more rapidly and subsequently decrease at an increasingly faster rate. At lower power levels, however, the concentration stabilizes at a steady-state value. This behavior may be attributed to the recombination of O_3 or its consumption in parallel reactions. The rate of this decline grows with both power and reaction time. Other authors report analogous phenomena with other plasma technologies [127–129]. At higher power levels, a transition occurs in the limiting reactions that control the process, shifting from being in the "ozone mode" at low power to the "transition mode" at medium power and finally to the "NO_x mode" [127]. This phenomenon will be discussed in greater detail later, with a simplified model proposed to fit the data based on certain assumptions.

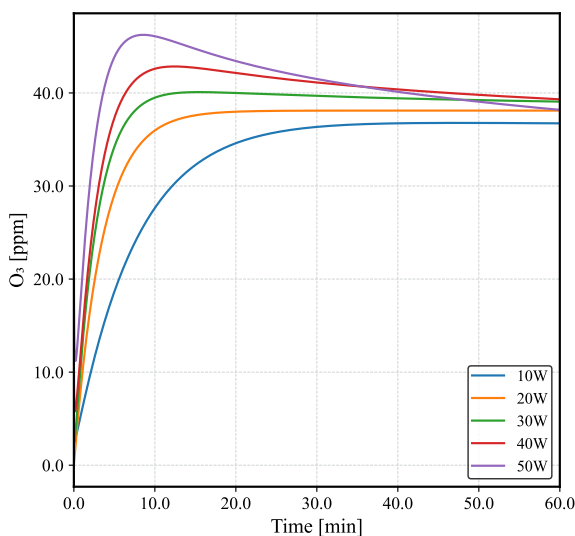


Figure 5.7: O_3 concentration over time at different power levels for 3 devices with 7 wires, 150 mm in length (N7H150), and a 3 mm gap.

Similarly, for NO_2 , the generation rate increases with higher power input. At higher power levels, NO_2 reaches a peak concentration before slightly declining to a steady-state value. In contrast, at lower power levels, the increase is less abrupt, and the steady-state is reached more gradually. This compound will also be included in the proposed modeling framework.

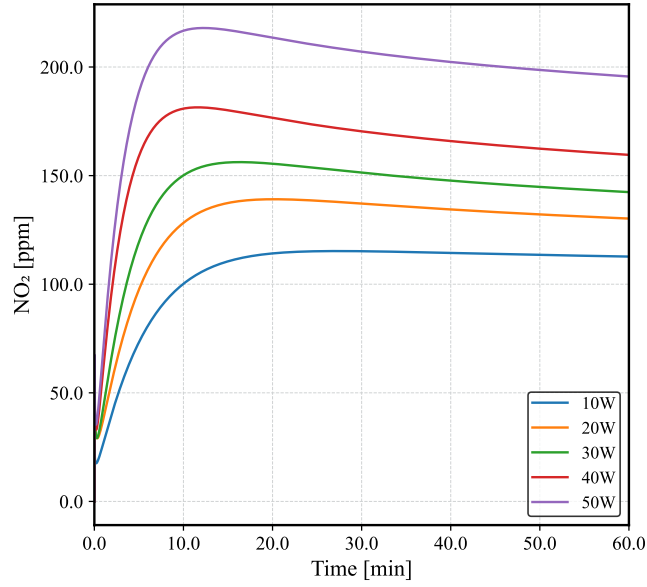


Figure 5.8: NO_2 concentration over time at different power levels for 3 devices with 7 wires, 150 mm in length (N7H150), and a 3 mm gap.

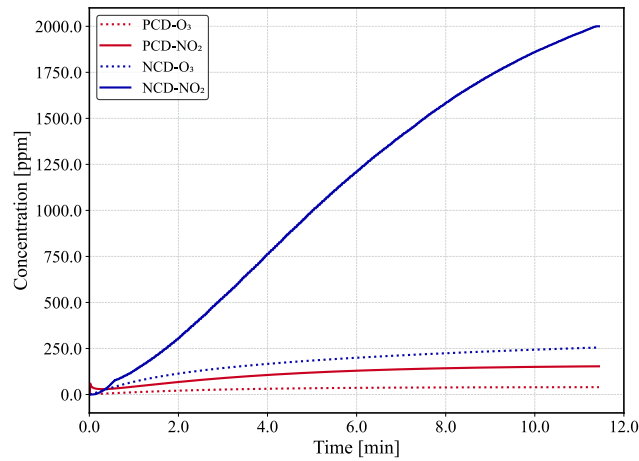


Figure 5.9: O_3 and NO_2 concentration over time at different power levels for positive corona discharge (PCD) and negative corona discharge (NCD) with 3 devices with 7 wires, 150 mm in length (N7H150), and a 3 mm gap at 30 W power.

An empirical model is proposed to fit the data obtained for ozone and nitrogen dioxide as a function of the time in positive corona discharge, as complete data are available for this mode. Intermediate species, mostly short-lived, are generated during the process. Although these species also play a role in the reaction dynamics, they are not quantified in this study as a result of the challenges associated with their short lifetimes.

To account for the participation of the evaluated compounds in other reactions, whether through recombination or consumption in parallel reactions, the empirical model incorporates certain assumptions. The accuracy of the model within the operating range analyzed is validated using the coefficient of determination (R^2) and the root mean square error (RMSE).

The proposed model simplifies the kinetics of O_3 and NO_2 by incorporating the effects of intermediate species, such as O (atomic oxygen) and NO (nitric oxide), into effective rate constants. This assumption is justified by the relatively constant concentrations of O and NO due to their rapid reactivity and low abundance compared to O_3 and NO_2 . Below, the governing reactions and equations are outlined.

Key Reactions Governing the System:

1. Ozone interaction with nitric oxide:



2. Nitrogen dioxide reaction with atomic oxygen:



3. Atomic oxygen generation from ozone:



4. Formation of nitric oxide by metastable nitrogen:



These reactions establish a dynamic cycle between O_3 , NO , and NO_2 , with O playing a crucial intermediary role.

Based on the proposed reactions, the following differential equations are proposed to model the dynamics of O_3 and NO_2 . Shimizu et. al [128] reports an analogous equation to the one proposed, introducing a term in the balance that varies with treatment time.

For O_3 :

$$\frac{d[O_3]}{dt} = k_1 - k_2[O_3] - \alpha t[O_3] \quad (5.5)$$

where:

- k_1 : Rate of O_3 generation.

- $k_2[O_3]$: Loss of O_3 due to reactions with plasma species, including NO.
- $\alpha t[O_3]$: Time-dependent cumulative loss of O_3 , accounting for processes such as diffusion and wall interactions.

For NO_2 :

$$\frac{d[NO_2]}{dt} = k_3[O_3] + k_4 - k_5[NO_2] \quad (5.6)$$

where:

- $k_3[O_3]$: Formation of NO_2 due to the reaction of O_3 with NO. Here, k_3 incorporates $[NO]$ as a constant, i.e., $k_3 = k_{3,\text{original}} \cdot [NO]$.
- k_4 : Additional sources of NO_2 from secondary plasma processes.
- $k_5[NO_2]$: Loss of NO_2 due to reactions with atomic oxygen or other processes.

All kinetic constants are assumed to be positive due to their physical interpretation as rates of generation or consumption. Specifically:

NO and $[O]$ are treated as constants due to their rapid equilibrium within the plasma.

- Their effects are incorporated into the effective constants:

$$k_3 = k_{3'} \cdot [NO] \quad (5.7)$$

$$k_5 = k_{5'} \cdot [O] \quad (5.8)$$

This simplification reduces the complexity of the model while retaining its physical accuracy.

Physical assumptions are made to solve and simplified the model:

1. The concentrations of O and NO are steady-state due to their rapid reactivity and low abundance compared to O_3 and NO_2 .
2. The primary dynamics of the system are dominated by O_3 and NO_2 , justifying the exclusion of NO and O as explicit variables.
3. Effective constants (k_3 and k_5) group the effects of O and NO, simplifying the model without significant loss of accuracy.

This model provides a simplified yet physically consistent representation of the key chemical processes in the plasma system. By incorporating $[O]$ and $[NO]$ into effective rate constants, it avoids unnecessary complexity while accurately describing the dynamics of O_3 and NO_2 . This approach is particularly useful for systems where O and NO are present at steady-state concentrations and the focus is on the evolution of O_3 and NO_2 .

The parameter adjustment for the model is performed using a nonlinear least-squares fitting method. This approach is used to determine the parameter values that minimize the discrepancy between the experimental data and the values predicted by the mathematical model. In this case, the kinetic model describes the dynamics of the concentrations of O_3 and NO_2 as functions of time, and the kinetic parameters ($k_1, k_2, k_3, \dots$) are adjusted to ensure the differential equations closely reproduce the observed experimental data.

The least-squares method minimizes a cost function representing the sum of squared errors between the model predictions (y_{model}) and the experimental data (y_{exp}):

$$\text{SSE} = \sum_{i=1}^N (y_{\text{model},i} - y_{\text{exp},i})^2$$

where: N is the total number of experimental data points, $y_{\text{model},i}$ is the value predicted by the model at point i , $y_{\text{exp},i}$ is the experimentally measured value at point i .

The goal of the nonlinear fitting process is to find the parameter values that minimize this cost function (SSE).

The model consists of a system of differential equations describing the concentrations of O_3 and NO_2 as functions of time. Since these differential equations are typically not analytically solvable, numerical methods (e.g., Runge-Kutta or `solve_ivp` in Python) are used to compute the model's solutions over time for a given set of initial parameters. In this case, `solve_ivp` in Python has been used.

The cost function for the fitting process is:

$$\text{SSE} = \sum_{i=1}^{N_{\text{O}_3}} ([\text{O}_3]_{\text{model},i} - [\text{O}_3]_{\text{exp},i})^2 + \sum_{j=1}^{N_{\text{NO}_2}} ([\text{NO}_2]_{\text{model},j} - [\text{NO}_2]_{\text{exp},j})^2$$

where: $[\text{O}_3]_{\text{model},i}$ and $[\text{O}_3]_{\text{exp},i}$ are the predicted and measured concentrations of ozone, respectively. $[\text{NO}_2]_{\text{model},j}$ and $[\text{NO}_2]_{\text{exp},j}$ are the predicted and measured concentrations of nitrogen dioxide, respectively.

A nonlinear optimization algorithm, adjusts the initial parameter values to minimize the cost function. The algorithm iteratively updates the parameters to reduce the value of SSE. At each iteration, the model is solved with the updated parameters, and the error between the results and experimental data is recalculated.

The process yields the optimal values that minimize the error. These values represent the parameters that best explain the experimental data within the framework of the proposed model.

The least-squares method applied to this kinetic model enables the determination of parameters that best describe the experimental data. This approach provides a quantitative and physically consistent representation of the system while leveraging numerical optimization to solve complex models.

Figure 5.10 presents the fitting curves for O_3 and NO_2 using the proposed model. Table 5.1 summarizes the results for the constants defined in the model, along with the RMSE and R^2 values for the curves of both species. As shown, the data aligns well with the model, achieving R^2 values exceeding 0.95.

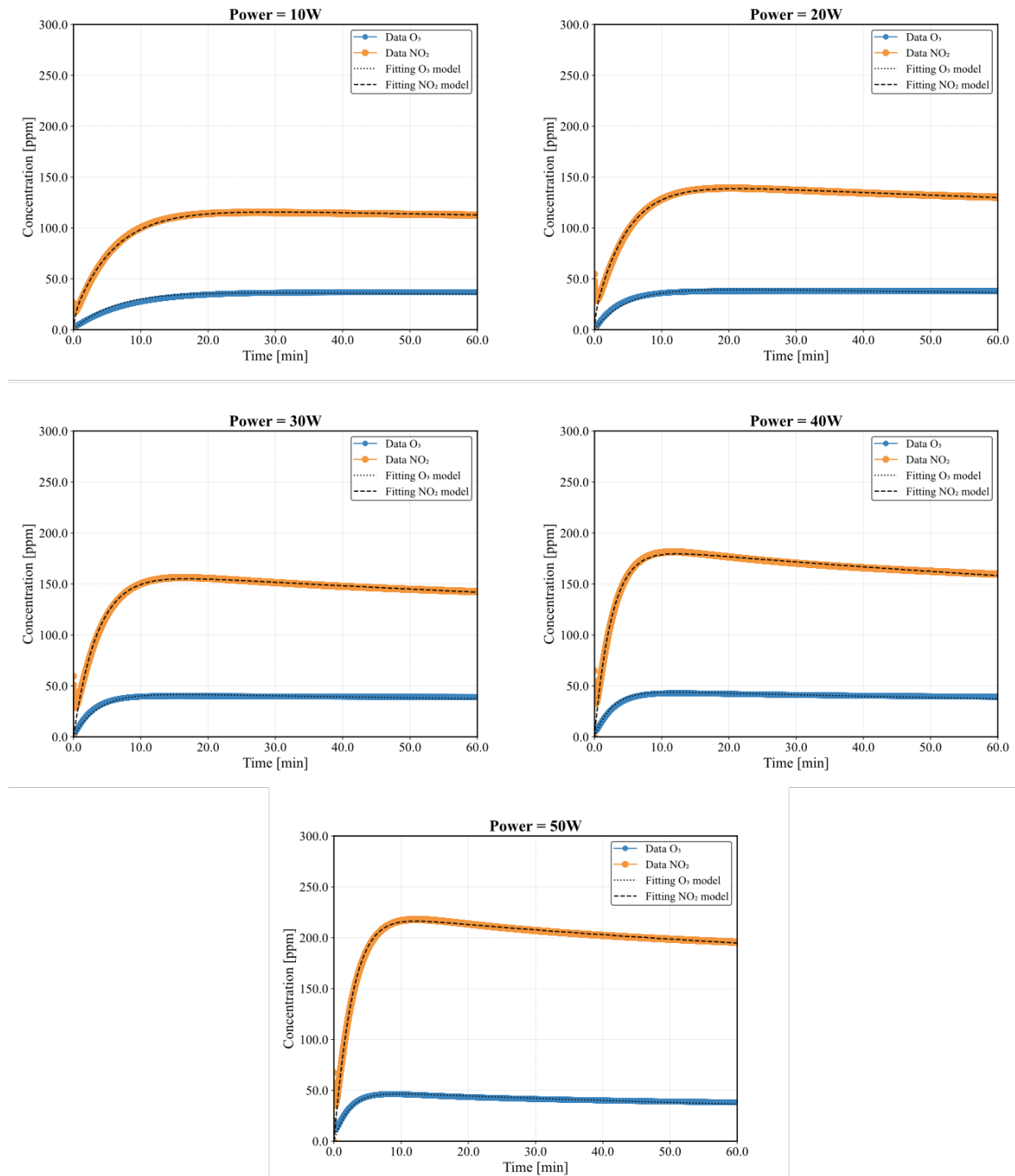


Figure 5.10: NO_2 concentration over time at different power levels for 3 devices with 7 wires, 150 mm in length (N7H150), and a 3 mm gap.

Table 5.1: Kinetic parameters and fitting metrics for different power levels.

Power [W]	k_1	k_2	k_3	k_4	k_5	α	RMSE O ₃	RMSE NO ₂	R ² O ₃	R ² NO ₂
10	6.207	0.166	10.025	66.069	3.704	0.0002	1.396	1.191	0.967	0.997
20	8.741	0.211	16.655	99.262	5.443	0.0005	1.131	1.972	0.966	0.992
30	12.143	0.279	6.180	45.388	1.946	0.0008	1.286	2.406	0.959	0.989
40	17.784	0.388	7.346	72.728	2.195	0.0015	1.146	5.928	0.962	0.988
50	21.700	0.440	1.519	76.232	0.678	0.0027	1.192	2.520	0.939	0.992

RMSE: root mean square error

5.3 Conclusion

This chapter presents a comprehensive analysis of the chemical behavior of plasma systems in closed chambers, aiming to develop practical models for optimizing plasma chemical composition. These systems are designed for ambient operation, using room temperature, atmospheric pressure, and typical humidity levels, without requiring additional chemical precursors. The ultimate goal is to evaluate their effectiveness as germicidal agents.

The study demonstrates that geometric parameters, such as the electrode gap, number of devices, and their configurations (series or parallel), affect the generation of reactive chemical species. Smaller electrode gaps result in higher steady-state and reaction rates of the concentrations of key reactive species, such as nitrogen dioxide (NO₂) and ozone (O₃), highlighting their critical role in germicidal applications. Negative corona discharge is found to produce approximately ten times more NO₂ than positive corona discharge, emphasizing its suitability for applications requiring enhanced reactive species concentrations.

The analysis shows that negative corona discharge generates higher concentrations of reactive species compared to positive discharge. The concentration of NO₂ increases significantly as the gap is reduced. For instance, a 2 mm gap yields higher NO₂ concentrations than larger gaps, reinforcing the importance of gap optimization. The research also reveals that configurations in series or parallel do not significantly influence steady-state reactive species concentrations but allow for higher total system power, enabling greater production rates of reactive species.

Higher power inputs result in increased production rates and concentrations of reactive species. Ozone concentrations rise rapidly at higher power levels but exhibit a subsequent decline due to recombination or parallel reactions, a behavior that intensifies with increased power. In contrast, nitrogen dioxide reaches peak concentrations at higher power before stabilizing at a steady-state value. These findings underline the necessity of balancing power and configuration to maximize efficiency in chemical species generation.

Modeling is an important consideration, as certain species may be suppressed beyond a specific power threshold. Moreover, power cannot be increased indefinitely relative to the device's surface area, inherently limiting the achievable power density based on the system's configuration. Consequently, the transition point at which ozone is entirely suppressed due to quenching, accompanied by a notable increase in nitrogen-derived species, is not observed within the scope of this study. This underscores the importance of identifying optimal operational limits to achieve the desired chemical composition.

An empirical kinetic model is proposed to describe the temporal evolution of O_3 and NO_2 concentrations under positive corona discharge conditions. The model integrates effective rate constants that account for intermediate species such as atomic oxygen (O) and nitric oxide (NO), which play a critical role in reaction dynamics. The model equations, derived from the governing chemical reactions, fit the experimental data with high accuracy ($R^2 > 0.9$), validating the assumptions made. Key reactions, including the interaction between O_3 and NO, and the generation of NO_2 , are effectively incorporated into the model, allowing accurate predictions of system behavior, which are useful to facilitate system design and performance optimization.

The findings of this study provide essential insights for the design and development of disinfection chambers tailored to specific requirements in various sectors. These include hospitals, pharmaceutical laboratories, and other environments that require high levels of sterility. By optimizing the configurations of the plasma system and the operating parameters, these chambers can be scaled for various industrial applications while ensuring efficiency, safety, and sustainability.

Moreover, this chapter underscores the importance of geometric and electrical optimization in plasma-based systems for germicidal applications. The correlation between chemical composition and microbial inactivation provides a robust foundation for designing efficient and scalable disinfection systems. These results are particularly relevant for the development of environmentally friendly solutions that operate under ambient conditions without chemical additives, supporting advancements in sustainable and safe sterilization technologies.

Chapter 6

Microbial inactivation by atmospheric corona discharge operating in an open system

This chapter² collects the microbial inactivation assays conducted on airborne microorganisms using atmospheric plasma systems that are fluidically and chemically modeled in Chapter 4 under an open system configuration. The efficacy of the systems is evaluated against viruses, including SARS-CoV-2, HCoV-229E, considering that these microorganisms present the highest risk of airborne transmission. The viral assays are performed at the National Center for Biotechnology of the Spanish National Research Council (SNRC).

²Ruiz-Trujillo, S., Vega-Martínez, P., Esparza, I., Usera, F., Sánchez-Soriano, P., van Raaij, M. J. and Díaz Lantada, A. (2023). Electrohydrodynamic devices for ionic wind generation and airborne positive-strand RNA viruses inactivation by nonthermal atmospheric plasma. *Plasma Processes and Polymers*, 20(10), e2300046. This publication is totally included.

6.1 Plasma device and operation parameters

The base configuration of the corona discharge plasma device used in these assays consists of three wires with a length of 50 mm and a diameter of 50 μm . The system operates under positive discharge within a voltage range of 3.5-6 kV DC and a gap of 3 mm.

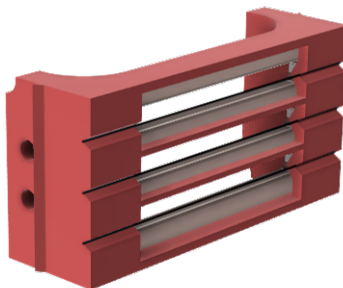


Figure 6.1: Three-dimensional design and prototype of corona discharge non-thermal plasma generation device.

In this case, assays with negative discharge are not performed to ensure the safety of personnel handling the microbiological samples, as negative discharge is inherently less stable. As previously described, the laboratory tests are conducted with microorganisms suspended in an aqueous medium containing salts, which can further affect the stability of the discharge.

The electrical, fluid, and chemical characteristics of the plasma prototype configuration used in the inactivation assays are detailed in the following sections, with the aim of understanding the conditions under which these assays are performed.

6.1.1 Electrical characterization

The electrostatic behavior of EHD-plasma device is justified from the characteristic voltage-current (CVCCs) and the onset voltage of the phenomenon [116]. Figure 6.2 shows the average of the performance power-voltage curves of the corona discharge devices tested. The voltage range is from 3.5 – 6 kV and the power is between 0 – 4W. The voltage value at power 0 is 3.5 kV and corresponds to the dielectric breakdown point of the medium where the discharge is made, air in this case, as a function of the distance between ground electrode planes and the gap. The relationship between the power and the voltage supplied to the plasma devices in the case study follows a parabolic trend (Figure 6.2).

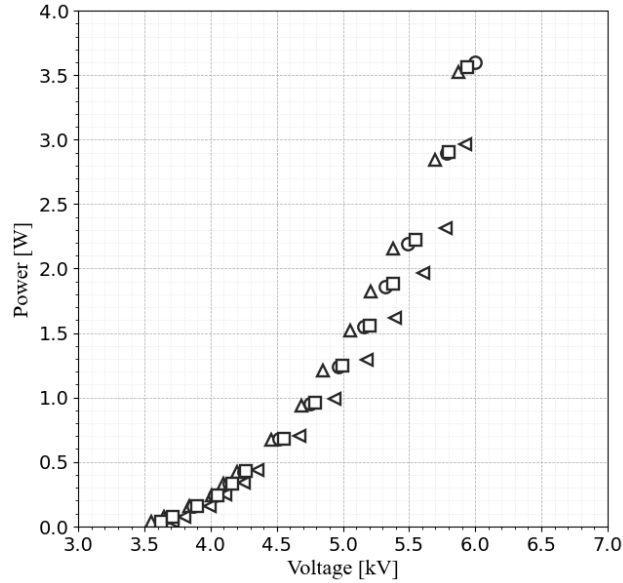


Figure 6.2: Power vs voltage curves of the tested plasma devices. The symbols refer to replicates of the same experiment.

6.1.2 Fluid dynamic characterization

Figure 6.3A shows the evolution of the airflow velocity as a function of the power. It is observed that the electrical power of the plasma is directly proportional to the velocity raised to 3, v^3 (Figure 6.3B). This correlation, as well as the influence of the geometry of the EHD-plasma devices, is investigated by our research group in previous studies [116], in view of obtaining optimal airflow, design, and scaling parameters.

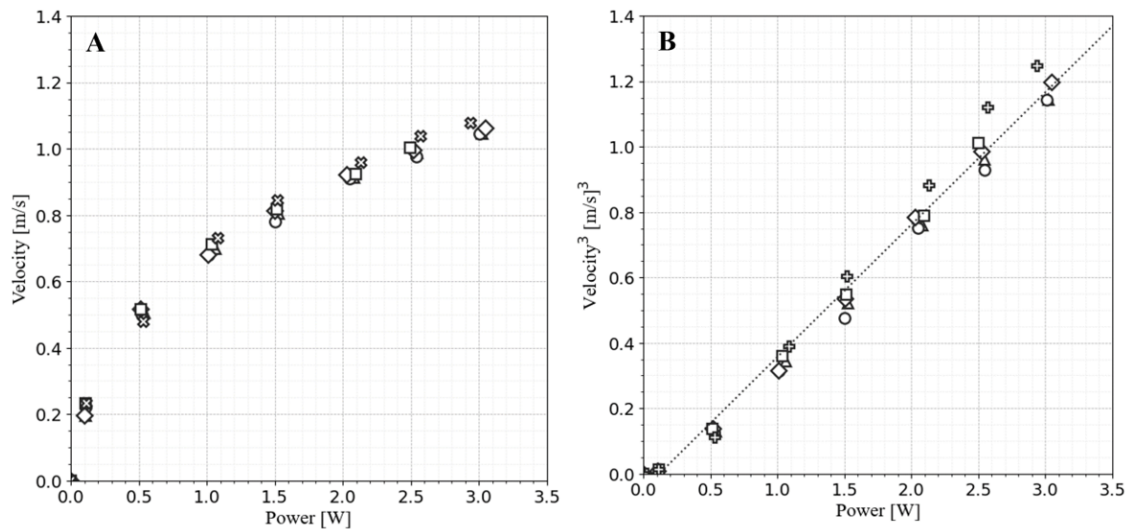


Figure 6.3: Relation between the electrical power and velocity for the plasma devices.

6.1.3 Long-lived chemical species

Ozone and nitrogen dioxide are taken as reference compounds, being long-lived species [130]. Figure 6.4 shows the concentration values obtained for each of the compounds evaluated as a function of devices power (Figure 6.4A ozone concentration and Figure 6.4B nitrogen dioxide). The concentration of nitrogen dioxide is slightly higher than ozone for the same power value of the EHD-plasma system. The maximum concentration achieved for O_3 in the power range studied in this test is 0.5 ppm, while that obtained for NO_2 is 0.6 ppm. These concentrations are very reduced compared to those described by other authors. Bisag et al. [131] reported ozone concentrations in the steady-state regime of 870 ppm and Baek et al. [132] recorded ozone values of 27 ppm.

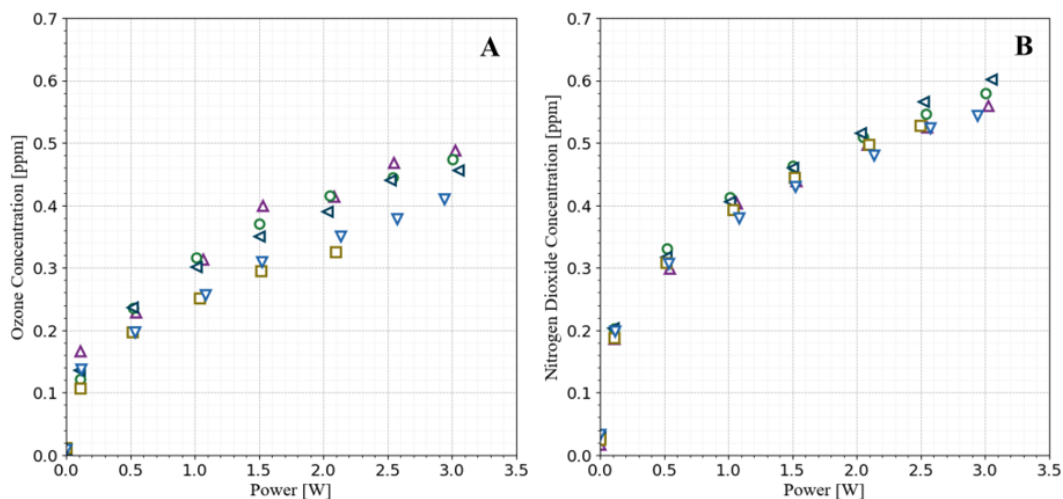


Figure 6.4: Concentration of ozone (A) and nitrogen dioxide (B) generated as a function of the power of the plasma devices.

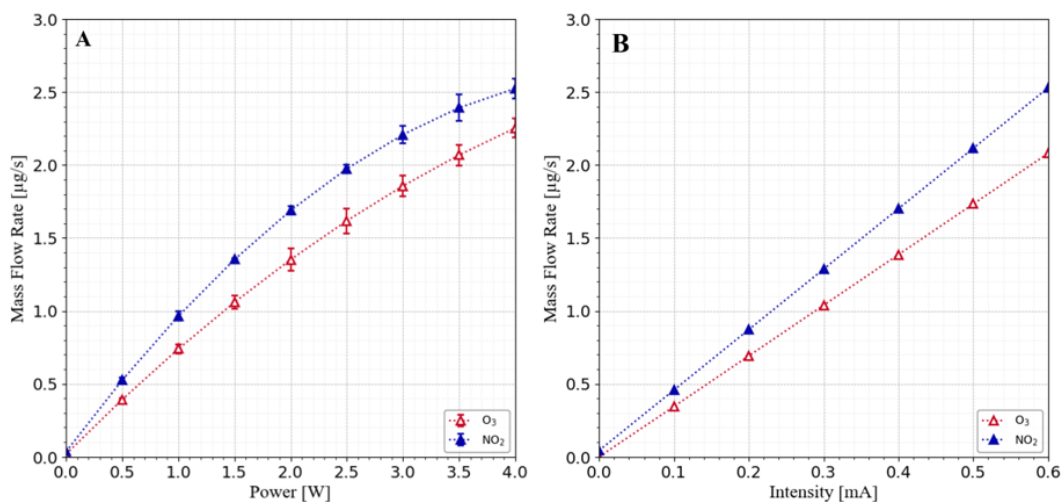


Figure 6.5: Relation between ozone and dioxide nitrogen mass flow rate and power (A) and linear dependence of the ozone and dioxide nitrogen mass flow rate with the current of the EHD-plasma devices (B).

By plotting the mass flow rates as a function of the electrical properties of the devices, the flow rates at each operating point can be known. Figure 6.5A shows the ozone and nitrogen dioxide mass flow rates as a dependence of the EHD plasma power. A linear relationship is observed between the current supplied and the calculated mass flow rates (Figure 6.5B). Nitrogen dioxide production is slightly higher than ozone generation.

6.2 Virus inactivation

This section reports the description of the operating parameters of the EHD device, and the results obtained in the inactivation test of coronavirus HCoV-229E and SARS-CoV-2 and Bacteriophage MS2 in an aerosol stream. Electrical, chemical, and fluid dynamic characterization of the ionic flow as a function of plasma device power is performed to determine the behavior of the system. In addition, the correlation of the working parameters of the system with the level of viral inactivation is presented. The higher initial MS2 concentration allowed a more accurate assessment of the plasma effect, reinforcing the results from the HCoV-229E and SARS-CoV-2 tests.

6.2.1 Analysis and results: HCoV-229E and SARS-CoV-2

The aim of this experimental campaign is to observe the effect of the atmospheric cold plasma against HCoV-229E and SARS-CoV-2 suspended in an airflow when it goes through the devices. The contaminated stream is treated in a single pass through the system.

Figure 6.6, 6.1 and 6.2 show the results of the inactivation of HCoV-229E (Figure 6.6A) and SARS-CoV-2 (Figure 6.6B) present in an airflow by the plasma devices at different power levels. The values at power 0 correspond to the control test. The initial viral titer is higher for HCoV-229E (10^5 pfu/mL) than for SARS-CoV-2 (10^4 pfu/mL). The difference between the initial and final concentration in the control tests gives information on the system losses. It can be seen that the losses are equal for both viruses, being 1.5-2 logarithmic reductions. These system losses are considered in the determination of viral titer reductions due to plasma treatment. The time of the experiment is 20 min for all cases.

The inactivation of the HCoV-229E and SARS-CoV-2 increases with the power of the EHD-plasma device. 2.4 log reductions are achieved for HCoV-229E and 1.20 log reductions for SARS-CoV-2 at 2W of power. In the case of 4W, no virus is detected in the collected samples, reaching log reductions > 3.40 and > 2.40 for HCoV-229E and SARS-CoV-2 respectively.

However, due to the low initial viral concentration of SARS-CoV-2, the degradation of the viral solution, and the losses of the system, it is hard to determine the inactivation curve of the EHD-plasma devices. Nevertheless, these experiments show the effectiveness of the atmospheric plasma produced by the devices against HCoV-229E and SARS-CoV-2 even at low power in just one pass.

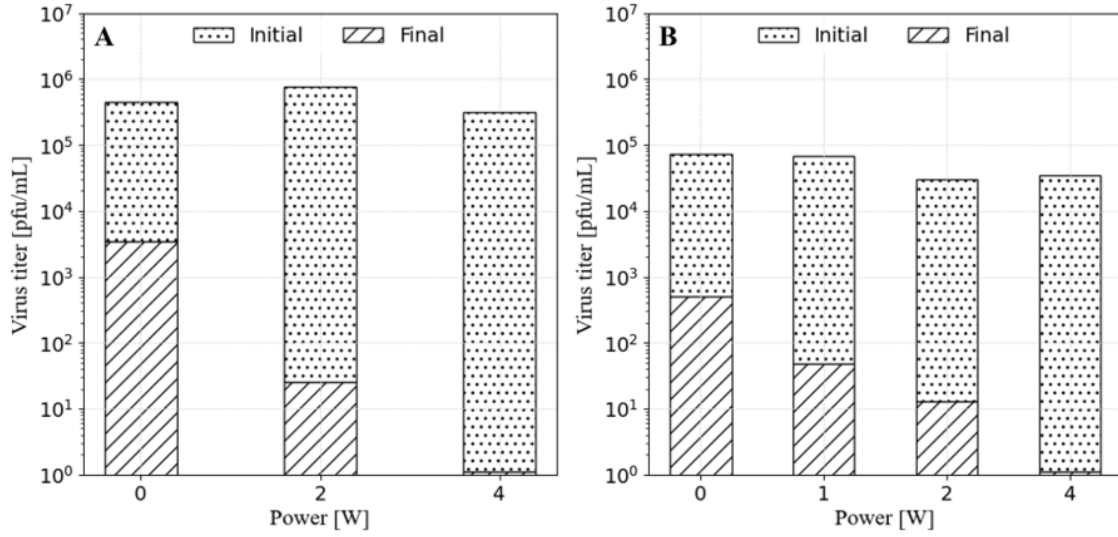


Figure 6.6: Experimental results regarding the inactivation of HCoV229E and SARS-CoV-2 using the plasma devices at different power levels (20 min). Non-detected (ND) values at 4W power in both cases.

Table 6.1: Initial and final concentrations of HCoV-229E at different plasma treatment powers at 20 min and the logarithmic reductions obtained.

Power Device	HCoV-229E				
	Initial [pfu/mL]	VT	Final [pfu/mL]	VT	Log. Red
0	$4.38 \cdot 10^5$		$3.44 \cdot 10^3$		-
1	-		-		-
2	$7.66 \cdot 10^5$		$2.50 \cdot 10^1$		2.38
4	$3.11 \cdot 10^5$		ND		> 3.39

ND: Non-detected.

Table 6.2: Initial and final concentrations of SARS-CoV-2 at different plasma treatment powers at 20 min and the logarithmic reductions obtained.

Power Device	SARS-CoV-2				
	Initial [pfu/mL]	VT	Final [pfu/mL]	VT	Log. Red
0	$7.27 \cdot 10^4$		$5.06 \cdot 10^2$		-
1	$6.87 \cdot 10^4$		$4.86 \cdot 10^1$		0.99
2	$2.98 \cdot 10^4$		$1.30 \cdot 10^1$		1.20
4	$3.50 \cdot 10^4$		ND		> 2.39

ND: Non-detected.

6.2.2 Analysis and results: MS2 Bacteriophage

An experimental campaign with airborne bacteriophage MS2 particles is carried out to more accurately determine the atmospheric plasma inactivation curve as a function of power.

Figure 6.7 and Table 6.3 show the effect of the atmospheric plasma produced by the devices on MS2-contaminated airflow at different power levels. The initial concentration of MS2 corresponds to $6.98 \cdot 10^{11}$ pfu/mL, being much higher than the HCoV-229E and SARS-CoV-2 stocks used. The time of all experiments is 20 min. System losses are also determined by comparing the initial and final titers for the control assay, being 1.5–2 log reductions. These losses are contemplated in the calculation of the reductions due to plasma treatment (see section 2.2.1).

The effect of EHD-plasma on the MS2-contaminated stream can be seen in detail due to the homogeneity of the initial titres and the very high concentration of the samples. The inactivation of MS2 by the plasma device increases with plasma power. 2.82 log reductions at 1.5 W power and 3.78 reductions at 3 W are achieved in a single pass through the device.

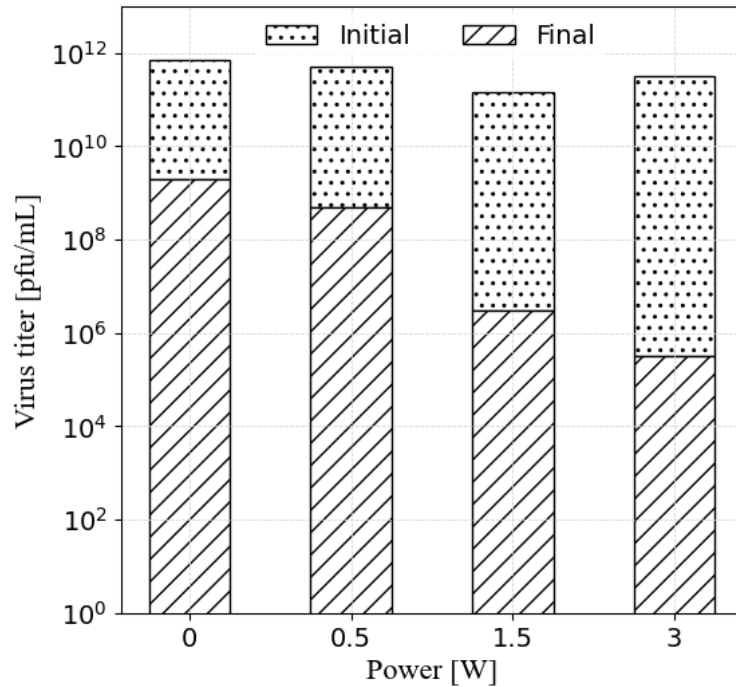


Figure 6.7: Positive corona discharge (PCD, red) and negative corona discharge (NCD, blue) at 30W for *E. coli* and *Listeria* at different treatment times, expressed in Log(N).

Table 6.3: Initial and final concentrations of bacteriophage MS2 at different plasma treatment powers at 20 min and the logarithmic reductions obtained.

Power Device	Bacteriophage MS2			
	Initial [pfu/mL]	VT	Final [pfu/mL]	VT Log. Red
0	$6.98 \cdot 10^{11}$		$1.93 \cdot 10^9$	-
0.5	$5.15 \cdot 10^{11}$		$4.83 \cdot 10^8$	0.60
1.5	$1.45 \cdot 10^{11}$		$2.92 \cdot 10^6$	2.14
3	$3.17 \cdot 10^{11}$		$3.23 \cdot 10^5$	> 3.43

6.2.3 Inactivation mechanisms and discussion

Figure 6.8 summarizes the results obtained in the present study, showing the comparison between the reductions obtained for the coronavirus HCoV-229E, SARS-CoV-2 and bacteriophage MS2 (left axis) and the ozone and nitrogen dioxide mass flow rates (right axis) as a function of the plasma device power presented. The viral reduction data and the system losses obtained in both studies are consistent, being 1.5-2 log reductions in all cases, so the proposed experimental installation is valid for the realization of the test.

The logarithmic reductions obtained in the atmospheric plasma inactivation test of HCoV-229E, SARS-CoV-2, and bacteriophage MS2 contained in an air stream increase as a function of the power supplied, in a single pass through the device. Similar trends can be seen in the reduction values achieved for the coronavirus HCoV-229E and for the bacteriophage MS2, which reinforces the results. The degree of inactivation reached for SARS-CoV-2 is lower. This may be due to the nature of the assay, as this virus degrades faster and the initial concentrations are lower than in the other case studies, or to the inactivation mechanism itself. However, the presence of SARS-CoV-2 is not detected from a plasma generation power of 4W, gaining logarithmic reductions greater than 2.40.

Ozone and nitrogen dioxide mass flow rates also increase with power (Figure 6.8). The generation of a higher concentration of chemical species at higher values of plasma device power may explain the improved viral inactivation rate.

A limited number of authors reported the use of non-thermal atmospheric plasma technology generated with different technologies, mainly by Dielectric Barrier Discharge (DBD), for the reduction of SARS-CoV-2, HCoV-229E, and bacteriophage MS2 in aerosols. The ozone concentrations described by them are higher than those obtained in the present study. In studies of SARS-CoV-2 treatment, Bisag et al. [131] reported concentrations of 870 ± 40 ppm generated by Dielectric Barrier Discharge. Baek et al. [132] gave inactivation efficiencies higher than 99.84% with ozone concentrations of 27 ppm.

O₃ levels measured in the present study are below 0.6 ppm, with NO₂ concentrations lower than 0.7 ppm. It could be expected that a significant contribution to the inactivation efficiency is made by the passage through the plasma region, electron-impact reactions, and short-lived species. It would be possible to reduce the concentration of the species generated at the outlet of the system by using activated carbon or manganese oxide catalysts. Xia et al. [133]

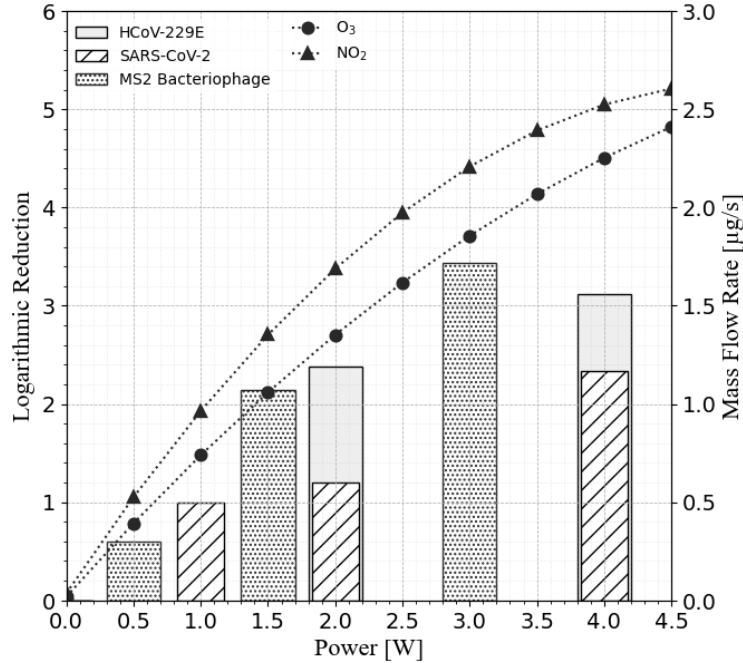


Figure 6.8: Logarithmic reductions of HCoV-229E, SARS-CoV-2 and Bacteriophage MS2 and ozone and dioxide nitrogen mass flow rate as a function of plasma devices power.

reported that 96% reductions in ozone generated are reached with a single layer of activated carbon.

Reductions > 2.4 -log have been achieved for SARS-CoV-2 and > 3.4 -log for HCoV-229E at 4W power with ozone and nitrogen dioxide mass flow rates of $2.3 \mu\text{g/s}$ and $2.5 \mu\text{g/s}$, respectively. Over 3.5-log reductions at 3W for bacteriophage MS2 are obtained at ozone mass flow rates of $1.85 \mu\text{g/s}$ and nitrogen dioxide mass flow of $2.2 \mu\text{g/s}$.

The treatment of a virus-contaminated stream with atmospheric non-thermal plasma (ANTP) combines physical and chemical mechanisms to destabilize viral structures and render them non-infectious. Physically, the plasma's electric field induces deformation and rupture of the viral envelope or capsid, while UV radiation damages nucleic acids and essential proteins. Chemically, reactive oxygen (ROS) and nitrogen species (RNS) generated by the plasma oxidize lipids, proteins, and nucleic acids, causing structural and functional damage. These effects are synergistic, as electric fields enhance ROS/RNS penetration and UV radiation intensifies genetic damage. ANTP's multifaceted approach ensures effective viral inactivation across diverse conditions. [76].

6.3 Conclusion

The chapter reports the results obtained from the study of the effect of EHD devices of non-thermal atmospheric plasma generated by corona discharge on an aerosol stream contaminated with SARS-CoV-2 or bacteriophage MS2 in a single pass. A reliable and reproducible system has been achieved, controlling all the variables of the process. The electrical, fluid-dynamic, and chemical characterization of these devices is also presented, in order to understand the behavior of the system and the correlation between the different parameters. The determination of the mass flow rate of the generated long-lived species allows correlating the characteristic parameters of the plasma devices as a function of their geometry and electrical properties. In addition, it enables evaluating the influence of these on the degree of inactivation and its scaling to real environments.

The proposed devices combine the effect of high efficiency against viruses present in an air stream with the generation of the airflow without the requirement of external drive systems, with low energy consumption and very low concentrations of chemical species. The reason for their high microorganism reduction efficiency of these systems resides in their ability to generate highly oxidizing compounds, mainly reactive oxygen, and nitrogen species, RONS, from air at ambient temperature and pressure. It is suggested that the high inactivation may be due to a physical process, by the impact of the viruses with the electrons and ions present in the plasma region, since the concentrations of long-lived chemical species are much lower than those reported by other authors. In addition, an increase in the system's power results in higher velocity and mass flow rate of the different generated species. The increase in flow rate causes a decrease in residence time. Regarding future research, this study has led us to new hypotheses that we aim to contrast in future studies, including exploring the effects of the different parameters involved in viral inactivation and the contribution of each of them to the described process. Also, the absence of moving parts and vibrations makes the system completely noiseless.

The viral inactivation effect is very evident, being the results consistent in all the cases of study. Different positive-strand RNA viruses, whose main mode of transmission is through the air, are tested. Reductions greater than 2.3 log-red are achieved for SARS-CoV-2 and HCoV-229E, respectively, at 4W of power, and efficiencies up than 3 log-red at 3W for the bacteriophage MS2 in a single pass through the device. Concentrations lower than 0.70 ppm for ozone and nitrogen dioxide are measured in all cases.

The technology could be implemented in poorly ventilated spaces (with the integration of catalysts to reduce health-damaging species), where viruses spread better in the presence of people, as it is designed to produce an airflow without the need for external elements. This is a major strategic advantage over other methods such as the use of gas-phase hydrogen peroxide or ultraviolet light. Treatment and air renewal times could be regulated by varying the power, size, and number of stages of the plasma device; furthermore, improving the degree of microbial inactivation. Activated carbon or manganese oxide catalysts could be used for the removal of the generated compounds. Pressure drops that occur when implementing catalytic systems in EHD-plasma devices can be compensated by selecting the optimal geometry for each case [115], resulting in a higher flow rate.

Chapter 7

Microbial inactivation by atmospheric corona discharge operating in a closed system

Chapter 7 explores the evaluation assays for microbial inactivation efficacy on inert surfaces, building upon the chemical compositions and configurations analyzed in Chapter 5. The study examines the influence of these factors on the elimination of viruses, specifically SARS-CoV-2 and HCoV-229E, as well as bacteria, including *Escherichia coli* and *Listeria monocytogenes*. Various conditions are considered regarding the suspension of biological samples in test specimens made from different materials. The focus is on determining the optimal chemical composition and configuration and exposure times required to achieve a specified degree of microbial reduction.

The viral assays are performed at the National Center for Biotechnology of the Spanish National Research Council (CSIC), while the bacterial assays are carried out at the Department of Hygiene and Food Technology at the University of León.

7.1 Plasma device and operation parameters

The analysis presented in this chapter is conducted using various corona discharge devices, based on a configuration featuring seven wires, each 150 mm in length. Three of these devices are positioned side by side on a support platform within a closed chamber with a volume of 17 L 7.2, as detailed in Chapter 3, Experimental set up section. These devices actively recirculate air inside the chamber, maintaining a uniform concentration throughout its interior. The performance and the inactivation efficacy of the corona discharge devices are evaluated in positive discharge in the case of the virus inactivation test and both operational modes, positive and negative discharge, in the bacterial test with a working voltage range of 0 to 6 kV.

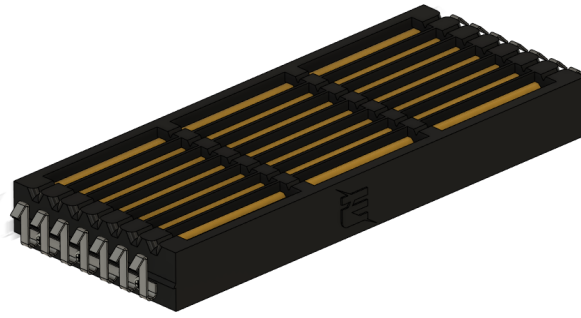


Figure 7.1: Three-dimensional design and prototype of corona discharge non-thermal plasma generation device with seven wires, each 150 mm in length.

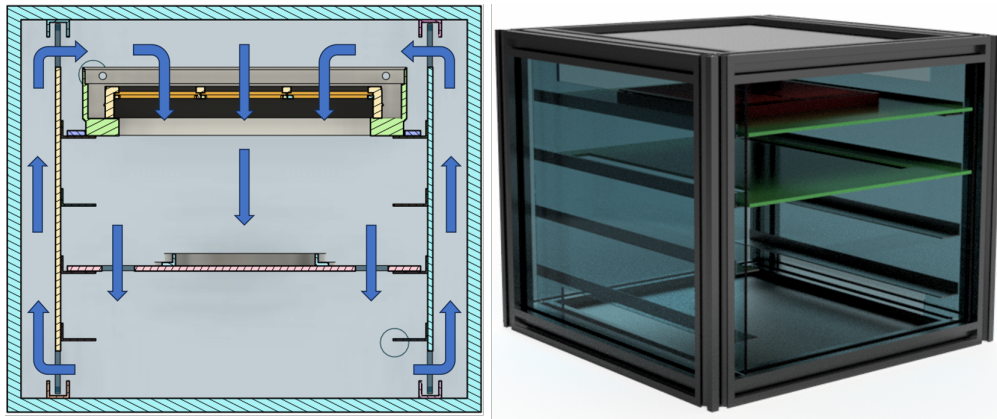


Figure 7.2: A hermetic chamber used for conducting experiments. On the right, a cross-section of the chamber is shown, illustrating the direction of airflow and recirculation within.

7.2 Virus inactivation

This section presents an analysis of the results obtained from the evaluation tests of the efficacy of corona discharge plasma devices against two coronaviruses, specifically HCoV-229E and SARS-CoV-2, on surfaces of inert material. Inactivation rates are assessed based on exposure time, the composition generated within the chamber by the plasma systems, and the electrical and geometric parameters of the devices. Preliminary control tests are conducted to determine the optimal testing conditions.

Four types of materials are analyzed, described in the Materials and Methods section in Chapter 3 of this document: stainless steel, glass, polypropylene, protective suit fabric (Type 3B), and the outermost layer of an FFP2 mask. Regarding the sample conditions, two deposition methods for the viral sample are evaluated. In the wet drop method, a known volume of the sample suspension is deposited directly onto the test specimen of each material and subjected to plasma treatment. In dry conditions, the same drop is air dried before being placed in the plasma treatment chamber. The results are compared with the control tests for each of the specimens analyzed.

7.2.1 Study of drying time on the viability of coronavirus HCoV-229E

To ensure an accurate assessment of the atmospheric plasma effect on the viruses under study, it is essential to account for the natural degradation of viruses on inert surfaces over time. Therefore, the initial step involved determining how drying time influences viability in the coronavirus HCoV-229E. A drying time curve is developed for this purpose, with measurements recorded at 15 min intervals.

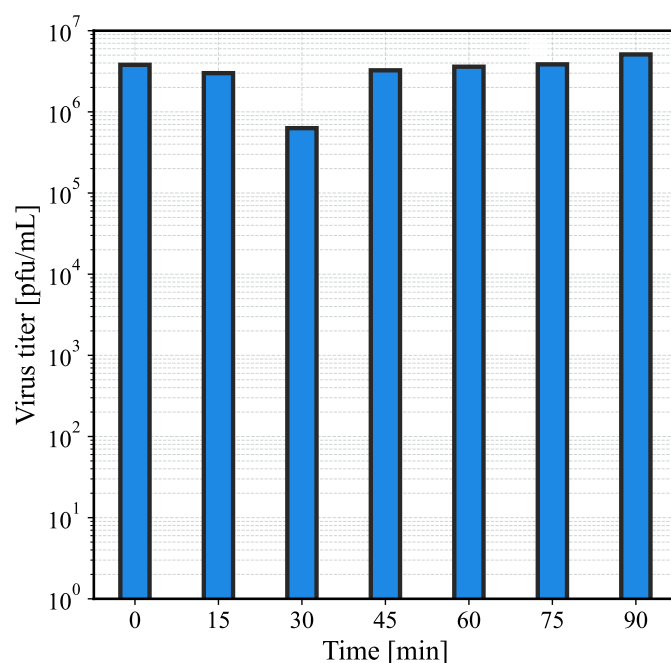


Figure 7.3: The effect of drying time on the viability of HCoV-229E.

There is no significant reduction in the initial viral titer in the drying time range from 0 to 90 min, as can be seen in Figure 7.3. Furthermore, in all cases, the volume of 300 μ L inoculated and spread on each plate is completely dried at 45 min. Therefore, this time was selected to prepare the samples for atmospheric plasma exposure assays.

7.2.2 Evaluation of the interfering effect of fetal bovine serum on HCoV-229E treatment

HCoV-229E viral stocks are obtained from the supernatant of infected cells, with viruses suspended in DMEM medium containing 2% fetal bovine serum (FBS). To evaluate the potential impact of the medium on nonthermal atmospheric plasma treatment, an experimental study is conducted because of the detection of possible shielding effects in previous assays. In order to select the best dilution to observe the plasma effect, an adjustment test is performed by sequentially diluting the initial viral titer with phosphate buffered saline (PBS) to gradually

reduce the FBS concentration.

Experimental conditions:

- Sample type: Dry viral suspension.
- Material tested: steel surface sample.
- Fixed plasma corona discharge device parameter: 50 W and 45 min

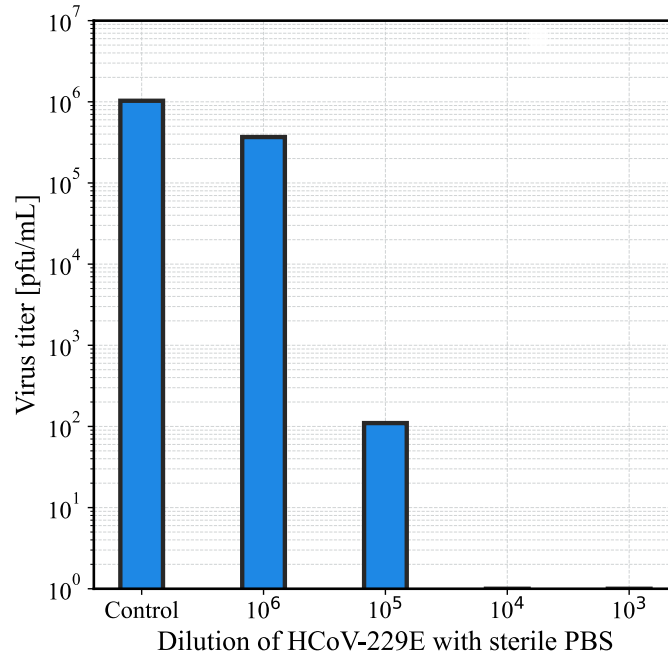


Figure 7.4: The effect of atmospheric plasma on serial dilutions of HCoV-229E.

Figure 7.4 presents the effect of atmospheric plasma on different serial dilutions of the viral titer of HCoV-229E. The first dot represents the untreated viral stock, and the next dot (10^6) represents the same treated viral stock.

As can be seen, no significant viral reduction is obtained in the presence of 2% FBS (10^6 vs. control), which induces plasma shielding by the presence of FBS. However, already with the first 1/10 dilution of FBS, a very significant reduction of the viral titer produced by atmospheric plasma is obtained, of 2.97 log cycles (reduction corrected for the dilution of the viral titer). This reduction is even greater in the following dilution, where the presence of the virus is no longer observed.

7.2.3 Effect of atmospheric plasma on HCoV-229E

Experimental conditions:

- Sample type: Dry and wet viral suspensions (applied dropwise) previously diluted 1:10 in PBS.
- Material tested: Stainless steel surface.
- Plasma device parameters: 50 W and 0, 15, 30, and 45 min.

As shown in Figure 7.5, atmospheric plasma generated at a power of 50 W caused a significant time-dependent reduction in the initial viral titer. At 45 min of exposure, reductions of 4.64 and 4.24 logarithmic reductions or greater are observed for dry and wet viral suspensions, respectively, both prepared on stainless steel surfaces. This demonstrates plasma effectiveness, with a gradual decrease in viral titers directly correlating with the duration of exposure.

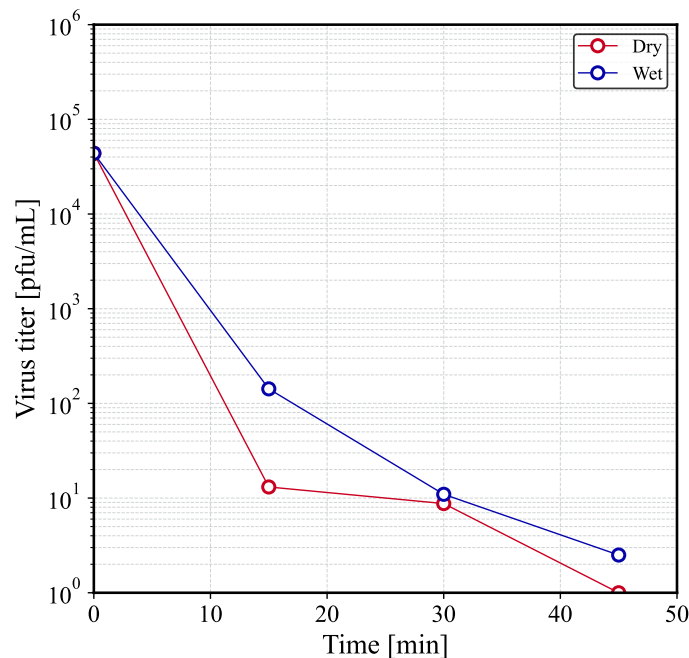


Figure 7.5: Effect of atmospheric plasma on dry and wet suspensions of HCoV-229E on stainless steel at 50W at different times.

7.2.4 Effect of atmospheric plasma on SARS-CoV-2

First, the effect of atmospheric plasma on the dry SARS-CoV-2 suspension deposited on stainless steel plates is evaluated. A power sweep of the plasma is performed with an exposure time of 15 min.

Experimental Conditions:

- Sample type: Dry viral suspension.

- Materials tested: Stainless steel
- Plasma device parameters: 0, 20, 30, 40 and 50 W and 15 min.

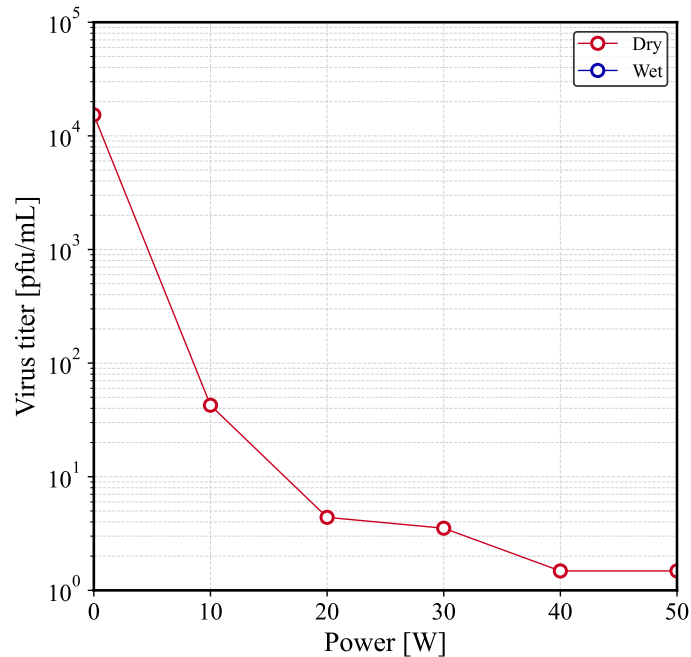


Figure 7.6: Effect of atmospheric plasma on dry suspensions of SARS-CoV-2 on stainless steel at 15 min at different powers.

Figure 7.6 presents the results obtained for different fixed power values. Logarithmic reductions of 2.66 are achieved at 10 W, reaching 4 log-red at 40 W power. Beyond this point, no detectable presence of the virus is observed, indicating that the reduction level could potentially be higher.

Second, various material substrates are contaminated with the virus to assess whether the properties of the substrate influenced the efficacy of atmospheric plasma treatment. The data presented have been normalized to provide a more precise comparison of the effects of treatment on different materials.

Experimental Conditions:

- Sample type: Dry viral suspension.
- Materials tested: stainless steel, glass, polypropylene, tyvek type 3B protective suit and outer layer of FFP2 mask filter fabric.
- Plasma device parameters: 50 W and 0, 15, 30, and 45 min.

The data presented in the graph in Figure 7.7 represent the mean of three assays performed in duplicate. As shown, the control viral titers at time 0 are within the same range in all materials, indicating consistent virus transfer efficiency. This consistency is highly advantageous, as it

allows for a more accurate comparison of relative efficacy among the different materials.

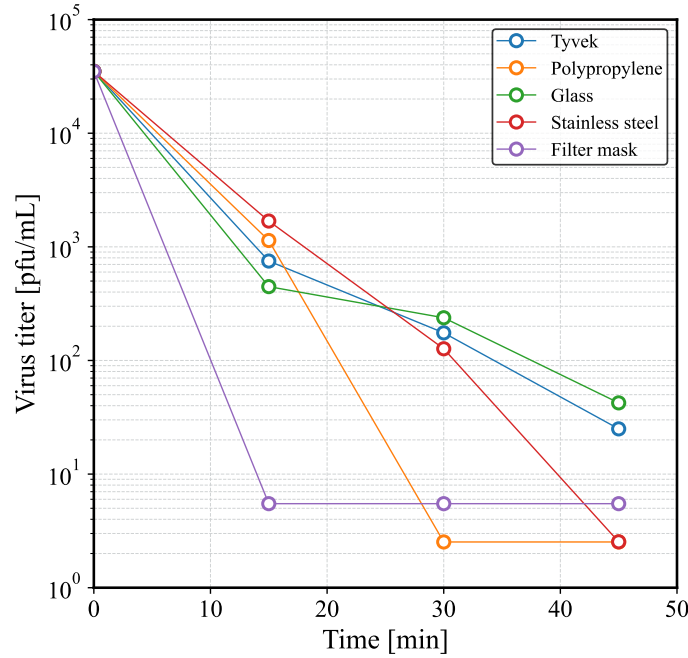


Figure 7.7: Effect of atmospheric plasma at different times on dry suspensions of SARS-CoV-2 on tyvek protective suit, polypropylene, glass, stainless steel, and filter mask.

In the case of protective suit fabric, glass, and stainless steel, a gradual reduction in viral titers is observed, reaching a maximum effect at 45 min with reductions ranging from 2.76 to 3.78 logarithmic reductions. In contrast, the reduction is faster on polypropylene, where no detectable viruses are observed at 30 min, corresponding to a reduction greater than or equal to 4.14 log-red. The most rapid effect is observed on the mask material, where complete viral inactivation is achieved in 15 min, with a reduction greater than or equal to 3.8 log-red.

7.2.5 Inactivation mechanism and discussion

The results of this study demonstrate that atmospheric pressure plasma at room temperature in a hermetic chamber exhibits a strong virucidal effect. This is observed in materials contaminated with both HCoV-229E (first phase) and SARS-CoV-2 (final phase). Using a plasma power of 50 W, the effect became evident after 15 min of exposure, with complete viral inactivation achieved for SARS-CoV-2 in the first filter layer of an FFP2 mask. For most other materials, the maximum effect is reached at 45 min, except for polypropylene contaminated with SARS-CoV-2, where total inactivation is observed at 30 min. Log reductions exceeded 4 log-red for HCoV-229E, while SARS-CoV-2 reductions ranged from 2.76 to 4.14 log-red. Chen et. al [134] report the total inactivation of SARS-CoV-2 on various types of materials (metal, plastic, cardboard) within 1 min. In this case, the treatment is performed with plasma directly exposed to the samples, meaning that the active component interacts with the virus,

creating a more aggressive environment. Instead of that, in our case study, the samples are treated with the generated ionic wind, composed mainly of neutral species. Capelli et al. [135] documented the near-complete inactivation of the virus after 10 min on food packages, at ozone concentrations around 1600ppm.

These findings suggest variability in the plasma effect between materials, particularly for SARS-CoV-2, but additional data would be needed to confirm these differences. Importantly, the trend of viral titer reduction is consistent in all materials tested, regardless of the type. The virucidal effect appeared faster for certain materials contaminated with SARS-CoV-2, such as polypropylene and FFP2 mask filters, potentially indicating a higher sensitivity of SARS-CoV-2 compared to HCoV-229E. However, more data are required to confirm this.

Plasma effectiveness is similar for dried and non-dried viral suspensions, as shown in materials contaminated with HCoV-229E, achieving reductions of 4.64 and 4.24 log reduction after 45 min. This consistency highlights the adaptability of atmospheric plasma as a disinfection agent for diverse materials and conditions.

In particular, the presence of 2% fetal bovine serum in the viral suspension significantly reduced the virucidal effect of plasma, as observed in initial tests with both viruses. Although this inhibitory effect is common to most virucidal agents, it underscores the importance of considering organic matter load when applying atmospheric plasma in practical scenarios.

7.3 Bacteria inactivation in enclosed chamber

This section explores the analysis of bacterial inactivation using atmospheric plasma devices generated by corona discharge. The study is conducted within a closed 17 L chamber, investigating both positive and negative corona discharges. Experiments are performed on bacteria deposited in petri dishes and on surfaces of various materials, assessing the inactivation kinetics under controlled conditions.

The correlation between bacterial inactivation and the dose of chemically active compounds generated during plasma treatment is examined in the case the study with agar in petri dish. Particular focus is placed on the temporal dynamics of ozone and nitrogen dioxide production, quantified as CT values (concentration multiplied by treatment time). This analysis aims to provide a comprehensive understanding of the relationships between plasma-generated chemical species, exposure time, and the efficiency of bacterial inactivation.

Two bacterial species with distinct structural and morphological characteristics are selected for this study: Gram-positive and Gram-negative bacteria. (For further details refer to Chapter 3, Materials and Methods). The aim is to identify potential differences in their resistance to atmospheric plasma treatment, attributed to structural differences in their cell walls. Gram-positive bacteria have a thick peptidoglycan cell wall, while Gram-negative bacteria possess a thinner cell wall layer covered by an outer lipid membrane. *Listeria monocytogenes* is selected as the Gram-positive model, and *Escherichia coli* as the Gram-negative model.

7.3.1 Validation on Agar-BHI dish

A preliminary evaluation is performed on BHI-agar plates using the same microorganisms as those employed in the probes: *E. coli* and *L. monocytogenes*. Initially, tests are conducted at different power levels with positive discharge, maintaining the same plasma device configuration (previously selected based on the chemical analysis in a closed environment) in all cases. The goal is to identify potential trends and differences between the two types of bacteria, enabling the selection of the most suitable configuration and conditions for a more in-depth study.

Figure 7.8 and 7.9 illustrate the inactivation curves for *E. coli* and *Listeria monocytogenes* as a function of exposure time across different plasma power levels (10, 20, 30, 40, and 50 W). No significant variations in the reduction of either bacterium are observed with increasing plasma power. This aligns with the chemical data, as the variations in the composition of reactive species generated by the plasma remain similarly negligible (see Chapter 4).

Plasma exposure results in 6-log reductions in 10 min for *L. monocytogenes* and 15 min for *E. coli*. The inactivation curve for *L. monocytogenes* decreases at a faster rate compared to *E. coli*, suggesting that it may be more susceptible to the applied plasma treatment.

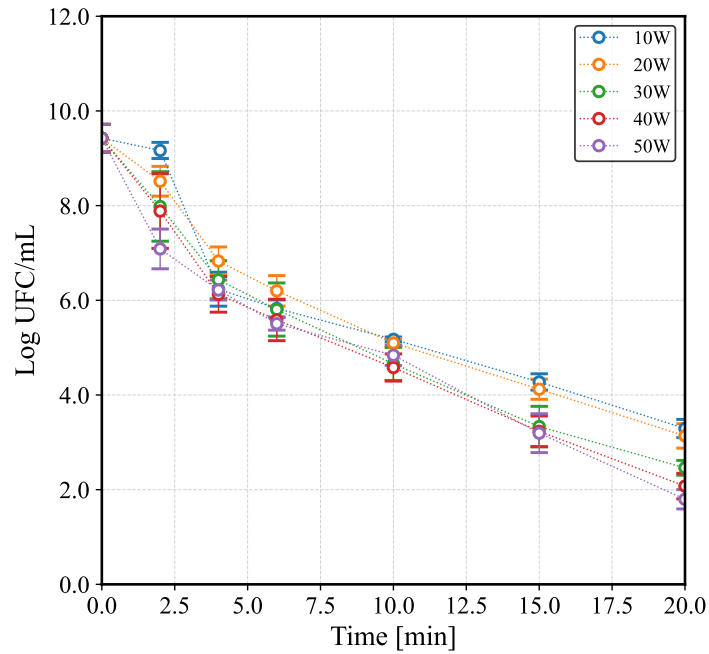


Figure 7.8: Effect of atmospheric plasma on *E. Coli* as a function of time at different power levels in positive corona discharge.

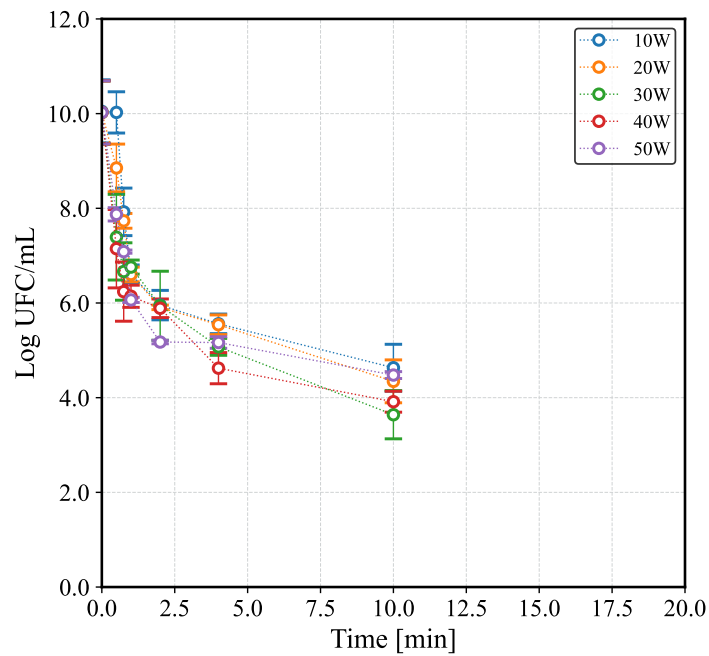


Figure 7.9: Effect of atmospheric plasma on *L. monocytogenes* as a function of time at different power levels in positive corona discharge.

The two proposed operating modes of corona discharge, positive and negative, are compared and analyzed to identify differences in the degree of inactivation achieved. As established in Chapter 4, the negative discharge generates a concentration ten times higher than the positive discharge. This study aims to assess the impact of this increased concentration on the inactivation curves. Given that no significant differences are observed with increasing power, 30 W is selected as the reference power for the analysis of both discharge modes, and experiments are conducted at different treatment times.

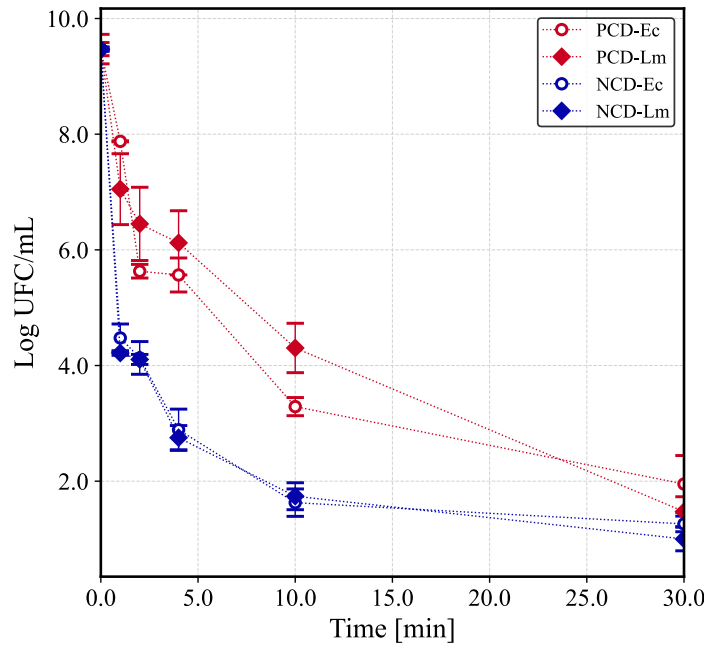


Figure 7.10: Positive corona discharge (PCD, red) and negative corona discharge (NCD, blue) at 30W for *E. coli* (Ec) and *L. monocytogenes* (Lm) at different treatment times, expressed in Log(N).

Figure 7.10 presents the normalized results for positive corona discharge (PCD) and negative corona discharge (NCD) at 30 W as a function of time for *E. coli* and *Listeria monocytogenes*. The NCD treatment shows a greater bacterial reduction effect for both types of bacteria at shorter exposure times compared to PCD. No significant differences are observed in the reduction levels of the two bacteria under NCD, effectively eliminating the resistance barrier between the two bacterial types. However, slight differences in the degree of reduction are noticeable under PCD.

For *E. coli*, 1.63 logarithmic reductions are achieved after 1 min of treatment with positive corona discharge, compared to 5.05 log-red with negative discharge. At 10 min, negative discharge achieves 7.94 log-red, exceeding 8 log-red beyond this point, which represents the maximum observable limit in the experiment. In contrast, positive discharge reaches 6.29 logarithmic reductions at 10 min and achieves 8 reductions after 30 min, which is also the maximum reduction observed in the tests conducted.

For *L. monocytogenes*, 2.67 log-red are achieved after 1 min of treatment with positive corona discharge. Similarly to the case of *E. coli*, negative discharge reaches near-maximum values within 10 min, achieving 7.73 log-red and exceeding 8 log-red beyond this time, compared to 5.34 log-red with positive discharge at the same interval. At the maximum treatment time of 30 min with positive discharge, 8.14 logarithmic reductions are achieved, matching the reductions observed with both types of discharge.

7.3.2 Effect on material surfaces: stainless steel

This study investigates the inactivation of *E. coli* and *L. monocytogenes* inoculated on stainless steel and glass surfaces using the ion wind generated within the chamber through both positive and negative corona discharges. The results reveal that microbial inactivation depends on the treatment time, the type of discharge applied, and the initial inoculation conditions prior to treatment.

As described in Chapter 3, section Materials and Methods, two conditions of the bacterial suspension deposited on the samples of both materials are analyzed. The dry condition, where a known volume of the suspension is deposited on the material and allowed to dry before treatment, and the wet condition, where the bacterial suspension is directly deposited on the surface and immediately placed in the chamber (without prior drying) for plasma treatment.

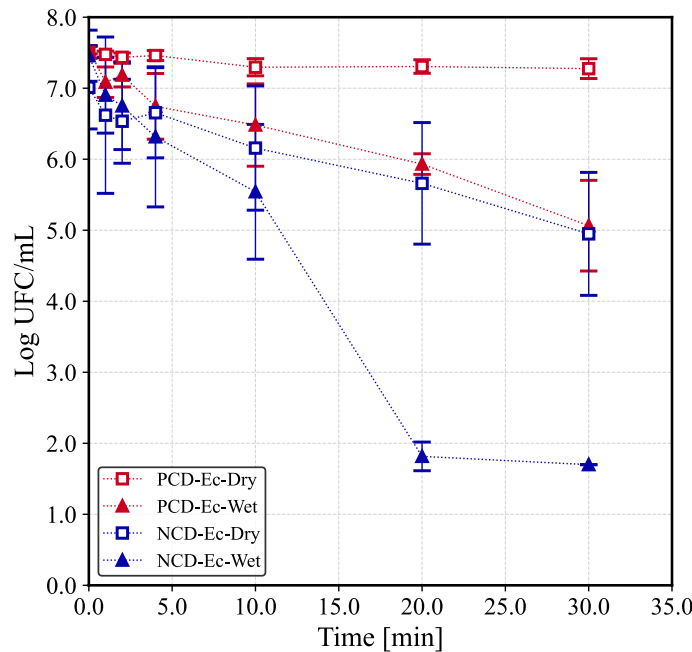


Figure 7.11: Positive corona discharge (PCD, red) and negative corona discharge (NCD, blue) at 30W for *E. coli* (Ec) in two suspension conditions: wet and dry at different treatment times on stainless steel.

The reductions of *E. coli* when treated in dry and wet conditions with both types of discharge

over time can be observed in Figure 7.11. It is evident that negative discharge and treating the inoculum without drying have greater effectiveness in bacterial inactivation, as higher logarithmic reductions are obtained. In 10 min of treatment under these two conditions, 2.05 logarithmic reductions are already achieved in *E. coli*. When treating the dry inoculum, barely any reductions are obtained with positive discharge, whereas with negative discharge, nearly 1 log-red is achieved in 10 min, reaching 2.06 log-red at the maximum treatment time (30 min). On the other hand, in the 30 min of treatment with the two most effective conditions (negative discharge and wet inoculum), up to 5.89 log-red are obtained.

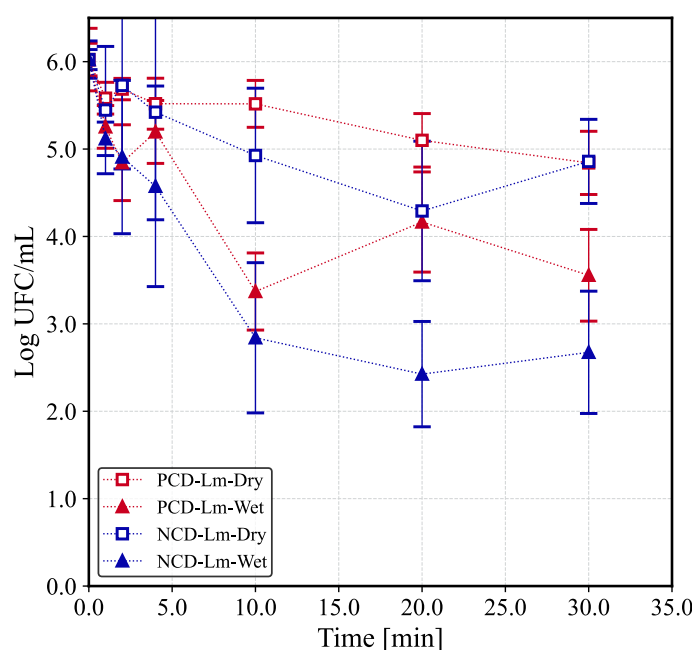


Figure 7.12: Positive corona discharge (PCD, red) and negative corona discharge (NCD, blue) at 30W for *L. monocytogenes* (Lm) in two suspension conditions: wet and dry at different treatment times on stainless steel.

Regarding *L. monocytogenes* (Figure 7.12), it is once again observed that negative discharge and treatment under wet conditions are more effective. However, negative discharge along with treatment in dry conditions also leads to better results than positive discharge, with 1.57 and 0.38 log-red achieved in just 1 min of treatment, respectively. With negative discharge and treatment under wet conditions, nearly 5 log-red (4.74) are obtained in 20 min of treatment. Practically double the reductions with negative discharge are achieved over the treatment times in both dry and wet conditions, concluding the 30 min dry treatment trial with 0.73 and 2.15 log-red for positive and negative discharge and in wet conditions with 2.14 and 4.51 log-red for positive and negative discharge, respectively.

7.3.3 Effect on material surfaces: glass

The aim of this study is to evaluate the degree of inactivation of *E. coli* and *L. monocytogenes* when exposed to the ionic wind generated in the chamber using positive and negative discharges, this time focusing on glass material samples. Negative discharge demonstrates a greater bactericidal effect than positive discharge, achieving higher logarithmic reductions across all treatment times. Furthermore, treating bacteria under wet conditions significantly increases reduction levels.

Figure 7.13 illustrates the inactivation of *E. coli* under dry and wet conditions for both types of discharge over the treatment duration. The effectiveness of both types of discharge is greater under wet conditions, particularly for negative discharge, which achieves 5.36 logarithmic reductions compared to 3.42 reductions with positive discharge after 30 min of treatment. In particular, after only 2 min of treatment under wet conditions, both discharges achieve a 1-logarithmic reduction. Conversely, under dry conditions, the bactericidal effects of both discharges are similar.

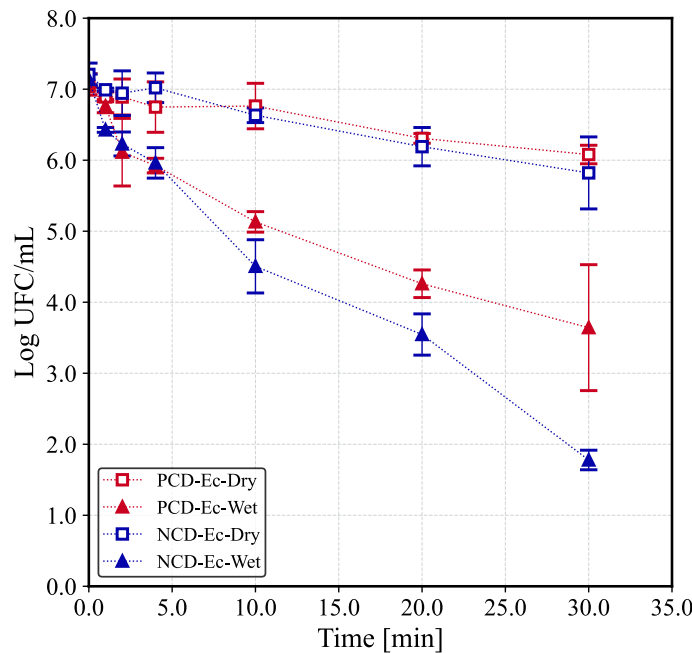


Figure 7.13: Positive corona discharge (PCD, red) and negative corona discharge (NCD, blue) at 30W for *E. coli* (Ec) in two suspension conditions: wet and dry at different treatment times on glass.

In Figure 7, it can observe the results of the treatment of *L. monocytogenes*, where it is once again evident that the effect of negative discharge is more detrimental, especially in wet conditions, achieving 4.04 logarithmic reductions after 30 min of treatment compared to 3.74 reductions with positive discharge. Already, 1.93 log-red are achieved in 2 min of treatment with negative discharge and wet conditions. However, when treating *L. monocytogenes* in dry conditions, a notable increase in reductions is observed when negative discharge is used after

5 min of treatment. At the maximum treatment time evaluated, negative discharge achieves 2.17 logarithmic reductions, approximately twice the 1.16 logarithmic reductions achieved with positive discharge.

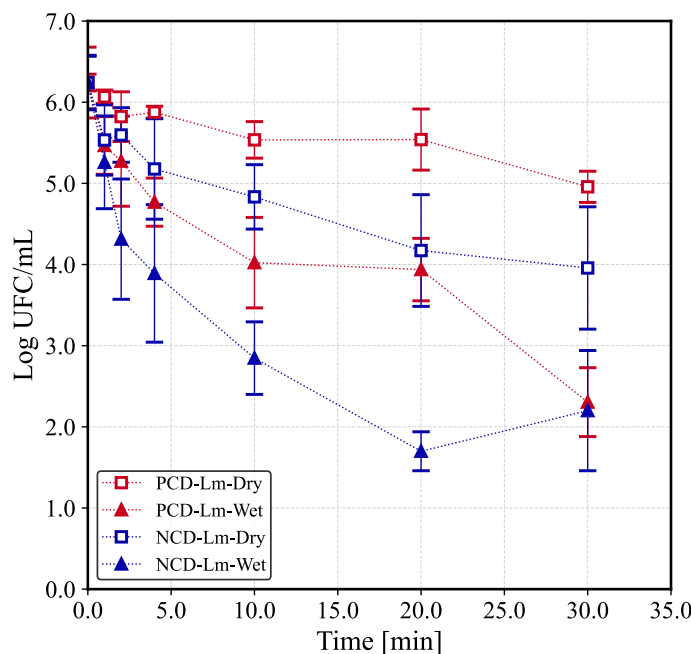


Figure 7.14: Positive corona discharge (PCD, red) and negative corona discharge (NCD, blue) at 30W for *L. monocytogenes* (Lm) in two suspension conditions: wet and dry at different treatment times on glass.

7.3.4 Inactivation mechanism and discussion

In this section, microbial inactivation kinetics for *E. coli* and *L. monocytogenes* are analyzed using theoretical models. The curves do not respond to linear functions, so it is necessary to use mathematical models capable of describing certain deviations in bacterial inactivation processes. The analysis is based on selected data considered most representative, specifically those obtained in agar medium. In addition, the degree of reduction for both bacteria are evaluated in relation to the dose of the chemical species used (CT value), with ozone (O_3) and nitrogen dioxide (NO_2) as reference compounds.

The dose (CT) of O_3 and NO_2 is defined as the accumulated concentration over each time interval required to achieve a specific level of inactivation, expressed in $[ppm \cdot min]$. These values are calculated by determining the area under the curve (AUC) for O_3 and/or NO_2 concentration versus time for each tested condition (geometric and electrical parameters and discharge mode). For positive corona discharge (PCD), the correlation is considered both O_3 and NO_2 , while for negative corona discharge (NCD), the analysis is focused on NO_2 , which is generated in significantly higher proportions.

Figures 7.15 and 7.16 display the fitting curves of various theoretical kinetic models applied to the data obtained for *E. coli* and *L. monocytogenes* under positive and negative corona discharge, respectively. The models used include the Weibull model, the biphasic model, and the biphasic with shoulder model, as described in Chapter 3. As shown, all the models provide a good fit to the experimental data.

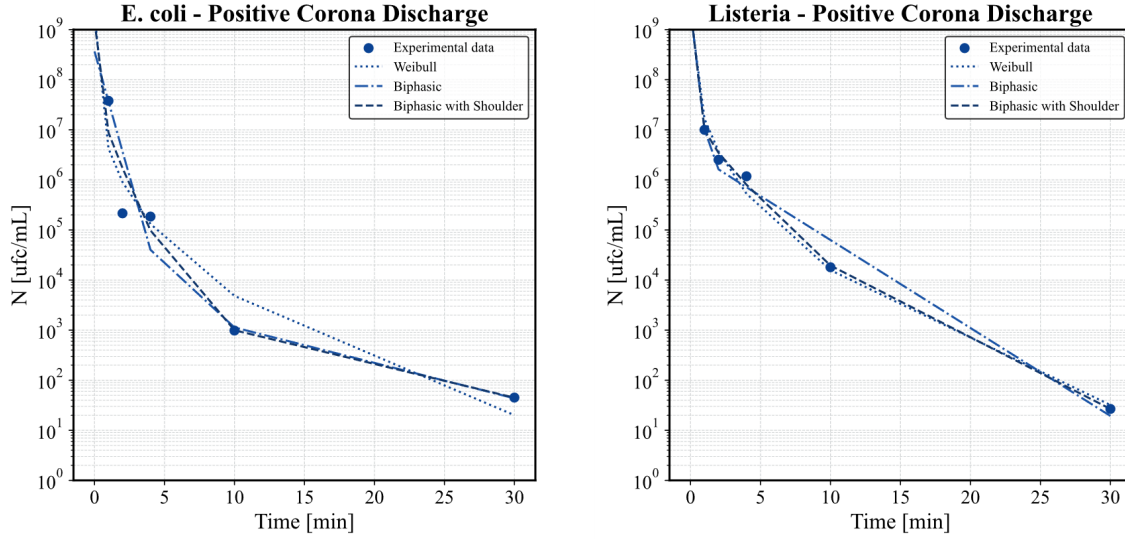


Figure 7.15: Fitting of mathematical models (Weibull, biphasic, and biphasic with shoulder) to inactivation curves of *E. coli* (left) and *L. monocytogenes* (right) in positive corona discharge operation mode at 30W in agar on a dish.

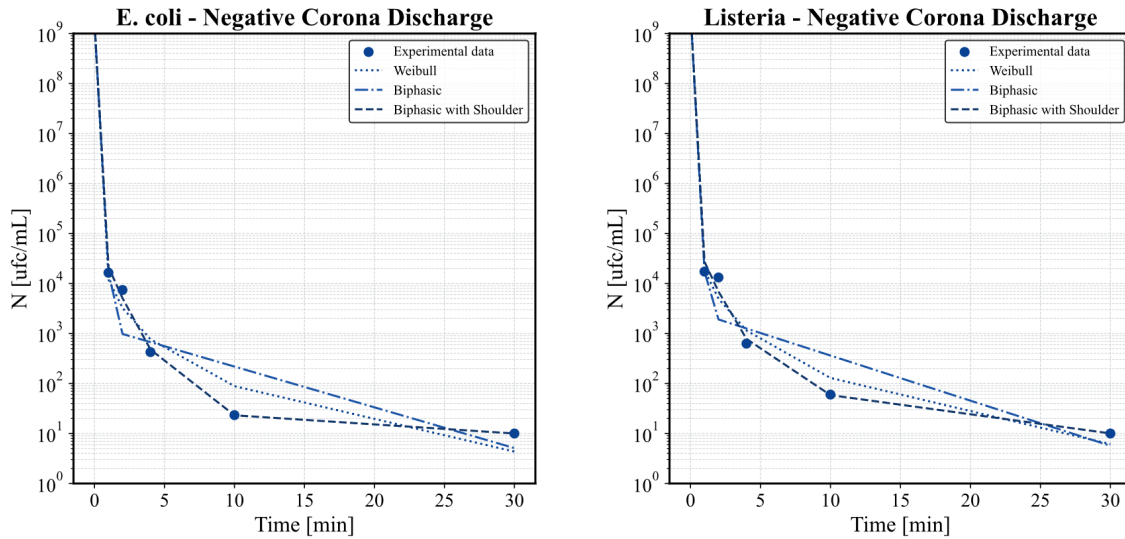


Figure 7.16: Fitting of mathematical models (Weibull, biphasic, and biphasic with shoulder) to inactivation curves of *E. coli* (left) and *L. monocytogenes* (right) in negative corona discharge operation mode at 30W in agar on a dish.

To assess the accuracy and reliability of the model fits, the root mean square error (RMSE) and the coefficient of determination (R^2) were calculated. Additionally, the "time to first decimal reduction" (D) was determined, representing the time required to achieve the first decimal logarithmic reduction in the bacterial population (e.g., from N_0 to $N_0/10$), as derived from each model. For the Biphasic models, although two D values are provided—one for the initial inactivation phase and another for the phase showing greater resistance to elimination—comparisons were made using the values from the first phase.

The equations used to calculate RMSE and R^2 are as follows:

Root Mean Square Error (RMSE):

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{n}} \quad (7.1)$$

where y_i represents the observed data, $\hat{y}_i$ represents the predicted values from the model, and n is the total number of observations.

Coefficient of Determination (R^2):

$$R^2 = 1 - \frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{\sum_{i=1}^n (y_i - \bar{y})^2} \quad (7.2)$$

where y_i represents the observed data, $\hat{y}_i$ represents the predicted values, and $\bar{y}$ is the mean of the observed data.

The estimated results are summarized in Table 7.1. All analyzed cases demonstrated strong fits, with R^2 values exceeding 0.93, indicating that these models are well suited to accurately fitting and modeling the experimental data.

The inactivation curves previously obtained for *E. coli* and *L. monocytogenes* in agar suspension medium are now analyzed against the chemical species dose, CT value delivered at each time interval for both positive and negative discharges at 30 W. The chemical generation curves of O_3 and NO_2 for each case study are used to estimate the areas under the curves (AUC) corresponding to each inactivation data point. The CT value (concentration x contact time) indicates that inactivation rates are determined by the product of concentration and exposure time rather than treating these factors independently. This means that a lower concentration applied over a longer contact time can achieve the same effect as a higher concentration over a shorter period, as long as the CT values are equivalent. This principle originates from the Chick-Watson model [136], which describes how the inactivation rate is influenced by the concentration of disinfectant and the exposure time, under the assumption of first-order kinetics. The CT value is calculated using the following equation:

$$CT = \int_{t_1}^{t_2} C(t) dt \quad (7.3)$$

where $C(t)$ is the concentration of the chemical species (O_3 or NO_2) at time t , and t_1 and t_2 represent the start and end times of the interval, respectively.

Table 7.1: Fitting parameters for microbial inactivation models applied to *E. coli* (Ec) and *L. monocytogenes* (Lm) under positive corona discharge (PCD) and negative corona discharge (NCD).

Bacteria	Operation Mode	Model	D [min]	RMSE	R ²
Ec	PCD	W	0.0464	0.5688	0.9500
		B	0.4348	0.6246	0.9397
		BS	0.7054	0.4649	0.9666
	NCD	W	0.00002	0.3365	0.9847
		B	0.0864	0.5541	0.9586
		BS	0.8582	0.0837	0.9990
Lm	PCD	W	0.1363	0.1876	0.9942
		B	0.1717	0.2581	0.9891
		BS	1.6555	0.0906	0.9987
	NCD	W	0.00002	0.2602	0.9910
		B	0.0820	0.4944	0.9676
		BS	0.9131	0.1453	0.9972

W: Weibull, B: biphasic, BS: biphasic with shoulder. RMSE: root mean square error

This approach allows for a detailed correlation between bacterial inactivation and the accumulated dose of reactive chemical species generated during plasma treatment.

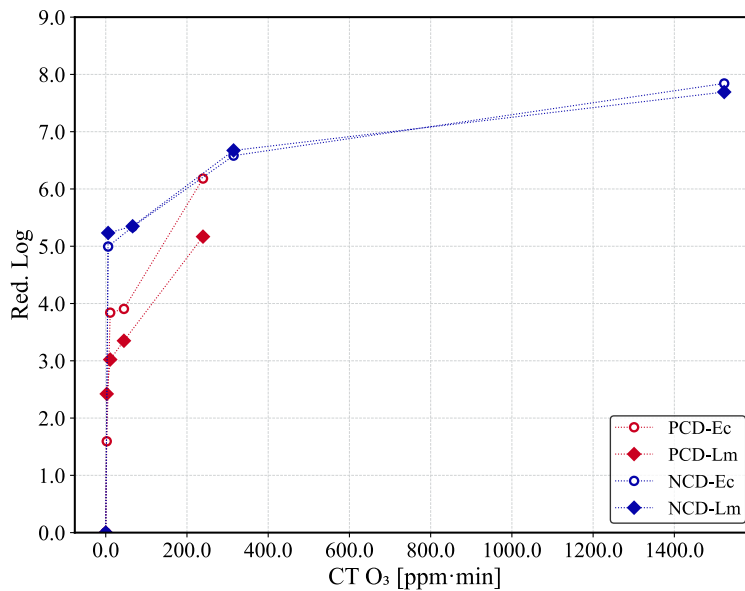


Figure 7.17: Positive corona discharge (PCD, red) and negative corona discharge (NCD, blue) at 30W for logarithmic reductions of *E. coli* (Ec) and *L. monocytogenes* (Lm) at different CT dose O₃ values.

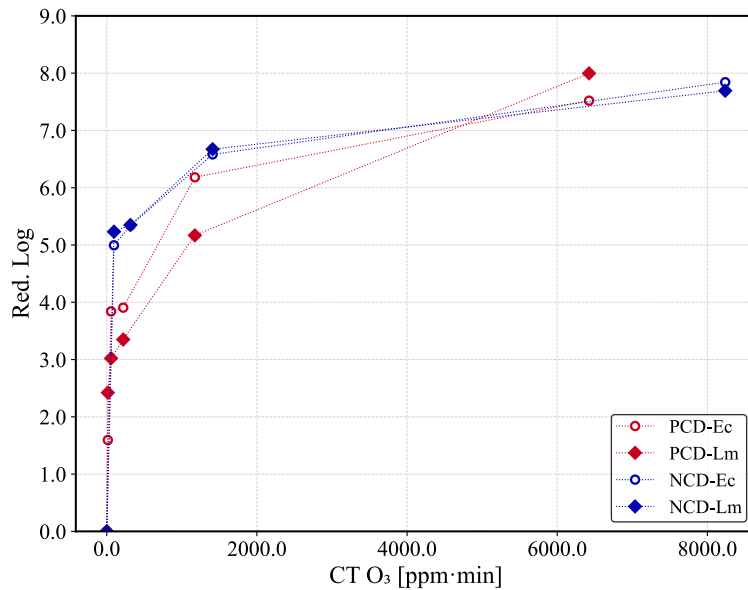


Figure 7.18: Positive corona discharge (PCD, red) and negative corona discharge (NCD, blue) at 30W for logarithmic reductions of *E. coli* (Ec) and *L. monocytogenes* (Lm) at different CT dose NO_2 values.

Figures 7.17 and 7.18 represent the accumulated doses CT of O_3 and NO_2 , respectively, for each logarithmic reduction point obtained for *E. coli* and *L. monocytogenes* under positive and negative corona discharges. The curves appear consistent, suggesting that the degree of inactivation may depend on the accumulated dose of each chemical species. This effect is very noticeable, where the curves almost overlap, with only a slight divergence observed for *Listeria* under positive discharge for the CT NO_2 dose value. In the case of the CT O_3 value, similar trends are observed. It should be noted that the results obtained in both types of bacteria in negative corona discharge are similar, while in positive corona discharge the reductions are slightly lower for the same TC value. This could be because there are other species that have not been quantified that are involved in the inactivation mechanisms, whether neutral or active.

Logarithmic reductions of 4-log are observed at approximately $CT \text{NO}_2 = 20 \text{ ppm} \cdot \text{min}$ and $CT \text{O}_3 = 45\text{--}50 \text{ ppm} \cdot \text{min}$. For 6 log-red, both bacteria require around $CT \text{NO}_2 = 1200 \text{ ppm} \cdot \text{min}$ and $CT \text{O}_3 = 240 \text{ ppm} \cdot \text{min}$. The maximum reductions observable in the experiment are achieved beyond $CT \text{O}_3 = 1000 \text{ ppm} \cdot \text{min}$ and $CT \text{NO}_2 = 4000 \text{ ppm} \cdot \text{min}$.

7.4 Conclusion

This study demonstrates the efficacy of atmospheric plasma generated by corona discharge as a microbial inactivation tool on inert surfaces contaminated with viruses (SARS-CoV-2 and HCoV-229E) and bacteria (*Escherichia coli* and *Listeria monocytogenes*) in an enclosed chamber. The results highlight several key findings regarding the influence of material properties, suspension conditions, and operational parameters.

One of the most significant milestones achieved is the attainment of reductions exceeding 6 log in materials after 30 minutes of treatment at 30 W of power under surface humidity conditions for both bacteria studied. This highlights the importance of further exploring this composition, as well as investigating the use of water at the surface level or in nebulize form. This approach could enhance interaction with the ionic wind, leading to greater species diffusion and facilitating potential scaling.

It would also be valuable to explore the replacement of current industrial processes, such as autoclave or nebulize hydrogen peroxide disinfection, with these plasma-based technologies.

The plasma effectiveness is consistent across dried and non-dried viral suspensions, making it versatile for various materials and conditions.

The virucidal effect of plasma is evident under both wet and dry conditions, achieving reductions greater than 4 log-red for HCoV-229E after 45 min at 50 W. For SARS-CoV-2, complete inactivation is observed as 15 min on polypropylene and FFP2 mask filter materials, while stainless steel and glass required up to 45 min to achieve maximum reductions. These findings indicate variability in the plasma effectiveness depending on the material, with polypropylene and mask materials being more susceptible. Furthermore, the presence of 2% fetal bovine serum significantly reduced plasma efficacy due to shielding effects; however, dilutions of the medium restored substantial virucidal activity. This may be attributed to the presence of organic compounds and others interacting with the species generated by the plasma system. Conducting further detailed studies could be valuable to better understand the interaction between plasma and organic matter, particularly from the perspective of surface decontamination. Moreover, during drying, substances such as inorganic salts may crystallize, potentially coating viruses and reducing their exposure to ionic wind. While these variables significantly impact atmospheric plasma disinfection, detailed studies on their effects on virus inactivation are currently limited [137].

In bacterial assays, the negative corona discharge (NCD) mode was significantly more effective than the positive corona discharge (PCD) mode, achieving faster and higher reductions. For *E. coli*, NCD reached 8 log reductions within 10 min, while PCD required 30 min to achieve similar results. Similarly, for *L. monocytogenes*, NCD achieved maximum reductions within 10 min, indicating its higher bactericidal potency. Wet inoculation conditions further enhanced the plasma effectiveness compared to dry conditions, particularly on stainless steel and glass surfaces. This may be due to the interaction of plasma-generated species with surface water, potentially producing nitrogen-derived acidic groups, ammonia, and highly oxidative hydroxyl groups. These reactions significantly enhance the germicidal effect. Further investigation into this phenomenon would be valuable, such as increasing the humidity within the chamber or

nebulize a controlled amount of water onto the samples prior to treatment.

Nonlinear inactivation kinetic models are also analyzed for the agar tests conducted on both types of bacteria studied. The models considered include Weibull, biphasic, and biphasic with shoulder. To assess the goodness of fit, the R^2 and RMSE (root mean square error) values were calculated, with R^2 values exceeding 0.93. These results confirm that such models are appropriate for describing the inactivation kinetics observed in the experiments. Among all cases, the BS model consistently shows the highest R^2 and the low RMSE values, indicating that it best describes the inactivation kinetics. This suggests the presence of an initial resistance phase (shoulder) and more than one microbial subpopulation, a phenomenon commonly observed in non-thermal treatments such as corona discharges [75, 138–140]. For the Weibull model, D-values are very low, which may lack practical meaning unless interpreted as a scale parameter. In contrast, the BS model yields higher D-values, which aligns with the concept of a shoulder phase or initial resistance.

The logarithmic reduction data for *E. coli* and *Listeria monocytogenes* on dishes are presented in relation to the concentration-time (CT) values of O_3 and NO_2 . The same levels of inactivation are observed for identical CT values for both gases. This establishes a concentration-time correlation, which serves as a baseline for scaling the systems effectively.

Logarithmic reductions of 4-log were achieved at approximately $CT\ NO_2 = 20\text{ ppm} \cdot \text{min}$ and $CT\ O_3 = 45\text{--}50\text{ ppm} \cdot \text{min}$, while maximum reductions were achieved beyond $CT\ NO_2 = 4000\text{ ppm} \cdot \text{min}$. This correlation underscores the importance of reactive species dosage in optimizing plasma-based disinfection processes. However, there are differences in the treatment in positive and negative corona discharge. Similar trends are detected in the degree of reduction in both bacteria in the case of negative discharge, being slightly lower in the positive discharge. This could be due to the possible presence of other neutral or active species that are participating in the inactivation mechanisms. For future studies, it is necessary to deepen the analysis of this phenomenon, quantifying other possible chemical compounds. Likewise, it would be valuable to isolate the effects of O_3 and NO_2 by using O_2 instead of air. Different oxygen concentrations could be tested to suppress reactions stemming from the presence of N_2 . However, as the goal in this case is to develop a simple system with minimal cost and energy consumption, air under ambient conditions was chosen for the experiments.

In this type of treatment, the germicidal effect of ANTP is mainly produced by the neutral species generated and transported by the ionic wind, mainly ozone and nitrogen dioxide, as well as possible by-products produced by air-water interaction in the case of wet samples. The most aggressive active species and free radicals remain in the areas close to the discharge.

In conclusion, atmospheric plasma is a highly effective, adaptable, and scalable disinfection technology. The variability in material response and the influence of organic matter suggest that further optimization of plasma parameters and pre-treatment conditions may enhance its applicability for real-world decontamination challenges.

Chapter 8

Conclusions and future work

8.1 Main conclusions

This PhD thesis has leveraged atmospheric non-thermal plasma systems through the research and industrial development of innovative devices capable of inactivating pathogens of surfaces and environments. Through the knowledge generated, unique devices for Cedrión are developed, along with a long-term collaboration on a new line for Universidad Politécnica de Madrid.

The research provides a comprehensive understanding of the key factors influencing the performance of atmospheric non-thermal plasma systems generated by corona discharge. Understanding the operating mechanisms of these systems is fundamental for their scaling and application in various industrial sectors.

Moreover, the type of development undertaken has provided skills in design, knowledge of additive manufacturing and prototyping, as well as the ability to develop and structure an experimental process.

The laboratory where these developments are conducted is set up and optimized, incorporating new characterization techniques that can be applied in future developments not only in the plasma field but also in other areas. For instance, the optical emission spectroscopy equipment used in this work can also be employed in material characterization, offering the potential to evaluate possible effects of the treatment on different materials analyzed.

The research demonstrates the critical role of electrical and geometric parameters in optimizing plasma systems for diverse applications, including disinfection, air treatment, and microbial inactivation.

The main advantage of the developed systems is their ability to combine a high degree of microbial reduction with the generation of ionic wind and a concentration of species that is appropriately designed for this purpose, showing low concentration levels (in the case of positive discharge) compared to other systems of the same type.

Regarding the chemical and fluid dynamics characterization, the main conclusions are sum-

marized as follows:

- The production of long-lived reactive species, such as ozone (O_3) and nitrogen dioxide (NO_2), is significantly influenced by electrical parameters. Mass flow rates correlate linearly with the discharge current, and the type of discharge (positive or negative) has a profound impact on species concentration. Negative discharge generates up to ten times more O_3 and NO_2 than positive discharge due to higher electron energy and ionization efficiency. Electrode geometry also affects species generation; smaller electrode gaps enhance chemical reaction rates, while larger gaps promote higher ionic wind velocities but dilute reactive species.
- The second positive system (SPS) of nitrogen is detected via optical emission spectroscopy in both positive and negative discharges, with the negative discharge being 10 times more intense. The highest intensity peaks are observed at 337.1 nm and 357 nm. These emissions are directly related to electron excitation processes, and their intensity exhibited a linear relationship with the system's supplied current. The intensity in the negative discharge is 4.5 times greater than in the positive discharge, correlating with higher electron energy and greater chemical activity. This is particularly interesting from the perspective of system application and modeling.
- The concentration of NO_2 is significantly higher than that of O_3 in closed-chamber experiments within the analyzed working range. A quenching effect on O_3 begins to be observed at higher total power levels. At this point, O_3 actively participates in the reactions leading to the formation of other compounds.
- A fitted kinetic modeling approach is proposed to describe the reaction rate of the species in a closed chamber, based on certain assumptions. The model demonstrates good fit quality, suggesting that the proposed approach effectively describes the data within the analyzed working range.

Regarding the evaluation of the effect of atmospheric plasma on microbial inactivation, the following main conclusions are presented:

- An experimental setup has been designed, fabricated, and implemented to evaluate plasma systems against a stream of viral aerosols.
- Complete inactivation of SARS-CoV-2 on FFP2 mask filters and polypropylene surfaces is achieved within 15 minutes at 50 W of power, while other materials, such as stainless steel and glass, required up to 45 minutes to achieve maximum reductions of over 4 log (99.99%). For HCoV-229E, reductions greater than 4 log were observed after 45 minutes under similar conditions. These results emphasize the versatility of plasma in disinfecting high-risk materials.
- Nonlinear bacterial inactivation kinetic models have been evaluated for *Escherichia coli* and *Listeria monocytogenes* on petri dishes, achieving good fit coefficients and validating the use of these models.
- Better inactivation results are obtained in the case of negative discharge in wet conditions. These findings are consistent with the chemical generation data, as higher concentrations

of oxidizing species are produced in shorter times. Moreover, the presence of surface water can promote the generation of highly oxidizing OH groups and nitrogen-derived acids, which could significantly impact the degree of inactivation.

- The reduction data for *E. coli* and *L. monocytogenes* are presented in relation to the accumulated concentration · time (CT) values of NO₂ and O₃. It is observed that the same degree of reduction is achieved for identical CT values under ideal conditions on agar, regardless of the operational mode.
- Up to 6 log reductions are achieved on stainless steel and glass for *E. coli* and *L. monocytogenes* under wet conditions and negative corona discharge after 30 min of treatment. This is an important achievement, as it reaches the level of material sterilization.

From a practical perspective, the system achieved rapid viral inactivation on critical surfaces such as mask filters and polypropylene, highlighting its applicability for high-risk scenarios. The absence of moving parts ensures noiseless operation, while its scalability and adaptability make it a promising technology for air treatment and disinfection in poorly ventilated spaces.

In parallel studies conducted within the company Cedrión, aimed at enabling the use of the optimized system for open air treatment, various catalytic phases have been analyzed. Suitable flow rate values have been achieved with concentrations below exposure limits (0.08 ppm in the case of ozone).

In conclusion, this study establishes a strong foundation for the design and optimization of scalable and energy-efficient NTP systems. By balancing electrical inputs and geometric configurations, these systems can be tailored to achieve high efficiency in chemical species generation and microbial inactivation. Future research focusing on interactions between plasma, organic matter, and microorganisms will further enhance their real-world applicability and efficiency in diverse disinfection challenges.

8.2 Future research lines

Non-thermal atmospheric plasma generated by corona discharge continues to demonstrate excellent performance across various applications. Moreover, new opportunities for adapting these systems to different sectors and uses are constantly emerging, making this an innovative and rapidly developing branch of science.

The large-scale industrial implementation of the proposed systems for sterilizing objects in sealed chambers would require compliance with the Medical Device Regulation (MDR) 2017/745 [141]. It would be necessary to develop and execute the relevant protocols to register these systems with the Spanish Agency of Medical Devices (AEMOS), as well as to conduct a comprehensive review of the requirements to be met and the steps to be undertaken. A review on this matter was initiated as part of the CIEN-INNMEDICAL project by CDTI, carried out in collaboration with Fagor Electrónica.

Despite the extensive research already conducted in this field, several mechanisms remain poorly modeled or insufficiently explained. Models that establish clear correlations between

chemical and fluidic processes are limited in the literature. Such models are essential for bridging the gap between laboratory-scale prototypes and fully industrialized products.

Following the developments achieved during this thesis, both areas of improvement and several interesting aspects have been identified as potential points of future development to continue advancing in the established line of research.

First, practical models that describe real-world applications are essential for subsequent scaling processes. These models should be based on extensive parametric analyses to provide a more accurate representation of the system's behavior and properties, allowing them to be adapted to other designs and scenarios. In this context, the use of artificial intelligence emerges as a promising approach to model large datasets efficiently, enabling faster and more precise scalability predictions.

To identify which compound has the greatest effect on the degree of inactivation, it is proposed to suppress the generation of certain compounds. For instance, ozone generation could be suppressed by achieving higher power densities (power/discharge area), as the ozone produced would be consumed in forming other nitrogen-derived species. Since current systems have limitations regarding the stability and total power that can be supplied, it is suggested to analyze configurations that increase power capacity, building on the designs already studied. Possible designs include three-point discharge, wire-to-grid discharge, and others. Additionally, experiments using pure O_2 instead of air could be conducted to reduce the concentration of N_2 and, consequently, minimize nitrogen-derived reactions.

The best results for microbial inactivation efficacy have been achieved under negative discharge operation mode and surface humidity conditions. Based on these findings, two main directions are proposed for further investigation. First, a more detailed analysis of the effect of humidity on microbial inactivation and its interaction with the ionic wind generated by the plasma. Second, an exploration of plasma systems that produce high reactive species concentrations in configurations other than negative discharge.

Generally, negative corona discharge tends to be less stable. While this is not a significant issue at low power levels, scaling up to significantly higher power levels may require analyzing alternative configurations to ensure system stability.

Regarding increased humidity, it could reduce the required concentrations of reactive species, thereby lowering electrical consumption. Studies are proposed to focus on water nebulization within the chamber to identify the optimal humidity level for water-ion wind interaction, achieving high microbial reductions.

In both cases, the materials used for the electrodes must be carefully reviewed, as the increased generation of oxidizing agents, including acidic groups, may lead to accelerated electrode degradation. Additionally, an increase in humidity could destabilize the devices.

An alternative approach could involve systems with a dedicated ionic wind generation zone, which directs the flow to a separate, isolated area where nebulization and microbial treatment take place. This configuration could mitigate potential stability issues while maintaining effectiveness.

On the other hand, Plasma-Activated Water (PAW) is gaining significant interest across various fields of development, including microbial inactivation. With a solid understanding of chemical modeling, processes can be developed to transfer the generated species into water and evaluate the distinct properties they acquire.

The interaction of NO_2 with water, along with other nitrogen-derived compounds, can produce nitrates and nitrites, which are particularly interesting from the perspective of fertilizer production.

Minimizing the oxidizing compounds generated in open systems could be advantageous for cooling applications, as these compounds may degrade the materials they come into contact with. Using small-sized corona electrodes combined with catalytic material coatings could help limit the production of such compounds.

Applying these processes to current systems would be particularly valuable, considering their existing use in cooling applications. This approach could enhance material longevity while maintaining system efficiency.

Another important aspect to investigate is whether materials subjected to plasma treatment for disinfection experience any degradation or alterations that could modify their properties over prolonged exposure times.

8.2.1 Research lines in progress

Based on the results obtained in this PhD thesis, several lines of research are currently underway. Some of them are:

- Evaluating the efficacy of ANTP for reducing volatile organic compounds (VOCs). A test bench has been designed and manufactured for this purpose (Figure 8.1).



Figure 8.1: Experimental setup for the evaluation of the efficacy of ANTP against VOCs.

- Integration of plasma systems in 3D printing manufacturing processes for the disinfection of biomedical materials.

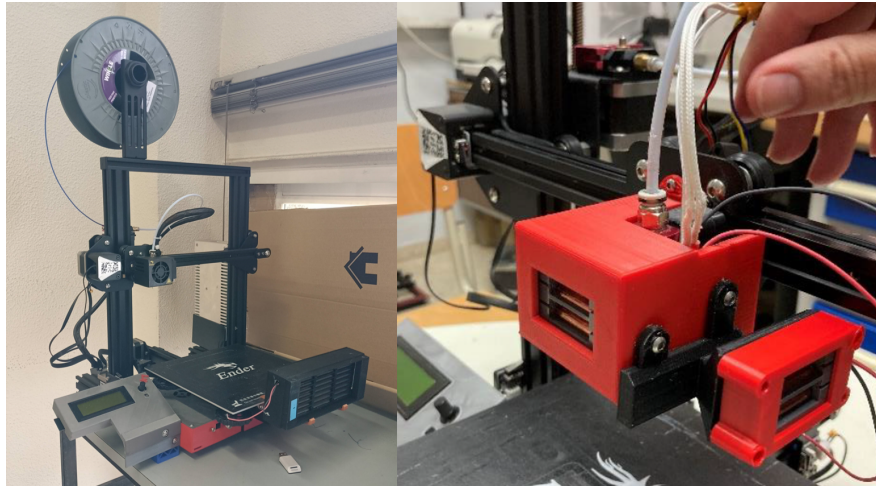


Figure 8.2: ANTP devices integrated into 3D printers.

- Use of catalysts as electrodes and their integration into the corona discharge plasma generation process.

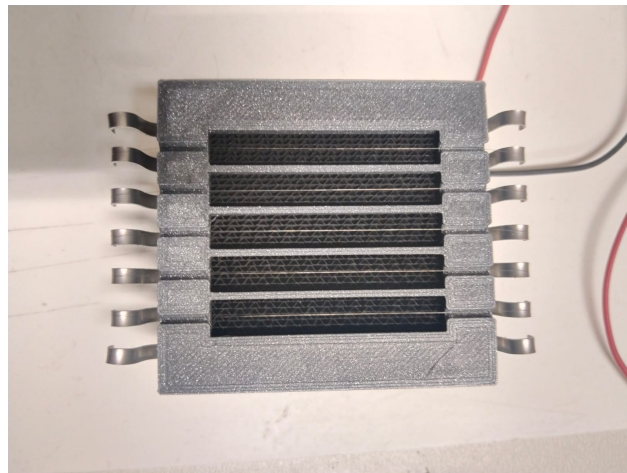


Figure 8.3: Prototype ANTP device with MnO_2 catalyst supported on corrugated glass fiber as a grounding electrode.

8.3 Benefits for UPM and Cedrión Consultoría Técnica e Ingeniería, S.L.

The benefits obtained by the entities whose collaboration made this thesis possible (Cedrión: Consultoría Técnica e Ingeniería, S.L. as the industrial environment and the Department of Mechanical Engineering of the UPM as the academic environment) are diverse and summarized as follows.

From the company's perspective, the project has established the foundation for opening several market niches, integrating cooling and processes of disinfection and sterilization. A range of products has been developed for implementation across various industrial sectors. In addition, new lines of research have been initiated to improve and expand the current applications of the systems:

1. Development of Cedrión's first product, commercially named "KIRION," an air treatment system using atmospheric plasma to inactivate viruses and bacteria while reducing particulate matter. It can be used in the presence of people as a result of efficient modeling and the study and use of catalysts to control the generated chemistry.
2. Application of KIRION in various sectors: offices, restaurants, the food industry, hospitals, etc.
3. Feasibility analysis of reducing ambient air pollutants using the developed systems. The aim is to build on the progress of the thesis and generate new projects.
4. Development of a chamber for object treatment in the healthcare sector, intended for implementation in the industry through the INNMEDICAL project by CDTI, in collaboration with Fagor Electrónica.
5. Opportunities in the pharmaceutical sector, which has strict disinfection requirements. Projects have been initiated with various companies to explore replacing current hydrogen peroxide Sterile Access Systems (SAS) with plasma-based systems. These systems are not only applicable to the pharmaceutical sector but also to research and healthcare environments with stringent protection standards. Air treatment proposals are also being considered.
6. Exploration of integrated disinfection in the customized manufacturing of medical devices using additive technologies (e.g., 3D printing). Interest has also grown in integrating plasma systems into food manufacturing processes via 3D printing to maintain sterility.
7. New development and innovation projects in the agri-food sector.

The academic impact, particularly regarding the new R&D&I lines of the Product Development Laboratory and the Department of Mechanical Engineering at the Polytechnic University of Madrid, has also been significant. Highlights include:

1. Establishment of a laboratory for conducting tests in controlled environments and for fluid-dynamic and chemical characterization.
2. Implementation and optimization of new spectrometric characterization techniques and surface analysis of materials, paving the way for new research, developments, and collaborations with various research groups.
3. Generation of knowledge and value in the area of non-thermal plasmas and their species, applicable to disinfection and sterilization of materials used in the manufacturing of medical equipment through additive technologies.
4. Development of a novel 3D printing technique, combining fused filament deposition and in-situ plasma sterilization, with significant medical applications.

5. Strategic alliances with the Gas Discharge Physics Laboratory (GDPL) of the Department of Nuclear and Quantum Engineering at KAIST (Korea Advanced Institute of Science and Technology). This collaboration enables future bilateral exchanges of researchers and professors with one of Asia's top universities.
6. Generation and dissemination of scientific and technical knowledge on atmospheric plasma technology and its applications, positioning the Department of Mechanical Engineering as a national reference in this field.

Additionally, a national PERTE (Strategic Projects for Economic Recovery and Transformation) project has been achieved, which is being executed by CEDRIÓN in collaboration with the Product Development Laboratory of UPM and the University of León. This reinforces the sustainability of the collaboration between industrial and academic environments. The project, named ALIMTECH: PP13 – "Research on Atmospheric Plasma Disinfection and Cleaning Applied to the Food Industry," continues the line initiated by the researcher, reinforcing the links generated and opening up new opportunities for future projects and knowledge transfer.

8.4 Summary of scientific-technological and industrial contributions

Articles in JRC-indexed journals

7. **Ruiz-Trujillo, S.**, Vega-Martínez, P., Esparza, I., Usera, F., Sánchez-Soriano, P., van Raaij, M. J. and Díaz Lantada, A. (2023). Electrohydrodynamic devices for ionic wind generation and airborne positive-strand RNA viruses inactivation by nonthermal atmospheric plasma. *Plasma Processes and Polymers*, 20(10), e2300046.
8. **Ruiz-Trujillo, S.** Vega-Martínez, P., Herrera-Arteaga, M. I. and Díaz Lantada, A. (2025). Experimental research on atmospheric non-thermal plasma devices under positive and negative discharge operating conditions: effects on ionic wind and generated species. *Plasma Processes and Polymers*, e70024.
9. Müller, T., Scholz, S., Ehrhardt, M., **Ruiz-Trujillo, S.**, Cogollo de Cádiz, M., Díaz Lantada, A., Guttman, M. (2025). Hybridizing 3D printing and electroplating for the controlled forming of metals within fused deposition modeled contours. *The International Journal of Advanced Manufacturing Technology*, 1-13.

Works presented in congresses and conferences

10. **Ruiz-Trujillo, S.**, Vega-Martínez, P., Díaz Lantada, A., (2023). Electrohydrodynamic atmospheric plasma system for efficient production and control of reactive oxygen and nitrogen species. 4th International Workshop on Plasma Agriculture, IWOPA4, Kwang Woon University in Seoul, Korea.
11. **Ruiz-Trujillo, S.**, Vega-Martínez, P., Fernández García, C, Díaz Lantada, A., López, M. (2023). Integration of atmospheric non-thermal plasma electrohydrodynamic devices generated by corona discharge in 3D food printers. 9th International Conference on Food Chemistry and Technology, FTC-2023, París, Francia.

Industrial technology transfer projects

12. Programa de Doctorados Industriales, Comunidad Autónoma de Madrid y Universidad Politécnica de Madrid, Reference: IND2020/IND-17525.
13. CIEN-INNMEDICAL CDTI Project: Tratamiento del ambiente del habitáculo de vehículos with Grupo Antolín. Automotive sector: 2020-2022.
14. CIEN-INNMEDICAL CDTI Project: Cámara de desinfección de objetos en el ámbito sanitario with FAGOR Electrónica. Medical sector: 2021-2023.
15. National Project PERTE (Proyectos Estratégicos para la Recuperación y Transformación Económica), in collaboration with Universidad Politécnica de Madrid and Universidad de León. Project: ALIMTECH: PP13 - Investigación de la desinfección y limpieza por plasma atmosférico aplicado a la industria alimentaria.

Final degree thesis

16. Co-direction with the Prof. Andrés Díaz Lantada and Eleonora Atzeni of the Master's Thesis of Mr. Antonio De Palois, Design, prototyping and testing of components for incorporating low-temperature plasma sterilizers to 3D printers, 2022.
17. Co-direction with the Prof. Jorge Rodríguez Chueca and Mr. Adrián López Arrabal of the Master's Thesis of Mr. Jesús Quintana Terriza, Activación de agua mediante plasma atmosférico con diferentes técnicas electromecánicas, 2022.
18. Co-direction with the Prof. Andrés Díaz Lantada of the Master's Thesis of Mrs. María Isabel Herrera Arteaga, Investigación del comportamiento fluídico-químico de dispositivos de plasma atmosférico aplicados a la purificación de aire, 2024
19. Co-direction with the Prof. Andrés Díaz Lantada of the Bachelor's degree final project of Mr. Pablo Moreno Serrano, Estudio del impacto en materiales de la integración de sistemas de plasma atmosférico en procesos de fabricación aditiva para desinfección in situ, 2024.

8.4.1 Other formative actions during the PhD thesis

As mentioned, the doctoral student completed a three-month international research stay at the Korea Advanced Institute of Science and Technology (KAIST). During this period, she was mentored by Prof. Dr. Wonho Choe, of the Department of Nuclear and Quantum Engineering at KAIST, a renowned expert in fusion and low-temperature plasma studies, including their modeling and application analysis. The tasks were carried out in the Gas Discharge Physics Laboratory (GDPL). Beyond the significant knowledge gained and the invaluable international experience, this stay enables the student to qualify for an international distinction.

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